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Temperature and Relative Humidity Drive Leaf Rust and Stripe Rust Incidence and Severity in Wheat Across Major Wheat-Growing Districts of Punjab, Pakistan

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Title: Temperature and Relative Humidity Drive Leaf Rust and Stripe Rust Incidence and Severity in Wheat Across Major Wheat-Growing Districts of Punjab, Pakistan

Running title: Climatic Drivers of Wheat Rust

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Abstract:

Wheat (*Triticum aestivum* L.) is the principal staple crop of Pakistan, with Punjab contributing approximately 75% of the country's total wheat production. Leaf rust (*Puccinia triticina*) and stripe rust (*Puccinia striiformis*) are among the most detrimental diseases affecting wheat yield in the country. This study aimed to assess the influence of temperature and relative humidity on the incidence and severity of both diseases in eight major wheat-producing districts of Punjab during the 2024–2025 cropping season. A field survey was conducted at 20 wheat fields with 160 locations at three critical growth stages of the wheat crop and the survey included the commercially cultivated wheat cultivars Akbar-19, MH-21, and Fareed-06. Disease and climatic data were analyzed using multiple linear regression models. The analysis revealed that temperature was the most influential climatic variable, and Regression coefficients indicated that disease incidence increased by approximately 4.3–12.7 percentage points per 1°C increase depending on district. The effect of relative humidity was insignificant or very limited in most of the district-level models. Disease responses varied among districts, reflecting regional differences in climatic conditions. Among the cultivars studied, Akbar-19 was the most susceptible cultivar while Fareed-06 showed lower levels of disease incidence and severity. Overall, these findings indicate that temperature plays a major role in driving wheat rust epidemics in Punjab highlight the need to integrate temperature-based forecasting models along with resistant cultivars for climate-smart wheat disease management in the context of future climate change scenarios.

Keywords: Climate change, Disease forecasting, Multiple linear regression, Rust epidemiology, Temperature, Wheat disease management

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the world's major cereal crops and serves as a staple food for more than one-third of the global population. Archaeological

evidence indicates that wheat was first domesticated in the Fertile Crescent between 10,000 and 8,000 BC, making it one of the earliest crops cultivated by human civilizations [1]. Currently, wheat is cultivated on approximately 215 million hectares worldwide and provides approximately 18% of total calories and around 20% of dietary protein consumed [2]. Owing to its wide adaptability to different agroclimatic regions and its use in many food and industrial products, wheat still plays a vital role in global food security, rural livelihoods, and agricultural economy. Nonetheless, ensuring the productivity of wheat is becoming more difficult because of climate change, environmental degradation, and increasing prevalence of destructive plant diseases [3,4].

Pakistan is among the world's leading wheat-producing countries in the world, where wheat is cultivated on more than 8.9 million hectares, which accounts for over a third of the total agricultural land, contributing about 2% of the GDP of the country [5]. Punjab is the major wheat-producing province of Pakistan, contributing approximately 75% of the country's total wheat production because of its fertile soils, extensive irrigation network, and favorable agro-climatic conditions [6,7]. Though it is a strategical crop in the country, the production of wheat in Pakistan faces multiple biotic and abiotic stresses, such as global warming, irregular rainfall, continuous droughts, lowering soil fertility, scarcity of water, and increasing threats of diseases [8,9,3]. Moreover, the existing challenges are likely to aggravate in light of climate change which would hence threaten sustainable wheat production and food security.

Rust diseases are among the most damaging biotic barriers in terms of the high rates of transmission, epidemic capability, and serious yield losses. Leaf rust and stripe rust are diseases caused by Puccinia triticina and Puccinia striiformis f. sp. tritici, respectively. These diseases are among the leading economically important fungal diseases affecting wheat production throughout the world [10,11]. Yield losses in susceptible wheat cultivars may reach up to 40% under favorable environmental conditions, while severe epidemics can result in substantially greater losses [12,13]. Rust outbreaks in Pakistan have been significantly damaging, particularly in Punjab, where favorable climatic conditions facilitate pathogen survival and disease spread [14,15]. Disease outbreaks are further intensified by the emergence of new pathogen races capable of overcoming host resistance genes.

Temperature, relative humidity, rainfall, and leaf wetness are major environmental factors governing wheat rust epidemiology. Environmental conditions

influence spore germination, infection efficiency, incubation period, sporulation, and disease dissemination. In historical terms, stripe rust was associated with cool conditions, whereas leaf rust was associated with warmer temperatures [16,17]. Nonetheless, studies have shown that different populations of *Puccinia striiformis* f. sp. *tritici* have adapted to higher temperatures and that this made possible propagation of disease under more severe thermal conditions. Consequently, this adaptation has altered the distribution, seasonality, and epidemic potential of stripe rust, which created new difficulties in terms of its forecasting and management [18,19].

The presence of host genetic resistance remains the most effective and environmentally sustainable strategy for managing wheat rust in an environmentally friendly and cost-effective way. More than 60 leaf rust resistance (Lr) genes and a number of others of stripe rust resistance (Yr) readily used in commercial varieties [20,21]. However, the rapid evolution of pathogen populations and genetic changes have led to the fact that race-specific resistance has often become ineffective thus leading to the emergence of outbreaks of the infection [22]. Therefore, in the process of efficient control of disease development, constant control of the pathogen population, identification of newly emerging traits of virulence, application of resistant plants, and application of data of climatology need to be achieved should be integrated into disease management programs.

Although numerous studies have investigated wheat rust epidemiology worldwide, limited information is available regarding the impact of climatic factors on disease occurrence and severity in the agro-climatic region of Punjab, Pakistan. Previous studies tend to concentrate on pathogen diversity, host resistance, and fungicide effectiveness, while limited investigation has focused on assessing the relationship between environmental factors and disease development. Such investigations are essential in spotting disease hotspots, climate-related factors of epidemics, thereby providing a scientific basis for region-specific forecasting, climate-smart disease management, and the development of durable resistance strategies under future climate change scenarios [19].

