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Stepwise Regression Models-Based Prediction for Leaf Rust Severity and Yield Loss in Wheat

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Abstract: Leaf rust is a devastating disease in wheat crop. The disease forecasting models can facilitate the economic and effective use of fungicides and assist in limiting crop yield losses. In this study, six wheat cultivars were screened against leaf rust at two locations, during three consecutive growing seasons. Subsequently, the stepwise regression analysis was employed to analyze the correlation of six epidemiological variables (minimum temperature, maximum temperature, minimum relative humidity, maximum relative humidity, rainfall and wind speed) with disease severity and yield loss (%). Disease predictive models were developed for each cultivar for final leaf rust severity and yield loss prediction. Principally, all epidemiological variables indicated a positive association with leaf rust severity and yield loss (%) except minimum relative humidity. The effectiveness of disease predictive models was estimated using coefficient of determination (R^2) values for all models. Then, these predictive models were validated to forecast disease severity and yield loss at another location in Faisalabad. The R^2 values of all disease predictive models for each of the tested cultivars were high, evincing that our regression models could be effectively employed to predict leaf rust disease severity and anticipated yield loss. The validation results explained 99% variability, suggesting a highly accurate prediction of the two variables (leaf rust severity and yield loss). The models developed in this research can be used by wheat farmers to forecast disease epidemics and to make disease management decisions accordingly.

Keywords: leaf rust; regression model; *Triticum astivum* L.; yield loss; disease forecast

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Citation: Ali, Y.; Raza, A.; Iqbal, S.; Khan, A.A.; Aatif, H.M.; Hassan, Z.; Hanif, C.M.S.; Ali, H.M.; Mosa, W.F.A.; Mubeen, I.; et al. Stepwise Regression Models-Based Prediction for Leaf Rust Severity and Yield Loss in Wheat. *Sustainability* **2022**, *14*, 13893. https://doi.org/10.3390/su142113893

Academic Editor: Emanuele Radicetti

Received: 28 July 2022
Accepted: 15 October 2022
Published: 26 October 2022

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1. Introduction

Leaf rust caused by *Puccinia triticina* Eriks is a devastating foliar disease of wheat in Pakistan and all the wheat-growing regions of the world [1]. The epidemic of leaf rust has caused severe yield losses in the past and continue to be a constant threat to future grain production. The disease appears in early March on the wheat crop, and it can spread quickly depending upon conducive environmental conditions and the availability of the vulnerable host [2]. The earlier disease onset, later sowing and maturation, warmer winter,

further cooling and moist days periods, emergence of novel virulent leaf rust races, lack of partial resistance in wheat germplasm, and favorable environmental conditions for the diffusion of pathogen increase the chances of severe epidemics of this disease that may significantly affect wheat production [3]

Plant genetic resistance is the key strategy to avoid severe disease outbreaks and reduce crop losses. The variations in pathogen virulence and the lack of slow rusting resistance increase the need for predictive disease models. The forecasting models are valuable to ensure economic and effective use of fungicides and limit yield losses due to novel pathogen variants [4]. In Pakistan's wheat-growing areas, leaf rust sustains on vulnerable wheat cultivars and grass hosts. The disease spreads by airborne spores, resulting in rust epidemics, especially in wheat crops cultivated in the hilly areas of the country [3,5].

Wheat yields associated with leaf rust were reported to be decreased by 5–15% in susceptible cultivars in Canada, 10–21% in the United States (during moderate to severe epidemic years) and up to 40% in Mexico in 1976–1977 [6]. In Argentina, nearly 2,800,000 tons of wheat were lost due to leaf rust from 1948 to 1958, and yields were decreased by 80 kg/ha for each 10% of leaf rust severity when gains production exceeded 1100 kg/ha [7]. The grain losses were 38.6–50.5% and 8.7% for early and late epidemics, respectively [7].

Crop models, remote sensing, and meteorological data analyses are powerful tools for predicting the incidence of a specific disease [8]. Leaf rust epidemics have been successfully forecasted using empirical and mechanistic models [9–11]. The empirical approaches were developed based on epidemiological factors alone [12,13] or in combination with biological factors [4,14,15]. Most of the former investigations developed disease predictive models by screening selected wheat genotypes in Europe and Argentina [16].

A stepwise regression model based on the severity of leaf rust during three successive growing seasons, i.e., 2016/2017, 2017/2018 and 2018/2019, was developed in Egypt, where winter temperature and precipitation are extremely important for predicting leaf rust severity. In Egypt, hours of dew and accumulated degree days over 14 °C in March explained >70% of the disease severity variation for many commercial cultivars [9]. A few empirical models have been developed previously in Pakistan to forecast the leaf rust epidemics. However, these models consist of a one-to-two-year data set and only characterize the environmental conditions conducive to disease development [17,18]. Still, studies regarding stepwise regression models based on epidemiological variables to predict leaf rust epidemics and yield losses simultaneously have not been conducted in Pakistan. Therefore, it is essential to investigate all epidemiological variables involved in a disease epidemic and validate them with new data sets. For this purpose, detailed information about the disease, the host, and the epidemiological variables that can cause an epidemic is crucial. Understanding leaf rust epidemiology allows an accurate forecast of its outbreak and precise timing of chemical application based on the most favorable environmental conditions. This would ultimately reduce pesticide use and enhance environmental friendly disease management, resulting in decreased yield losses. The current three-year study was conducted to determine the association between leaf rust severity and various epidemiological variables. Moreover, we aimed to estimate yield loss caused by leaf rust epidemics and formulate stepwise regression equations for predicting both leaf rust severity and yield loss in selected wheat cultivars. Further, this study also presents the validation of disease forecast regression models to predict leaf rust severity and yield loss under natural field conditions.

2. Materials and Methods

Six wheat cultivars were sown in Faisalabad, Pakistan at two different locations: University of Agriculture Faisalabad (UAF), and Plant Pathology Research Institute (PPRI), Faisalabad (Figure 1), Pakistan during three growing seasons, viz. 2016–2017, 2017–2018 and 2018–2019. Another research trial was conducted at the third location i.e., Wheat Research Institute (WRI), Ayub Agriculture Research Station-Faisalabad, during the crop

season 2018–2019 and 2019–2020 to validate disease prediction models for estimating leaf rust severity percentage and related yield losses (Table 1).