To our knowledge, no comprehensive district-scale assessment has simultaneously quantified the influence of temperature and relative humidity on both leaf rust and stripe rust incidence and severity across the major wheat-growing regions of Punjab using multiple linear regression analysis. Therefore, the current study was conducted to determine the incidence and severity of leaf rust and stripe rust across

major wheat-growing regions of Punjab, Pakistan, and assess the influence of temperature and relative humidity on the disease development using regression analysis. As a result, scientific knowledge of wheat rust epidemiology in the local agro-climatic conditions is expected to be increased, climate-related factors of disease outbreaks identified, and a scientific foundation for region-specific disease forecasting and integrated disease management.

2. Materials and Methods

Survey Design and Study Locations

A field survey was conducted during the 2024–2025 Rabi growing season (November 2024 to April 2025) to evaluate the influence of temperature and relative humidity on the incidence and severity of leaf rust (*Puccinia triticina*) and stripe rust (*Puccinia striiformis* f. sp. *tritici*) in wheat (*Triticum aestivum* L.) across major wheat-growing districts of Punjab, Pakistan.

Eight districts were selected based on the following criteria: (i) historical occurrence of wheat rust diseases, (ii) wheat cultivation area, (iii) agro-climatic diversity, and (iv) accessibility for repeated field surveys. The surveyed districts included Mandi Bahauddin and Kasur (Northern Punjab); Faisalabad and Toba Tek Singh (Central Punjab); and Lodhran, Bahawalpur, Bahawalnagar, and Sadiqabad (Rahim Yar Khan District) in Southern Punjab. The selected districts represented contrasting climatic conditions, enabling the evaluation of disease development under different environmental regimes. These areas reflected a spectrum of agro-climatic conditions, facilitating a comprehensive spatial analysis of rust epidemiology across the province.

Experimental Layout and Sampling

A systematic random sampling was used across selected districts. A total of 20 wheat fields were surveyed in each district, resulting in 160 surveyed fields. Fields were selected randomly while maintaining a minimum distance of 5 km between sampling sites to avoid repeated observations within the same production system. Surveys were conducted throughout the 2024–2025 wheat-growing season, from November to April, to capture seasonal variations. In each field, disease assessment was conducted using a W-shaped sampling pattern. Five sampling points were selected along the transect, and twenty consecutive plants were examined at each point, giving a total of one hundred plants per field.

Sampling was aligned with the three critical growth stages of wheat:

- Tillering stage (GS 20–29)
- Stem elongation to heading (GS 30–59)
- Flowering to grain filling (GS 60–79)

Disease Assessment Methodology

The assessment of disease incidence and severity for leaf rust and stripe rust, was performed using standardized procedures recommended by the International Maize and Wheat Improvement Center (CIMMYT). These protocols ensured consistency in field evaluation and comparability across different regions and wheat varieties. Disease severity was measured using the Modified Cobb Scale, which assigns a percentage value from 0 to 100% based on the proportion of leaf area covered by rust pustules. The Modified Cobb Scale enabled quantitative estimation of disease pressure experienced by each variety under natural field conditions. In addition to severity, disease incidence was recorded as the percentage of plants infected in each surveyed field, reflecting the extent of disease spread within the population.

Environmental Data Collection

Daily meteorological data were obtained from the nearest Automatic Weather Stations (AWS) of the Pakistan Meteorological Department (PMD) or the respective agricultural research stations located near each surveyed district. These stations recorded key meteorological variables essential for understanding disease development, including daily mean temperatures, and relative humidity. The continuous monitoring ensured a high-resolution temporal dataset aligned with crop phenological stages and disease progression periods. This environmental data was then integrated with field-level disease assessments to establish correlations and analyze the influence of temperature and humidity on the incidence and severity of wheat rusts across the surveyed districts. The climatic dataset was integrated with field disease observations and subsequently used for correlation, regression, and multivariate analyses to determine the influence of temperature and relative humidity on wheat rust development. Disease incidence and severity were assessed following the standard wheat rust assessment protocols described by CIMMYT [23].

$$\text{Disease Incidence } (\%) = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Disease severity was visually estimated using the Modified Cobb Scale [24], which estimates the percentage of leaf area covered by rust pustules on individual

plants. Mean disease severity for each field was calculated by averaging the disease severity values recorded from all assessed plants.

Statistical Analysis

Statistical analyses were performed using Minitab® version 21 (Minitab LLC, State College, PA, USA). Data were tested for normality using the Anderson–Darling test and homogeneity of variance using Levene's test before analysis. Differences among means were considered statistically significant at $P \leq 0.05$. Multiple linear regression was used to examine the relationship between disease parameters and environmental factors such as temperature and humidity. The performance and reliability of the regression models were evaluated using key statistical metrics such as R^2, to ensure the robustness and predictive performance of the interpretations drawn from the data.

$$Y=\beta 0+\beta 1T+\beta 2RH+\varepsilon$$

where

Y = disease incidence (%) or disease severity (%), $\boldsymbol{\beta_0}$ = intercept, $\boldsymbol{\beta_1}$, $\boldsymbol{\beta_2}$ = regression coefficients, **T** = mean temperature (°C), **RH** = relative humidity (%) and **ε** = random error term

3. Results

Effect of Temperature and Relative Humidity on Leaf Rust Incidence Across Punjab Districts

The influence of temperature and relative humidity on leaf rust incidence was evaluated using multiple linear regression analysis across eight major wheat-growing districts of Punjab. The regression models were statistically significant for Faisalabad, Bahawalpur, Kasur, Lodhran, Mandi Bahauddin, and Toba Tek Singh ($P < 0.05$). The models for Bahawalnagar and Rahim Yar Khan (Sadiqabad) were not statistically significant overall but still indicated that temperature was the primary weather influence on disease occurrence.

Temperature showed a positive and statistically significant relationship with leaf rust meaning that increase in temperature facilitated the development of disease in different agro-climatic zones. The magnitude of this effect was different in the regions as shown by the sizes of the regression coefficients, which were the highest for Kasur ($\beta = 12.61$), Toba Tek Singh ($\beta = 12.17$), Faisalabad ($\beta = 11.57$), Mandi Bahauddin ($\beta = 10.72$), Lodhran ($\beta = 9.88$), Bahawalpur ($\beta = 9.29$), Bahawalnagar ($\beta = 8.00$), and Rahim Yar Khan (Sadiqabad) ($\beta = 7.15$).

On the other hand, relative humidity did not have a statistically significant effect on the leaf rust incidence in any of the areas surveyed (with a P-value greater than 0.05). The regression coefficients for humidity were very small and inconsistent in direction, which implies that humidity did not contribute significantly towards the spatial variations in the occurrence of leaf rust during the study period. The explanatory capacity of the regression models varied between moderate and high, as the coefficients of determination (R^2) ranged from 55.38% and 74.43%. The best-performing model was for Kasur (74.43%), while Toba Tek Singh had the second-best performance with R^2 of 72.34%, followed by Faisalabad (71.44%) and Mandi Bahauddin with an R^2 of 69.83% The performance of the model was lower yet acceptable for Bahawalpur (64.50%), Lodhran (67.33%), Bahawalnagar (58.69%), and Rahim Yar Khan (55.38%).

Variance Inflation Factor (VIF) values were found between 1.19 and 1.55 in every regression model, confirming the lack of multicollinearity between the variables. The results show that there was an effect of temperature on the occurrence of leaf rust in Punjab, while the effect of relative humidity was insignificant given the prevailing conditions (Figure 1; Table 1).

Table 1: Summary of multiple linear regression analysis for leaf rust incidence across eight wheat-growing districts of Punjab, Pakistan.

District	β (Temperature)	β (Humidity)	Model P-value	R^2 (%)
Faisalabad	11.57	-0.43	0.023	71.44
Bahawalpur	9.29	-0.71	0.045	64.5
Bahawalnagar	8	-0.5	0.054	58.69
Kasur	12.61	0.11	0.017	74.43
Lodhran	9.88	-0.42	0.035	67.33
Mandi Bahauddin	10.72	-0.44	0.027	69.83
Rahim Yar Khan (Sadiqabad)	7.15	-0.46	0.089	55.38
Toba Tek Singh	12.17	-0.33	0.021	72.34

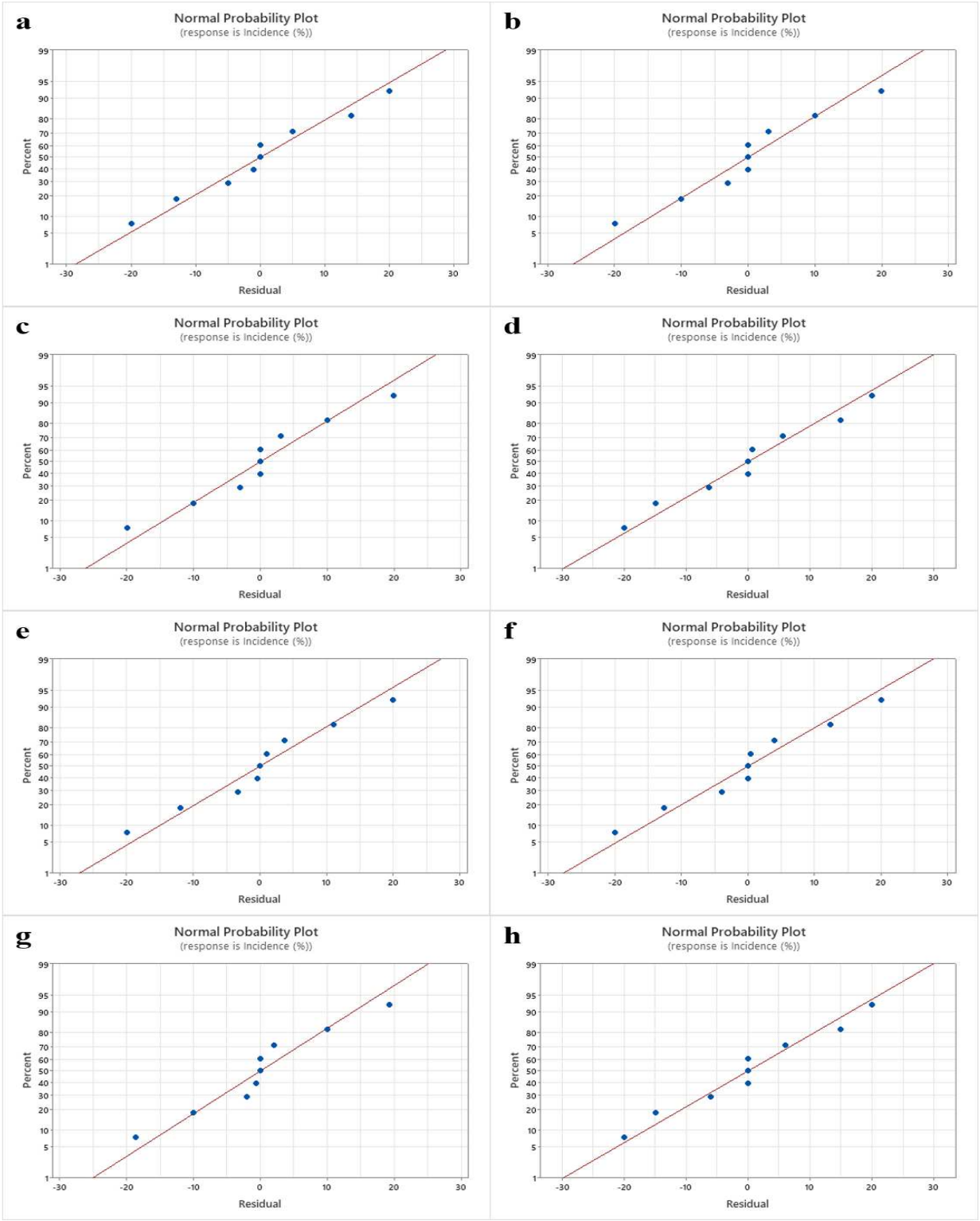


Figure 1. Relationship between temperature and leaf rust incidence across eight wheat-growing districts of Punjab, Pakistan. Panels: (a) Faisalabad, (b) Bahawalpur, (c) Bahawalnagar, (d) Kasur, (e) Lodhran, (f) Mandi Bahauddin, (g) Rahim Yar Khan (Sadiqabad), and (h) Toba Tek Singh.

Effect of Temperature and Relative Humidity on Leaf Rust Severity Across Punjab Districts

Multiple linear regression analysis was performed to evaluate the influence of temperature and relative humidity on the severity of leaf rust in wheat in Punjab. For disease severity, the temperature was established as the main climatic factor while the relative humidity did not show any significant association ($P < 0.05$).