Table 1. Physical detail of study locations in Faisalabad–Pakistan.

Location	Date of Sowing	Soil Classification	Lat. (°N)	Long. (°E)	Elevation (m)	Average Temp. (°C)	Average Rainfall (mm)	Average Relative Humidity (%)
*UAF	25 November 2017–2019	Sandy clay loam	31°25′	73°4′	185	19.8	25.2	58.2
*PPRI	26 November 2017–2019	Clay loam	31°41′	73°12′	184	20.2	25.9	58.9
*WRI	2 December 2018–2019	Clay loam	31°40′	73°04′	184	20	25.9	58.9

*UAF = University of Agriculture Faisalabad; *PPRI = Plant Pathology Research Institute (PPRI), Faisalabad; *WRI = Wheat Research Institute, AARI-Faisalabad.

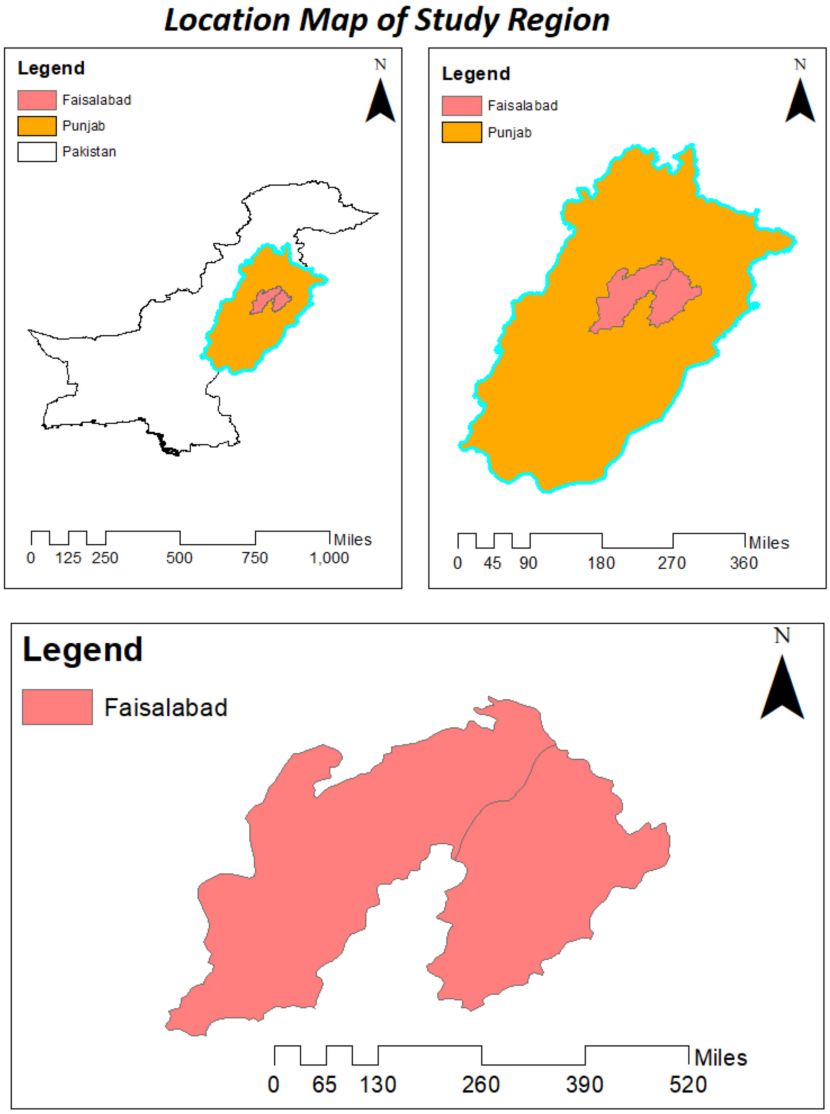


Figure 1. Map of Pakistan showing the study region of wheat cultivation.

The experiments were conducted in two locations in a split-plot design with three replications, where the main plot were divided into infected and protected plots, while the six cultivars were distributed randomly in each of the main plot. The six wheat cultivars, namely Ujala-15, Pak-13, Rustam-12, Punjab-11 Millat-11 and SH-2, exhibiting moderately

resistant to moderately susceptible response against leaf rust severity, were distributed randomly in each plot. The plot size was 3 × 3.5 = 10.5 m^2, and the wheat seeds were drilled in rows. Each plot contained seven rows with 6.5 m long and 30 cm between rows. Standard agronomic practices, including irrigation schedule and application of recommended fertilization doses, were followed. A highly susceptible rust spreader variety Morocco was sown around each plot to produce leaf rust pressure. To save the protected plots from *P. triticina* infection, the standard dose of protectant fungicide Tilt was sprayed @ 25 cm^3/100 L of water twice a week from mid of February to the end of March.

2.1. Field Inoculation and Data Recording

An inoculum of the mixture of leaf rust TKTRN, KSR/JS, PGRTB, PHTTL and TKTPR races collected from the farmers' fields of the Murree, Kaghan, Punjab, and rust trap nursery planted at Wheat Research Institute, Faisalabad was sprayed at a rate of 250 mg urediniospores/L of distilled water plus 2–3 drops of Tween-20 [3]. To ensure successful infection, the inoculum was sprayed on the nursery with a pressure of 1.1 kg/cm^2 by using a knapsack sprayer [19]. The fresh urediniospores of leaf rust races were mixed with talc powder and dusted on wheat plots in the early evening. The inoculation of plots was carried out at the booting stage (GS47, Zadoks scale) at the end of February, according to the method described by Tervet and Cassell [20].

The data for leaf rust severity was recorded in each plot at a weekly interval (on 1, 7, 14, and 21 March) during the three growing seasons of investigation by using a modified Cobb's scale proposed by Peterson et al. [21]. The final leaf rust severity was recorded when the highly susceptible check variety, i.e., SH-2, became 70–80% infected [22]. The six-leaf rust severities percentage data for each cultivar were used to develop the disease predictive model, and only the final leaf rust severity percentage was used to compare the resistance level for each tested genotype.