Positive correlation between temperature and leaf rust severity was found in all districts while overall regression results differed in terms of their significance. The highest influence of temperature coefficient was shown in Kasur ($\beta = 7.78$) district followed by Toba Tek Singh ($\beta = 7.00$), Faisalabad ($\beta = 6.38$), Mandi Bahauddin ($\beta = 5.67$), Lodhran ($\beta = 5.19$), Bahawalpur ($\beta = 4.86$), Bahawalnagar ($\beta = 4.14$), and Rahim Yar Khan ($\beta = 3.48$). The results suggest that every rise in temperature for 1°C causes increase in leaf rust severity along the lines of 3.5–7.8%, which implies considerable regional variation in response due to temperature change in different regions.

In comparison, relative humidity did not show a significant effect and remained statistically insignificant throughout all models. The unavailability of a significant humidity impact brings out the role of temperature as the primary climatic factor influencing disease severity under the conditions during the period of data collection.

The explained variation of the regression models varied from moderate to high, ranging from 48.59% to 68.86% as represented by the R^2. The highest R^2 was for Kasur (68.86%), followed by Toba Tek Singh (67.86%), Faisalabad (66.74%), Bahawalpur (62.27%), Mandi Bahauddin (62.05%), Lodhran (60.85%), Bahawalnagar (53.44%), and Rahim Yar Khan (Sadiqabad) (48.59%). Several models were somewhat significant, but temperature remains important in determining leaf rust severity.

The variance inflation factor (VIF) values were from 1.19 to 1.55, indicating the absence of multicollinearity between temperature and relative humidity. Overall, these results showed that temperature rises significantly affect leaf rust severity, while relative humidity does not play a significant role in disease development under field conditions (Figure 2; Table 2).

Table 2. Summary of multiple linear regression analysis for leaf rust severity across eight wheat-growing districts of Punjab, Pakistan.

District	β (Temperature)	β (Humidity)	Model P-value	R^2 (%)
Faisalabad	6.38	-0.62	0.037	66.74
Bahawalpur	4.86	-0.64	0.054	62.27
Bahawalnagar	4.14	-0.86	0.101	53.44
Kasur	7.78	-0.39	0.03	68.86
Lodhran	5.19	-0.54	0.06	60.85
Mandi Bahauddin	5.67	-0.5	0.055	62.05
Rahim Yar Khan (Sadiqabad)	3.48	-0.57	0.136	48.59
Toba Tek Singh	7	-0.5	0.033	67.86

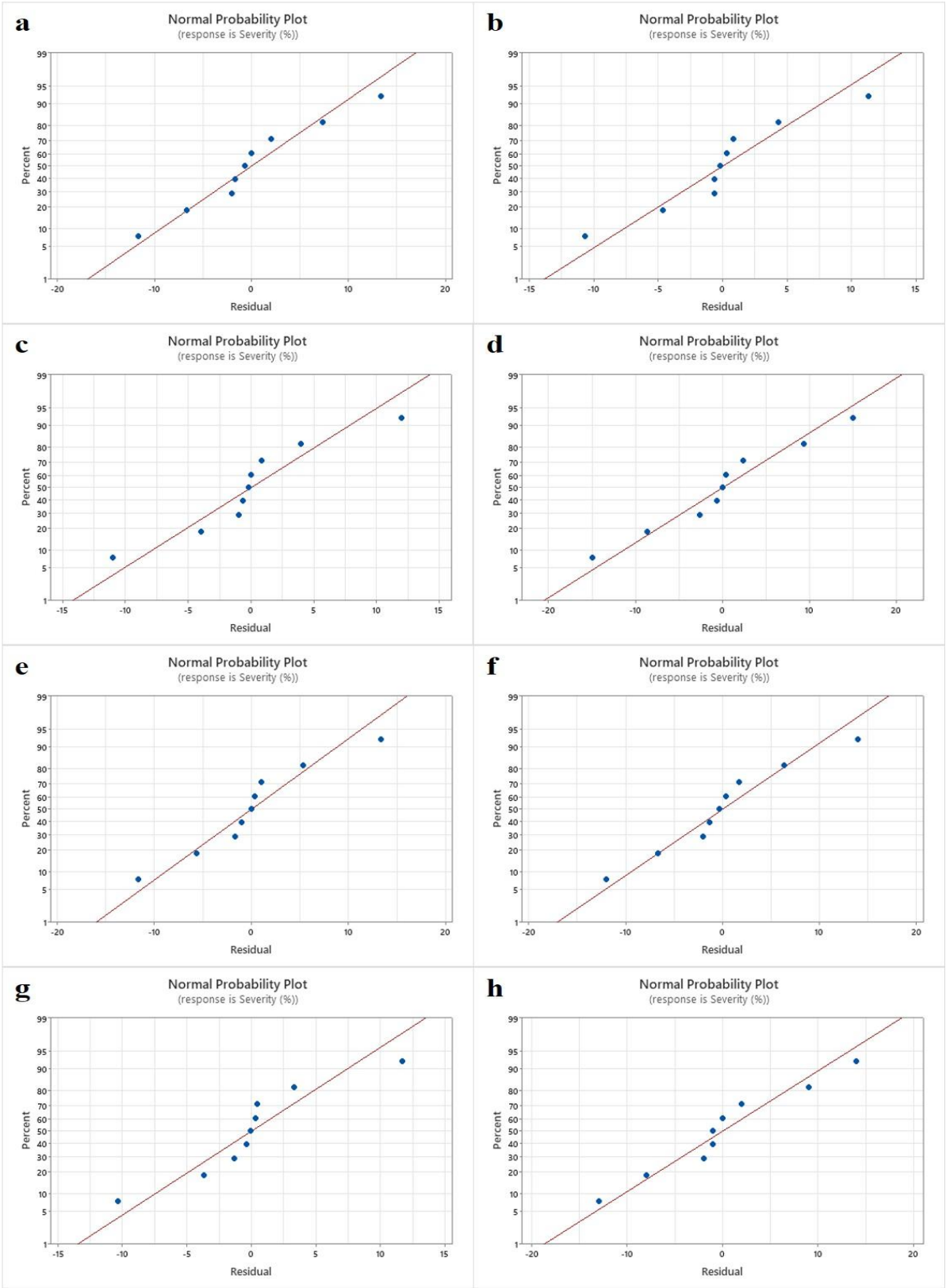


Figure 2. *Relationship between temperature and leaf rust severity across eight wheat-growing districts of Punjab, Pakistan.* Panels: (a) Faisalabad, (b) Bahawalpur, (c) Bahawalnagar, (d) Kasur, (e) Lodhran, (f) Mandi Bahauddin, (g) Rahim Yar Khan (Sadiqabad), and (h) Toba Tek Singh.

Effect of Temperature and Relative Humidity on Stripe Rust Incidence Across Punjab Districts

Through multiple linear regression analysis, this study investigated the effect of temperature and relative humidity on stripe rust infection in 8 wheat-growing districts of Punjab. Statistically significant overall models were found for Faisalabad (P = 0.040), Lodhran (P = 0.028), Mandi Bahauddin (P = 0.028), and Toba Tek Singh (P = 0.030) while models for Bahawalpur (P = 0.084), Bahawalnagar (P = 0.083), Kasur (P = 0.064), and Rahim Yar Khan (Sadiqabad) (P = 0.072) were marginally non-significant. However, temperature was found to be the most important climatic factor associated with stripe rust incidence in all districts included in this analysis.

A significant positive association was found between temperature and stripe rust incidence in all the districts studied. The highest value of β was obtained for Mandi Bahauddin (β = 12.67, P = 0.025) followed by Toba Tek Singh (β = 12.70, P = 0.016), Kasur (β = 12.43, P = 0.026), Faisalabad (β = 11.77, P = 0.020), Lodhran (β = 9.40, P = 0.023), Bahawalnagar (β = 7.70, P = 0.040), Bahawalpur (β = 5.50, P = 0.033), and Rahim Yar Khan (Sadiqabad) (β = 4.29, P = 0.028). This indicates that rising temperature positively influenced stripe rust incidence in the across Punjab, but its impact was variable in different areas.

On the contrary, the effect of relative humidity was less noticeable on stripe rust severity in various districts with P-values from 0.681 to 0.976. The regression coefficients were relatively low and inconsistent as they ranged from −0.83 in Bahawalpur to 3.67 in Mandi Bahauddin indicating that humidity had little impact on the spatial distribution of stripe rust disease under certain climatic conditions.

The explanatory power of the regression models was considered moderate to high. The highest R^2 value (69.72%) was achieved in the case of Mandi Bahauddin showing the following districts – Toba Tek Singh (69.03%), Lodhran (69.57%), Faisalabad (65.67%), Kasur (59.96%), Rahim Yar Khan (58.32%), Bahawalnagar (56.44%) and Bahawalpur (56.18%). Although the values of predicted R^2 were significantly lower (1.40–31.87%), the models sufficiently explained the observed differences in stripe rust severity.

The values of the Variance Inflation Factor (VIF) varied in all regression models from 1.00 to 1.33 and confirmed that there was no multicollinearity when it comes to temperature and relative humidity. Thus, it can be concluded that, generally, the results

indicate that temperature is a leading climate factor for the occurrence of stripe rust disease in Punjab while the impact of relative humidity is not significant (Figure 3; Table 3).

Table 3. Summary of multiple linear regression analysis for stripe rust incidence across eight wheat-growing districts of Punjab, Pakistan.

District	β (Temperature)	β (Humidity)	Model P-value	R^2 (%)
Faisalabad	11.77	0.93	0.04	65.67
Bahawalpur	5.5	-0.83	0.084	56.18
Bahawalnagar	7.7	-0.47	0.083	56.44
Kasur	12.43	1.93	0.064	59.96
Lodhran	9.4	-0.27	0.028	69.57
Mandi Bahauddin	12.67	3.67	0.028	69.72
Rahim Yar Khan (Sadiqabad)	4.29	-0.71	0.072	58.32
Toba Tek Singh	12.7	1.87	0.03	69.03