2.2. Data Recording of Yield and Yield Loss (%)

When the moisture content was 14% at crop maturity, the spikes of each tested cultivar were harvested by hand and threshed. Grain weight from the threshed spikes was measured with an electronic balance (4 digits) to estimate grain yield per plot. The grain yield was measured (kg) for each cultivar in each plot. The percentage of grain yield losses was calculated by using the expression given below [23].

$$Loss\ (\%) = 100 - [(yd/yh) \times 100]$$

where *yd* = yield of diseased plants and *yh* = yield of healthy plants.

The yield loss percentage for each genotype was transferred from the yield loss in plots to the feddan (Feddan = 4200 m^2), because the variation between the loss (%) in plots for each cultivar was very small to develop the predictive disease models for yield loss.

2.3. Meteorological Data

The data of meteorological variables viz. minimum temperature (X_1), maximum temperature (X_2), minimum relative humidity (X_3), maximum relative humidity (X_4), rainfall (X_5) and wind speed (X_6) of three locations: UAF, PPRI, and WRI, Faisalabad were obtained from the Meteorological Station of Department of Crop Physiology, Ayub Agriculture Research Institute, Faisalabad and then evaluated for efficacy in predicting yield loss and leaf rust severity percentage. All meteorological data used in the present study was converted into average weekly data for March, during which leaf rust severity for the tested genotypes was recorded (Figure 2)

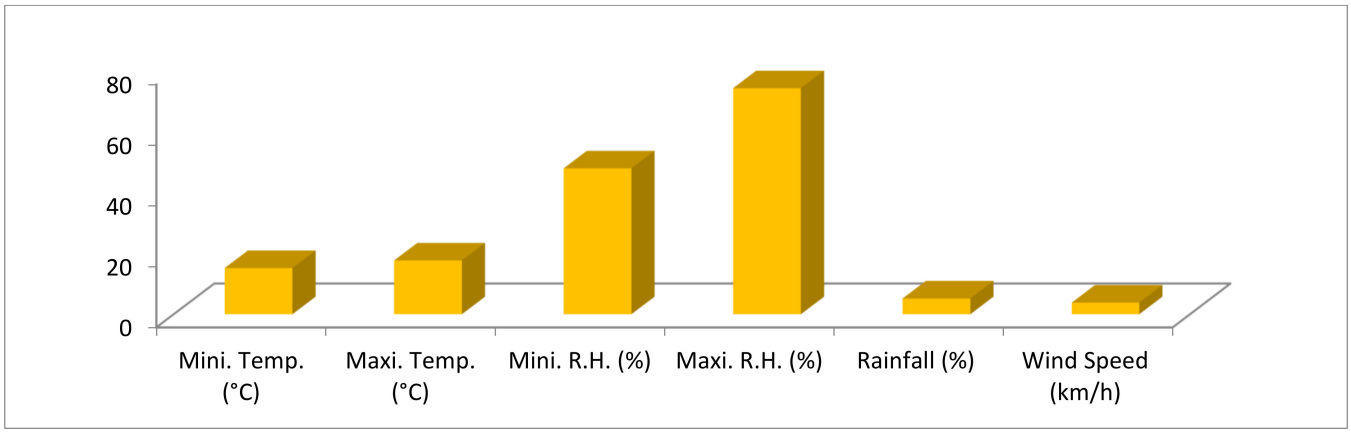


Figure 2. Summary of average weekly data of meteorological variables used during prediction of leaf rust and yield loss (%).

2.4. Development of Stepwise Regression Models

The correlation coefficients (r) and coefficients of determinations (R^2) were determined among leaf rust severity (%), yield loss (%) and environmental data by using statistical software SPSS v.17. The correlation coefficients and coefficients of determinations were also ascertained between the actual and predicted yield loss percentage and the actual and predicted leaf rust severity percentage using Minitab v.17. (Minitab Inc., State College, PA, USA). The stepwise regression models for different wheat cultivars under study were developed based on meteorological variables to explain the maximum variations in leaf rust severity and yield loss percentage. Moreover, the linear regression models were developed using the actual leaf rust severity and yield loss percentage data at the two locations, *viz*. UAF and PPRI for three growing seasons of the study to predict leaf rust severity and yield loss (%) [12].

2.5. Model Validation

The model validation and accuracy of prediction models were analyzed by comparing the values of observed (actual) and predicted leaf rust severity (%) and yield loss (%) of six cultivars sown at the WRI experimental site during the 2018–2019 and 2019–2020 study periods. These data were not used in stepwise and linear regression models for leaf rust severity and yield loss percentage. The yield loss (%) and leaf rust severity (%) were calculated by using the mean of three replicates of yield loss (%) and leaf rust severity (%) at the WRI location during crop seasons 2018–2019 and 2019–2020. The predicted values of yield loss and leaf rust severity (%) were estimated from stepwise regression models for each cultivar using the data of epidemiological factors that were present at the WRI location when the actual data were recorded. The values of the average meteorological variables at the WRI site were minimum temperature (X_1) = 15.03 °C, maximum temperature (X_2) = 21.34 °C, minimum relative humidity (X_3) = 47.34%, maximum relative humidity (X_4) = 71.67%, rainfall (X_5) = 7.03 mm and wind speed (X_6) = 3.14 km/ha.

3. Results

3.1. Evaluation of Wheat Cultivars against Leaf Rust Severity

Data in Table 2 exhibited that wheat cultivars Ujala-15, Pak-13, and Rustam-12 had low values of final leaf rust severity percentage (2.65–14.34) at UAF and PPRI during three growing seasons of investigation. Moreover, the genotypes Punjab-11 and Millat-11 showed a moderate level of resistance (13.69–21.00), whereas the SH-2 (check) wheat cultivar exhibited the highest leaf rust severity percentage ranging from 76.67 to 80.00 at both locations during all growing seasons of the investigation (Table 2).

Table 2. Final leaf rust severity (%) of all tested wheat genotypes under natural environmental conditions at UAF and WRI locations during the 2016–2017, 2017–2018 and 2018–2019 crop seasons.

Cultivar	Seasons/Locations/Disease Severity (%)					
	2016–2017		2017–2018		2018–2019	
	UAF	PPRI	UAF	PPRI	UAF	PPRI
Ujala-15	2.65 ± 0.964	4.26 ± 1.97	5.92 ± 2.78	7.74 ± 1.61	5.88 ± 3.37	10.06 ± 2.18
Pak-13	6.78 ± 2.00	9.89 ± 2.37	5.14 ± 3.37	5.07 ± 1.55	7.53 ± 4.04	7.84 ± 2.28
Rustam-12	5.00 ± 3.00	4.63 ± 3.68	7.00 ± 5.03	10.41 ± 1.91	14.33 ± 4.04	14.34 ± 3.51
Punjab-11	13.69 ± 2.00	13.11 ± 1.94	11.67 ± 6.11	20.43 ± 4.54	16.16 ± 5.48	22.26 ± 4.80
Millat-11	19.51 ± 2.35	18.26 ± 2.24	20.00 ± 5.03	18.00 ± 5.00	21.67 ± 10.41	21.00 ± 7.00
SH-2 (Check)	76.67 ± 3.51	77.78 ± 7.51	66.67 ± 7.64	77.34 ± 7.51	67.77 ± 10.41	80.00 ± 5.00
LSD of cultivars (V) at 5%	5.14					
LSD of seasons (S) at 5%	NS					
LSD of locations (L) at 5%	NS					
LSD of V × S at 5%	NS *					
LSD of V × L at 5%	8.34					
LSD of S × L at 5%	NS					
LSD of V × S × L at 5%	NS					

UAF = University of Agriculture Faisalabad; PPRI = Plant Pathology Research Institute; NS * = non-significant at $p \leq 0.05$.

3.2. Effect of P. triticina Infection on the Loss (%) of Grain Yield

The effect of leaf rust infection on the tested wheat cultivars was assessed during all three growing seasons based on the impact of leaf rust severity on grain yield per plot. Three cultivars, including Ujala-15, Pak-13, and Rustam-12 showed lower values for the loss (%) of grain yield per plot (2.63–9.30), while the other two cultivars, namely Punjab-11 and Millat-11, demonstrated moderate mean values for loss (%) of grain yield per plot (4.27–16.53) at both locations during all three growing seasons of the study. The check cultivar SH-2 indicated the maximum mean values of loss (%) for grain yield per plot (2.98–36.44) at two locations during the three growing seasons of the study (Table 3).

3.3. Correlation between Meteorological Variables and Leaf Rust Severity (%)

All meteorological variables showed a positive relationship with leaf rust severity except for minimum relative humidity (Table 4). The correlation of minimum temperature with leaf rust severity was positive in all six tested wheat cultivars. The wheat cultivars Pak-13 and Punjab-11 showed a significant response with an increase in minimum temperature (r = 0.67 and 0.70, respectively). On the other hand, the wheat cultivars Millat-11 and Ujala-15 indicated the lowest correlation coefficient viz., r = 0.44 and 0.52, respectively (Table 4). The contribution of minimum temperature in the prediction of leaf rust severity ranged from 27.7 to 62.4%. The relationship between maximum temperature, maximum relative humidity, rainfall and wind speed was positive with leaf rust severity in all six wheat cultivars. The cultivars showed a significant response with an increase in maximum temperature, maximum relative humidity, rainfall, and wind speed, as indicated by their correlation coefficient (r) values (Table 4). The contribution of maximum temperature, maximum relative humidity, rainfall, and wind speed in the prediction of leaf rust severity were in the ranges of 71.6–81.1%, 41.6–99.5%, 58.0–87.5%, and 57.6–99.7%, respectively. Conversely, the relationship between minimum relative humidity and leaf rust severity was negative for all six cultivars (r = –0.23 to –0.54). The contribution of minimum relative humidity in the prediction of leaf rust severity ranged from 5.6 to 23.9%. Our findings indicated that the minimum relative humidity was also associated with leaf rust severity (%) (Table 4).

Table 3. Effect of leaf rust infection on yield plot weight (kg) of 6 wheat cultivars at UAF and PPRI locations during 2016–2017, 2017–2018 and 2018–2019 crop seasons.

Cultivar	Infected Yield						Protected Yield						Yield Loss (%)					
	2016–2017		2017–2018		2018–202019		2016–2017		2017–2018		2018–2019		2016–2017		2017–2018		2018–2019	
	UAF	PPRI	UAF	PPRI	UAF	PPRI	UAF	PPRI	UAF	PPRI	UAF	PPRI	UAF	PPRI	UAF	PPRI	UAF	PPRI
Ujala-15	6.54 ± 1.68	6.11 ± 2.33	6.06 ± 2.28	6.51 ± 1.78	6.37 ± 1.75	6.49 ± 1.30	6.74 ± 1.84	6.62 ± 1.90	5.86 ± 2.47	6.02 ± 2.43	7.03 ± 3.19	5.59 ± 2.29	9.30 ± 1.95	6.84 ± 3.45	10.71 ± 3.15	8.29 ± 1.99	8.25 ± 1.61	4.74 ± 1.01
Pak-13	6.85 ± 0.91	7.18 ± 1.21	7.14 ± 1.24	7.21 ± 1.01	6.34 ± 0.84	6.11 ± 0.77	7.45 ± 2.08	7.25 ± 1.15	7.00 ± 1.37	6.70 ± 1.24	6.15 ± 0.94	6.93 ± 1.53	6.08 ± 1.98	4.92 ± 2.00	10.26 ± 1.96	6.99 ± 2.00	7.59 ± 1.33	2.63 ± 1.00
Rustam-12	7.63 ± 1.52	7.54 ± 2.41	7.21 ± 1.90	7.67 ± 2.77	6.97 ± 2.23	7.63 ± 2.27	8.23 ± 1.47	8.60 ± 1.49	8.14 ± 0.88	7.62 ± 1.27	8.04 ± 0.88	7.01 ± 1.30	8.50 ± 1.91	4.29 ± 2.00	6.41 ± 2.00	7.44 ± 3.07	7.69 ± 2.45	4.48 ± 2.00
Punjab-11	4.27 ± 2.07	5.44 ± 0.84	5.35 ± 0.84	5.00 ± 0.79	6.15 ± 2.28	6.16 ± 1.85	7.04 ± 1.95	6.34 ± 2.74	5.78 ± 1.96	6.12 ± 2.37	5.53 ± 2.38	6.37 ± 2.74	16.34 ± 4.00	11.37 ± 2.01	13.24 ± 2.11	13.71 ± 2.20	13.41 ± 2.11	16.53 ± 3.99
Millat-11	6.67 ± 2.00	6.56 ± 2.18	5.89 ± 2.63	5.23 ± 2.06	5.63 ± 1.64	5.93 ± 1.96	7.16 ± 1.69	7.62 ± 1.01	7.45 ± 1.55	7.74 ± 1.81	6.05 ± 1.63	6.99 ± 1.82	16.46 ± 1.86	16.56 ± 2.34	17.74 ± 1.55	15.89 ± 2.76	16.31 ± 2.75	15.37 ± 2.84
SH-2 (Check)	3.29 ± 1.06	3.55 ± 1.08	3.47 ± 1.12	2.98 ± 1.83	3.56 ± 1.61	3.85 ± 2.08	4.23 ± 1.62	4.60 ± 1.09	4.71 ± 1.21	4.23 ± 0.89	4.93 ± 1.46	5.11 ± 1.71	32.04 ± 3.01	28.56 ± 6.95	34.55 ± 3.95	33.75 ± 4.15	36.44 ± 3.94	33.56 ± 3.78
LSD of cultivars (V) at 5%										0.42								
LSD of seasons (S) at 5%										0.17								
LSD of locations (L) at 5%										NS								
LSD of V × S at 5%										NS *								
LSD of V × L at 5%										NS								
LSD of S × L at 5%										NS								
LSD of V × S × L at 5%										NS								