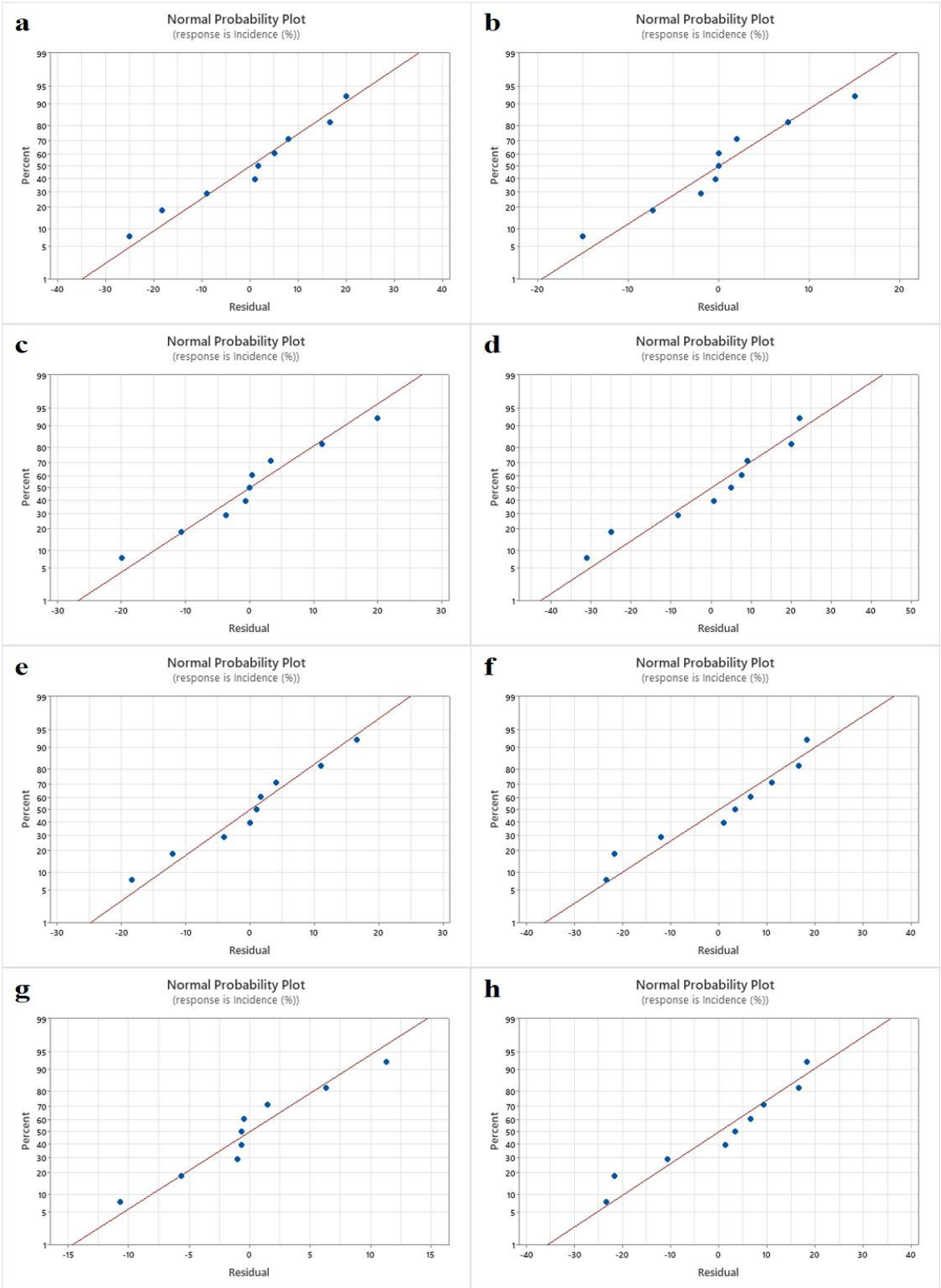


Figure 3. Multiple linear regression showing the relationship between temperature and stripe rust incidence across wheat-growing districts of Punjab, Pakistan. Panels: (a) Faisalabad, (b) Bahawalpur, (c) Bahawalnagar, (d) Kasur, (e) Lodhran, (f) Mandi Bahauddin, (g) Rahim Yar Khan (Sadiqabad), and (h) Toba Tek Singh.

Effect of Temperature and Relative Humidity on Stripe Rust Severity Across Punjab Districts

The effect of temperature and humidity on stripe rust severity was examined through the use of multiple linear regression analysis in Punjab's eight wheat-growing districts. Among the eight districts, only Toba Tek Singh had a statistically significant regression model (P = 0.037) while the ones for Faisalabad (P = 0.051), Lodhran (P = 0.054), and Mandi Bahauddin (P = 0.054) had marginally non-significant models. Bahawalpur (P = 0.122), Bahawalnagar (P = 0.107), Kasur (P = 0.086), and Rahim Yar Khan (Sadiqabad) (P = 0.128) showed no statistically significant regression model. Temperature was shown to be the main factor for the severity of stripe rust across all districts. The temperature was found to positively related to the severity of the stripe rust in every district, showing six out of eight districts with statistically significant results. The best temperature effect was found in Mandi Bahauddin (β = 8.06, P = 0.035) followed by Toba Tek Singh (β = 7.73, P = 0.016) and Kasur (β = 6.63, P = 0.040) respectively. The weakest effect was found in Rahim Yar Khan (Sadiqabad) (β = 1.31, P = 0.053). The results indicate that an increase in temperature leads to a significant increase in the severity of the stripe rust.

According to the results, the role of relative humidity in all regression models was statistically insignificant (P-value = 0.621 to 0.992). Humidity regression coefficients range from -0.72 to 0.13, emphasizing their very little yet inconsistent effect on the severity of stripe rust. These results thus indicate that relative humidity is of negligible role in explaining the variability in the severity of stripe rust in the existing conditions. Moderate R^2 values obtained in regression models indicate moderate explanatory power; for instance, the determined coefficient R^2 is 49.62% for Rahim Yar Khan (Sadiqabad) and up to 66.70% for Toba Tek Singh. Intermediate R^2 was observed for Faisalabad (62.96%), Lodhran (62.09%), Mandi Bahaudding (62.11%), Kasur (55.92%), Bahawalnagar (52.58%), and Bahawalpur (50.45%). The predicted R^2 values are relatively low (0.00–25.07%), thus reflecting low predictive power, but the models have always indicated that temperature remains the leading climatic factor that impacts stripe rust severity.

The results of the Variance Inflation Factor (VIF) analysis varied from 1.00 to 1.33 in all districts, which confirmed no multicollinearity between temperature and relative humidity. Overall, the analysis indicated that temperature acts as the major environmental factor influencing the incidence and severity of stripe rust in Punjab

while relative humidity has no significant independent effect on the development of the disease (Figure 4; Table 4).

Table 4. Summary of multiple linear regression analysis for stripe rust severity across eight wheat-growing districts of Punjab, Pakistan.

District	β (Temperature)	β (Humidity)	Model P-value	R^2 (%)
Faisalabad	5.8	0.13	0.051	62.96
Bahawalpur	1.94	-0.72	0.122	50.45
Bahawalnagar	2.81	-0.35	0.107	52.58
Kasur	6.63	-0.03	0.086	55.92
Lodhran	3.7	-0.47	0.054	62.09
Mandi Bahauddin	8.06	0.11	0.054	62.11
Rahim Yar Khan (Sadiqabad)	1.31	-0.36	0.128	49.62
Toba Tek Singh	7.73	-0.6	0.037	66.7