UAF = University of Agriculture Faisalabad; PPRI = Plant Pathology Research Institute; NS *= non-significant at $p \leq 0.05$.

Table 4. Correlation between average weekly meteorological variables and their contribution on leaf rust severity of six wheat genotypes.

Cultivar	Mini. Temp. (°C) (X_1)		Maxi. Temp. °C (X_2)		Mini. R.H. (%) (X_3)		Maxi. R.H. (%) (X_4)		Rainfall (mm) (X_5)		Wind Speed (km/ha) (X_6)	
	r	R^2	r	R^2	r	R^2	r	R^2	r	R^2	r	R^2
Ujala-15	0.52 (0.00)	27.7	0.84 (0.00)	71.6	−0.23 (0.38)	5.6	0.64 (0.03)	41.6	0.76 (0.02)	58.0	0.82 (0.01)	67.5
Pak-13	0.67 (0.00)	50.1	0.53 (0.00)	28.8	−0.48 (0.04)	23.9	0.99 (0.00)	99.5	0.93 (0.00)	87.5	0.80 (0.01)	64.7
Rustam-12	0.60 (0.00)	31.8	0.76 (0.00)	58.9	−0.35 (0.05)	12.9	0.95 (0.00)	91.0	0.88 (0.00)	77.6	0.99 (0.00)	99.7
Punjab-11	0.70 (0.00)	52.3	0.89 (0.00)	79.1	−0.31 (0.03)	9.8	0.85 (0.00)	73.8	0.90 (0.00)	81.1	0.89 (0.00)	79.2
Millat-11	0.44 (0.00)	19.5	0.81 (0.00)	65.7	−0.28 (0.02)	7.8	0.79 (0.01)	63.8	0.82 (0.00)	68.0	0.86 (0.00)	75.3
SH-2 (Check)	0.81 (0.00)	62.4	0.90 (0.00)	81.1	−0.54 (0.05)	19.8	0.86 (0.00)	74.4	0.91 (0.00)	83.6	0.75 (0.03)	57.6

3.4. Stepwise Regression Models for Leaf Rust Severity (%)

The disease predictive models to forecast leaf rust severity, including epidemiological factors, are illustrated in Table 5. All six regression models showed a high value of the coefficient of determination (R^2 > 70%), which is a good indicator for the forecasting model. The maximum variations were recorded in leaf rust severity (%) in the wheat cultivars SH-2 (R^2 = 93.44) and Millat-11 (R^2 = 91.66), which was followed by models in wheat cultivars Punjab-11 (R^2 = 89.19), Ujala-15 (R^2 = 83.44), Pak-13 (R^2 = 77.76) and Punjab-11 (R^2 = 70.71) (Table 5). The regression model in Morocco explained 93.44% of the leaf rust severity variations and included minimum temperature, minimum relative humidity, maximum relative humidity, rainfall and wind speed. Meanwhile, the regression model in Millat-11 elucidated 91.66% leaf rust severity (%) variation by including the variables minimum temperature, maximum relative humidity and rainfall (Table 5).

Table 5. Disease predictive models for leaf rust severity (%) in six wheat cultivars based on average weekly environmental conditions using a stepwise regression analysis.

Cultivar	Model	R^2 (%)	S.E.	F-Value	*p*-Value
Ujala-15	Y [a] = −3.00 + 0.225 [b] X_5 + 1.902 X_6	83.44	1.48	37.80	0.000
Pak-13	Y = −23.2 − 1.84 X_1 + 3.48 X_2 − 4.80 X_6	77.76	1.85	16.32	0.000
Rustam-12	Y = 28.6 + 1.340 X_3 − 0.798 X_4 + 4.25 X_5	89.19	2.25	34.16	0.000
Punjab-11	Y = −0.8 + 6.74 X_1 + 3.36 X_6	70.71	4.38	2.83	0.041
Millat-11	Y = −13.95 + 3.795 X_1 − 0.167 X_4 − 1.703 X_5	91.66	1.61	51.30	0.000
SH-2 (Check)	Y = −51.24 + 2.701 X_1 − 0.567 X_3 + 1.017 X_4 − 4.486 X_5 + 0.33 X_6	93.44	8.32	17.76	0.000

Y [a] = Leaf rust severity (%); [b] The leaf rust prediction variables (X) included in the regression models were minimum temperature (X_1), maximum temperature (X_2), minimum relative humidity (X_3), maximum relative humidity (X_4), rainfall (X_5) and wind speed (X_6).

3.5. Correlation between Meteorological Variables and Yield Loss (%)

The relationship of minimum temperature with leaf rust severity was positive in all six tested wheat cultivars. The wheat cultivar SH-2 exhibited a significant yield loss with an increase in minimum temperature (r = 0.42), whereas Ujala-15 and Millat-11 showed the lowest correlation coefficient for this parameter with r = 0.06 and 0.25, respectively. The contribution of minimum temperature in predicting yield loss (%) ranged from 0.8 to 13.8%. The relationship between maximum temperature, maximum relative humidity, rainfall and wind speed was also positive, with yield loss (%) in all the six wheat cultivars. These parameters contributed significantly to the prediction of yield loss percent by recording 2.3–10.8%, 53.2–90.2%, 0.7–6.7%, and 0.5–4.5% contribution by maximum temperature, maximum relative humidity, rainfall, and wind speed, respectively (Table 6). On the other hand, the minimum relative humidity demonstrated a negative relationship with yield loss in all six wheat cultivars (r = –0.44 to –0.67). The contribution of minimum relative humidity in predicting yield loss was 1.7 to 17.4%. A positive relationship was observed between the final leaf rust severity (%) and wheat yield loss in all six wheat cultivars. All six wheat cultivars viz. Ujala-15, Pak-13, Rustam-12, Punjab-11, Millat-11, and SH-2 exhibited significant yield loss with an increase in final leaf rust severity (r = 0.89, 0.91, 0.94, 0.92, 0.97 and 0.99, respectively). The contribution of final leaf rust severity in predicting yield loss ranged from 79.8 to 98.2% (Table 6).

Table 6. Correlation of average weekly meteorological variables and final leaf rust severity (%) and their contribution on yield losses of six wheat cultivars.

Cultivar	Mini. Temp. (X_1)		Maxi. Temp. (X_2)		Mini. R.H. (X_3)		Maxi. R.H. (X_4)		Rainfall (X_5)		Wind Speed (X_6)		[a] FLRS (%) (X_7)	
	r	r^2	r	r^2	R	r^2	r	r^2	r	r^2	r	r^2	r	r^2
Ujala-15	0.06 (0.95) [b]	0.8	0.18 (0.88)	7.7	−0.48 (0.68)	3.0	0.80 (0.00)	64.5	0.06 (0.48)	1.2	0.14 (0.45)	3.8	0.89 (0.02)	79.8
Pak-13	0.17 (0.88)	3.6	0.05 (0.96)	2.3	−0.49 (0.67)	3.6	0.71 (0.01)	53.2	0.09 (0.47)	1.9	0.12 (0.46)	2.9	0.91 (0.00)	83.8
Rustam-12	0.22 (0.85)	5.6	0.06 (0.95)	3.1	−0.53 (0.63)	5.7	0.83 (0.00)	68.5	0.05 (0.48)	1.0	0.09 (0.47)	2.1	0.94 (0.00)	89.3
Punjab-11	0.25 (0.83)	7.1	0.16 (0.89)	8.7	−0.59 (0.59)	10.0	0.91 (0.00)	82.5	0.02 (.049)	0.7	0.06 (0.48)	1.3	0.92 (0.00)	85.6
Millat-11	0.14 (0.90)	2.4	0.05 (0.96)	2.3	−0.44 (0.70)	1.7	0.88 (0.00)	77.2	0.14 (0.45)	2.3	0.02 (0.49)	0.5	0.97 (0.00)	94.8
SH-2 (Check)	0.42	13.8	0.25 (0.83)	10.8	−0.67 (0.53)	17.4	0.95 (0.00)	90.2	0.21 (0.43)	6.7	0.16 (0.44)	4.5	0.99 (0.01)	98.2

[a] LRS = Final leaf rust severity (%); [b] = level of probability.

3.6. Stepwise Regression Models for Yield Loss (%)

Six stepwise regression models were developed based on meteorological variables and leaf rust severity (%) to predict yield loss (%) and explain the different extent of variations in yield loss. The best predictive disease models to predict yield loss (%) encompassing meteorological variables are presented in Table 7. The coefficient of determination (R^2) values for all yield loss forecasting models were greater than 70. The regression models for wheat cultivars Ujala-15, Pak-13, Rustam-12, Punjab-11, Millat-11, and SH-2 exhibited R^2 values of 75.52, 70.16, 75.25, 77.78, 70.03, and 76.22, respectively, indicating the good efficacy of these models for yield loss prediction (Table 7).

Table 7. Disease predictive models for yield loss (%) in six wheat cultivars based on average weekly epidemiological variables using a stepwise regression analysis.

Cultivar	Stepwise Regression Models	R^2 (%)	S.E.	F-Value	*p*-Value
Ujala-15	Y [a] = 123.77 − 0.829 [b] X_2 + 0.261 X_4 + 0.396 X_5 + 2.16 X_6	75.52	1.53	10.03	0.001
Pak-13	Y = 275.54 − 0.094 X_1 − 0.272 X_2 + 0.268 X_5 + 1.884 X_6	70.16	0.96	7.29	0.003
Rustam-12	Y = 272.98 + 0.184 X_1 − 0.446 X_2 + 0.1344 X_4 + 0.1396 X_5 + 0.779 X_6	75.25	0.55	7.30	0.002
Punjab-11	Y = 551.011 − 0.4561 X_1 + 0.2587 X_5 + 1.193 X_6	77.78	0.63	16.34	0.000
Millat-11	Y = 839.52 − 0.336 X_1 + 0.3213 X_5 + 0.600 X_6	70.03	0.49	6.36	0.006
SH-2 (Check)	Y = 974.25 + 0.016 X_1 − 0.345 X_2 − 0.1287 X_3 + 0.212 X_4 + 0.134 X_5 + 0.746 X_6	76.22	0.58	5.88	0.006

[a] Y = Yield Loss (%); [b] The yield loss (%) prediction variables (X) included in the regression models were minimum temperature (X_1), maximum temperature (X_2), minimum relative humidity (X_3), maximum relative humidity (X_4), rainfall (X_5) and wind speed (X_6).

3.7. Models Validation

The six models developed for the prediction of leaf rust severity and yield loss (%) were validated by forecasting the leaf rust severity and yield loss (%) and comparing the results with the actual values of these parameters as observed at Wheat Research Institute (WRI), Faisalabad during the 2018–2019 and 2019–2020 growing seasons (Tables 8 and 9 and Figures 3 and 4). The disease predictive leaf rust severity and yield loss (%) models were close to the actual leaf rust severity and yield loss (%) for each genotype. These disease predictive models were therefore considered accurate to predict leaf rust severity and yield loss (%). The coefficient of determination value of association between actual and predicted leaf rust severity and yield loss (%) was greater than 0.99. Thus, the predictive models proposed in this study are highly accurate, having a prediction capacity of 99%.