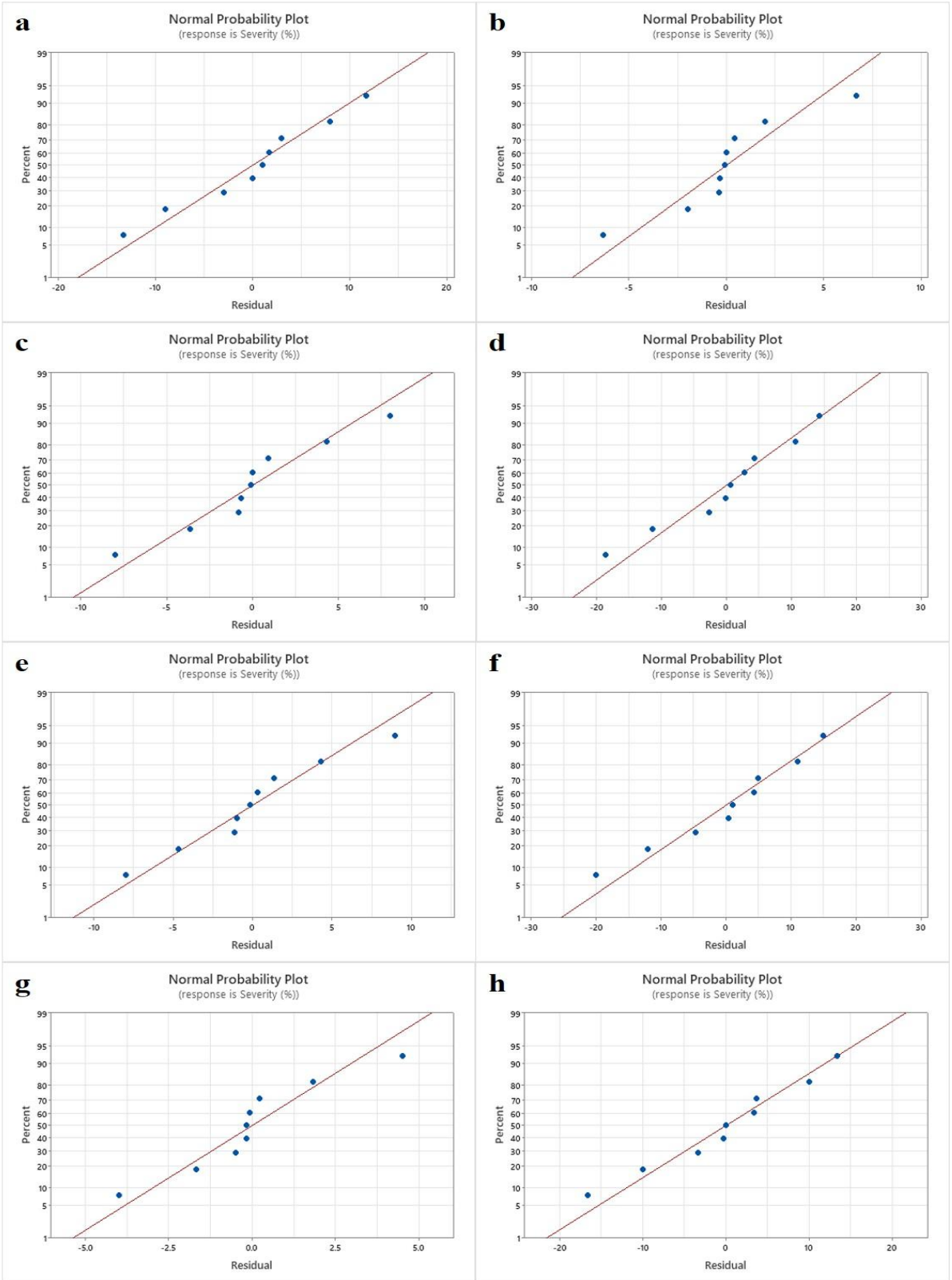


Figure 4. Multiple linear regression illustrating the relationship between temperature and stripe rust severity across eight wheat-growing districts of Punjab, Pakistan. Panels represent (a) Faisalabad, (b) Bahawalpur, (c) Bahawalnagar, (d) Kasur, (e)

Lodhran, (f) Mandi Bahauddin, (g) Rahim Yar Khan (Sadiqabad), and (h) Toba Tek Singh.

Effect of Temperature and Relative Humidity on Leaf Rust Development Among Wheat Cultivars Across Punjab

An analysis using multiple linear regression was carried out to determine the impact of climate conditions, in particular temperature and relative humidity, on the leaf rust incidence and their severity among three varieties of wheat (Akbar-19, MH-21 and Fareed-06) in all the regions of Punjab that were studied. All the regression analyses had high significance levels ($P < 0.001$) since the climate parameters collectively accounted for a huge part of the fluctuations in the level of crop disease incidence.

As for the performance of the model for leaf rust occurrence, temperature and humidity were significant predictors of the disease level in all varieties. The strongest impact can be traced to Akbar-19 variety (the coefficient is equal to $\beta = 5.53$, $P < 0.001$), followed by MH-21 ($\beta = 3.84$, $P = 0.001$) and Fareed-06 ($\beta = 2.18$, $P = 0.003$). In this way, humidity also played a positive role as it had coefficients of 4.06 for Akbar-19, 3.06 for MH-21, and 2.04 for Fareed-06 (all values are significant $P < 0.001$). The obtained regression equations managed to explain approximately 56.02-58.54% of the variations in contagion of the disease and $R^2 = 44.44$-48.15%.

A similar trend was recorded for the case of leaf rust severity where both climatic factors had highly significant effects on disease development in all cultivars. For the temperature coefficient, maximum values were recorded in Akbar-19 ($\beta = 3.13$) compared to the lowest values in MH-21 ($\beta = 1.93$) and Fareed-06 ($\beta = 0.93$). Similar tendencies were observed with humidity coefficients which were equal to 2.33 for Akbar-19, 1.55 for MH-21, and 0.90 for Fareed-06 ($P < 0.01$). The used regression functions obtained can explain about 51.80-55.88% of the total disease severity variation. The predictive R^2 value ranges from 38.81 and 44.26% showing a certain medium level of predictability of regression models.

The observed values for the Variance Inflation Factor (VIF) were the same (1.75) in all models of leaf rust establishing the absence of multicollinearity between temperature and humidity. As a whole, Akbar-19 was found to be the most susceptible cultivar to climatic variables, followed by MH-21, while Fareed-06 has the least

regression coefficients confirming that it is not very sensitive to the changes in environmental conditions (Figure 5; Table 5).