Table 8. Comparisons between predicted leaf rust severity (%) from stepwise regression models for each cultivar and observed leaf rust severity (%) at WRI location during 2018–2019 and 2019–2020 crop seasons.

Cultivar	Stepwise Regression Models	Rust Severity (%)	
		[b] Observed	[c] Predicted
Ujala-15	[a] Y = −3.00 + 0.225 [d] X_5 + 1.902 X_6	5.92	5.58
Pak-13	Y = −23.2 − 1.84 X_1 + 3.48 X_2 − 4.80 X_6	7.84	6.88
Rustam-12	Y = 28.6 + 1.340 X_3 − 0.798 X_4 + 4.25 X_5	14.33	14.27
Punjab-11	Y = −0.8 + 6.74 X_1 + 3.36 X_6	16.16	17.25
Millat-11	Y = −13.95 + 3.795 X_1 − 0.167 X_4 − 1.703 X_5	22.34	21.40
SH-2 (Check)	Y = −51.24 + 2.701 X_1 − 0.567 X_3 + 1.017 X_4 − 4.486 X_5 + 0.33 X_6	71.66	74.45

[a] Y = Leaf rust severity (%); [b] Mean of five replicates; [c] Determined by using the constant a and predictors X_5 and X_6; [d] The leaf rust severity (%) prediction variables (X) included in the regression models were minimum temperature (X_1), maximum temperature (X_2), minimum relative humidity (X_3), maximum relative humidity (X_4), rainfall (X_5), and wind speed (X_6).

Table 9. Comparisons between predicted yield loss from stepwise regression models for each cultivar and observed yield loss (%) at WRI location during 2018–2019 and 2019–2020 crop seasons.

Cultivar	Stepwise Regression Models	Yield Loss (%)	
		[b] Observed	[c] Predicted
Ujala-15	[a] $Y = 123.77 - 0.829$ [d] $X_2 + 0.261\ X_4 + 0.396\ X_5 + 2.16\ X_6$	143.11	138.83
Pak-13	$Y = 275.54 - 0.094\ X_1 - 0.272\ X_2 + 0.268\ X_5 + 1.884\ X_6$	280.08	278.87
Rustam-12	$Y = 272.98 + 0.184\ X_1 - 0.446\ X_2 + 0.1344\ X_4 + 0.1396\ X_5 + 0.779\ X_6$	283.24	282.44
Punjab-11	$Y = 551.011 - 0.4561\ X_1 + 0.2587\ X_5 + 1.193\ X_6$	551.50	550.65
Millat-11	$Y = 839.52 - 0.336\ X_1 + 0.3213\ X_5 + 0.600\ X_6$	840.42	838.40
Morocco (Check)	$Y = 974.25 + 0.016\ X_1 - 0.345\ X_2 - 0.1287\ X_3 + 0.212\ X_4 + 0.134\ X_5 + 0.746\ X_6$	983.34	981.46

[a] Y = Yield loss (%); [b] Mean of five replicates; [c] Determined by using the constant a and predictor X_2, X_4, X_5, and X_6; [d] The yield loss (%) prediction variables (X) included in the regression models were minimum temperature (X_1), maximum temperature (X_2), minimum relative humidity (X_3), maximum relative humidity (X_4), rainfall (X_5), and wind speed (X_6).

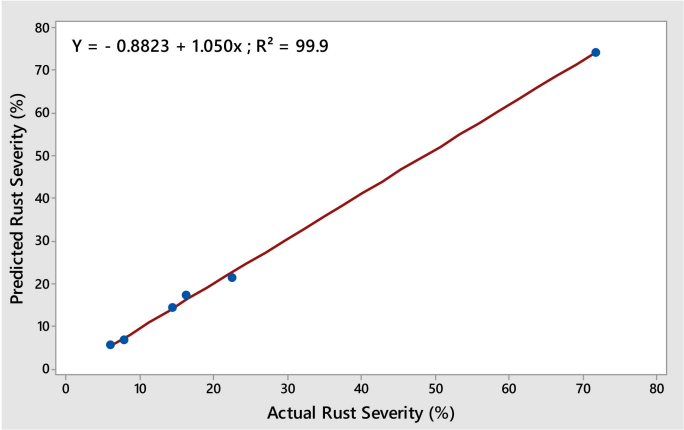


Figure 3. Relationship between predicted and observed leaf rust severity (%) as predicted by disease predictive models for tested wheat cultivars.

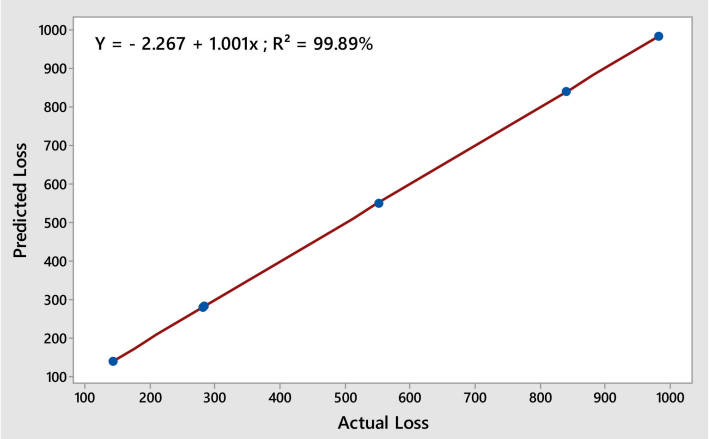


Figure 4. Relationship between predicted and observed yield loss (%) as predicted by disease predictive models for tested wheat cultivars.

4. Discussion

Leaf rust is a damaging disease of the wheat crop in Pakistan, as it occurs as a severe epidemic at the anthesis stage of growth when the kernel filling is in progress. The disease is promoted by the optimum temperature (15–25 °C) and relative humidity (60–80%) for

more than three hours continuously on the plant's surface [24]. In the present investigation, six wheat cultivars were screened for resistance to leaf rust at two different sites, viz., the University of Agriculture Faisalabad (UAF) and Plant Pathology Research Institute (PPRI), Faisalabad, during three consecutive seasons, i.e., 2016–2017, 2017–2018 and 2018–2019. All six tested cultivars indicated vulnerable field response with different levels of disease resistance. Primarily, the susceptibility of genotypes could be attributed to the constant emergence of novel virulent races of leaf rust [1] as well as the impact of conducive environmental conditions in Pakistan. All cultivars showed different levels of slow rusting or partial resistance except SH-2. The partial resistance might be due to the existence of some partial resistance genes in these genotypes, as described in a former investigation from Pakistan [25–27].

Epidemiological variables, in general, have a significant role in plant diseases, particularly leaf rust of wheat. The monitoring of environmental conditions in relation to disease development can help forecast future disease epidemics so that control measures should be taken to minimize crop losses due to pathogen infection [28]. The main focus of the present investigation was to determine the role of some epidemiological variables conducive to leaf rust development. A positive relationship was observed among leaf rust severity (%), yield loss (%), and all epidemiological variables, i.e., minimum temperature, maximum temperature, maximum relative humidity, rainfall, and wind speed. Only one variable, viz., minimum relative humidity, indicated a negative relationship between disease severity and yield loss (%). The earlier investigation of Vallavieillie et al. [29] described that the efficacy of pathogen infection is enhanced by as high as twelve times under optimal temperature and relative humidity.

During all growing seasons of investigation, at two sites, the University of Agriculture Faisalabad and Plant Pathology Research Institute, Faisalabad, the maximum relative humidity was the main epidemiological variable that played a critical role in developing optimal environmental conditions for disease development and its onset. Hence, the yield loss (%) was severely influenced by the maximum relative humidity. The significant association of relative humidity with disease severity and yield loss (%) is explained by the fact that it plays a key role in the penetration of haustorium of *P. triticina* by making the host leaves tender [30]. The duration of the leaf wetness period regulates the number and amount of germinated urediniospores and facilitates the successful *P. triticina* infection [16]. In the case of wind speed, it does not directly influence the yield loss and leaf rust severity (%) but plays a vital role in urediniospores dispersal at both long and short distances [31]. It was observed from former investigations that urediniospores spread to a longer distance at high wind speed; whereas low wind speed agitates the leaves of wheat plants with each other, resulting in drying the canopy of plants that facilitates the spread of spores from uredinia [31].

Our findings were in agreement with the reports of Khan and Trevathan [32], who developed a predictive disease model using stepwise regression analysis. The researchers employed wind speed, rainfall, relative humidity, and maximum and minimum temperature as independent variables, while disease severity was used as the dependent variable. The leaf rust severity data were collected from three sites of Mississippi i.e., Holy Springs, Starkville, and Poplarville. All epidemiological variables showed an association with disease development.

The predictive disease model based on total rainfall and minimum air temperature from March to May at Holy Springs fit the data well. Similarly, Khan [33] studied fifteen wheat cultivars for partial resistance with respect to epidemiological variables. Various cultivars indicated partial resistance. The epidemiological variables, i.e., minimum and maximum temperature, and relative humidity were in the ranges of 16–18 °C, 22–28 °C, and 77–78%, respectively. A linear regression model best explained the relationship between partial resistance and different epidemiological variables.

Under present investigation, the regression models for disease severity and yield loss (%) for each cultivar contained two to three epidemiological variables for five test

genotypes (Ujala-15, Pak-13, Rustam-12, Punjab-11, and Millat-11), whereas the check cultivar SH-2 contained five to six meteorological variables. It could be attributed to the fact that all five test cultivars possess higher resistance than the check cultivar SH-2, as reported previously [34,35]. Therefore, the impact of the environmental attributes on resistant cultivars was smaller compared to the vulnerable cultivar. Thus, more meteorological variables were present in the prediction model of SH-2 compared to other cultivars. In stepwise regression models of yield loss (%), the main predictor in all disease predictive models was final leaf rust severity, and its contribution to the prediction of yield loss ranged from 70.03 to 76.22%. Previous studies support the findings of the present investigation that yield loss (%) mainly depends upon the final leaf rust severity (%) [9,36,37].

A disease predictive model tested and developed for a particular cultivar is suitable for disease prediction of that genotype only, as different genotypes may possess different leaf rust-resistance genes. Therefore, there is an immense need to establish prediction models for major genotypes cultivated at understudied sites based on leaf rust severity (%) and related yield loss. In this study, the values of coefficient of determination (R^2) of all disease predictive models for each of the six widely cultivated wheat cultivars were high. Hence, the results revealed that our regression models can be employed to predict leaf rust disease severity and yield loss. The models developed in this research can be used by wheat growers to forecast disease epidemics and to make disease management decisions

5. Conclusions

The significant epidemiological variables, including minimum temperature, maximum temperature, maximum relative humidity, rainfall and wind speed, showed a positive relationship with leaf rust severity and yield loss. The stepwise regression models were validated to predict leaf rust severity and yield loss (%). The validation results explained 99% variability for accurate prediction of the two variables: leaf rust severity and yield loss. Thus, these prediction models can assist in early predicting leaf rust severity and probable yield loss at the crop's anthesis stage. The accurate prediction models can help wheat-growers choose apposite cultivars and arrange early control measures accordingly.

Author Contributions: Conceptualization, Y.A., A.R. and I.M.; methodology, Y.A., A.R., H.M.A. (Hafiz Muhammad Aatif) and A.A.K.; software, Y.A., S.I. and I.M.; validation, Y.A., A.R., Z.H. and C.M.S.H.; formal analysis, Y.A. and H.M.A. (Hafiz Muhammad Aatif); investigation, Y.A., A.R., A.A.K., Z.H. and C.M.S.H.; resources, Y.A., A.R., H.M.A. (Hayssam M. Ali), W.F.A.M. and L.S.-P.; data curation, Y.A. and S.I.; writing—original draft preparation, Y.A., A.R. and C.M.S.H.; writing—review and editing, S.I., H.M.A. (Hayssam M. Ali), W.F.A.M. and L.S.-P., supervision, A.R., I.M. and H.M.A. (Hayssam M. Ali); project administration, A.R. and H.M.A. (Hayssam M. Ali); funding acquisition, A.R., H.M.A. (Hayssam M. Ali), W.F.A.M. and L.S.-P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Researchers Supporting Project number (RSP-2021/123) King Saud University, Riyadh, Saudi Arabia.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to extend their sincere appreciation to the Researchers Supporting Project (RSP-2021/123) King Saud University, Riyadh, Saudi Arabia.

Conflicts of Interest: The authors declare no conflict of interest.

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