Table 5: Summary of regression models for leaf rust among wheat cultivars

Cultivar	Disease parameter	β (Temperature)	β (Humidity)	Model P-value	R^2 (%)
Akbar-19	**Incidence**	5.53	4.06	<0.001	58.52
Akbar-19	**Severity**	3.13	2.33	<0.001	53.25
MH-21	**Incidence**	3.84	3.06	<0.001	58.54
MH-21	**Severity**	1.93	1.55	<0.001	55.88
Fareed-06	**Incidence**	2.18	2.04	<0.001	56.02
Fareed-06	**Severity**	0.93	0.9	<0.001	51.8

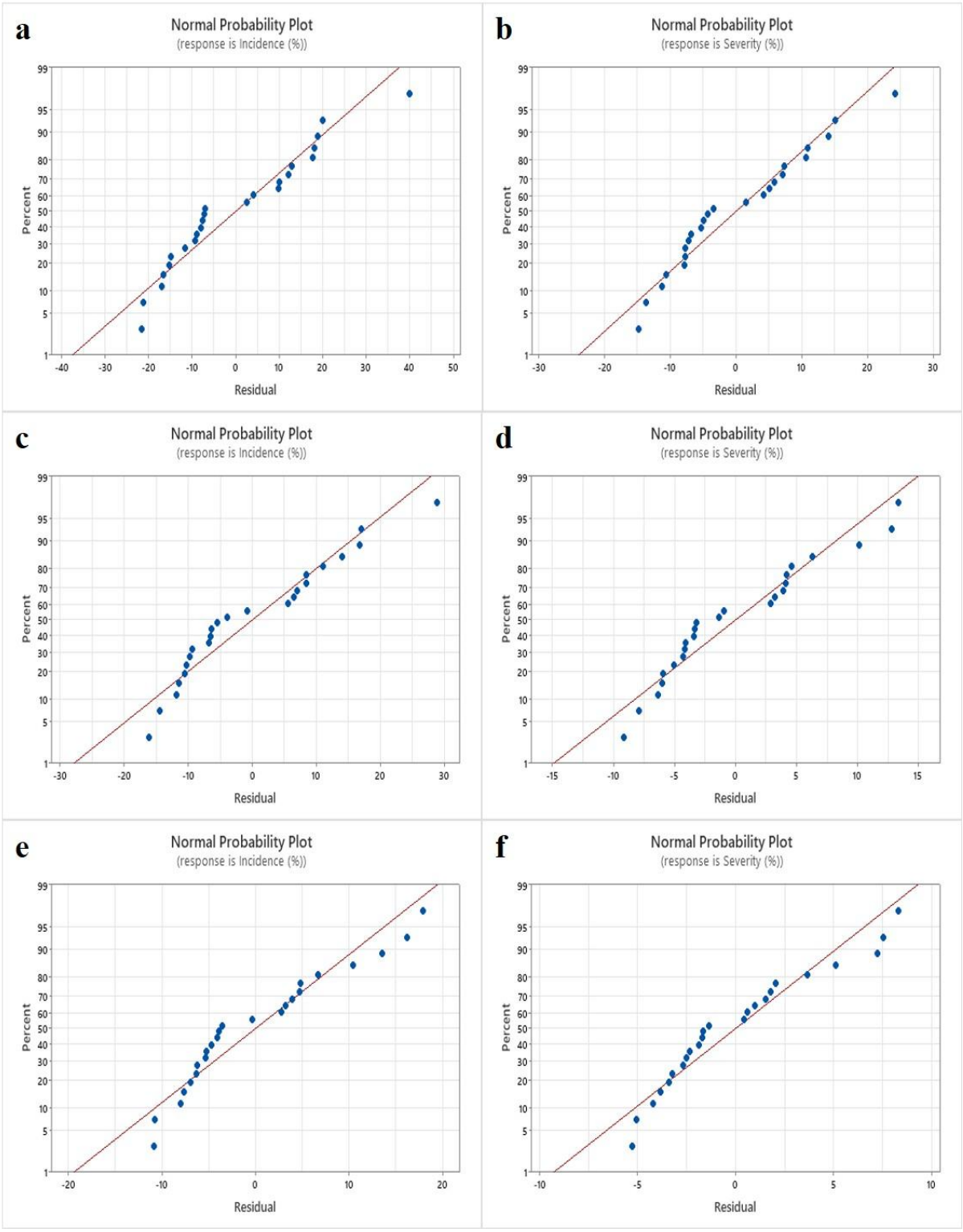


Figure 5. Relationships between temperature, relative humidity, and leaf rust development in three wheat cultivars across Punjab. Panels: (a) Akbar-19 incidence, (b) Akbar-19 severity, (c) MH-21 incidence, (d) MH-21 severity, (e) Fareed-06 incidence, and (f) Fareed-06 severity.

Effect of Temperature and Relative Humidity on Stripe Rust Development Among Wheat Cultivars Across Punjab

In order to assess the effect of temperature and relative humidity on stripe rust incidence and severity on different cultivars of wheat namely Akbar-19, MH-21, and Fareed-06 across various districts of Punjab, the multiple linear regression analysis was deployed and the all regression models were highly significant ($P < 0.001$) implying that the climatic factors explained a good percentage of variance in the process of cultivation of stripe rust in cereals. Both temperature and relative humidity were highlighted as highly significant predictors.

For the occurrence of stripe rust on wheat, Akbar-19 had the greatest sensitivity to climate changes with respect to occurrence of the disease having the coefficients worth 9.46 (temperature) and 7.66 (humidity), respectively. The model could explain 88.69% of variance in disease occurrence, being the most revealing one among the evaluated cultivars. MH-21 had the coefficients of 7.71 and 6.52, accounting for 84.86% of variance. The third variety Fareed-06, on the other hand, exhibited less sensitivity to climate changes (temperature = 4.36, humidity = 3.76) and while still being significant ($P < 0.001$), the variance could be explained at 77.01%. The predictions represented the R2 values varying between 70.18 and 85.61%.

Just like the first case, stripe rust severity was similarly affected by the temperature and relative humidity of the environment in each one of the cultivars investigated. Akbar-19 showed the highest sensitivity with the regression coefficients being 4.86 for the temperature and 4.02 for the humidity, which makes it possible to explain 80.17% of the observed variability of severity of the disease. MH-21 ranked second with the coefficients equal to 3.58 for temperature and 3.06 for humidity (R^2=74.09%). Fareed-06 exhibited the lowest regression coefficients (1.55 for temperature and 1.37 for humidity), even despite the fact that both defined climatic factors have highly significant statistical significance ($P<0.001$) in the model that has a coefficient of determination equal to 69.98%. The final high adjusted and predicted R^2 values guarantee the model accuracy and adequacy of the results.

VIF values for temperature and humidity for all models performed similarly with values equal to 5.06, suggesting moderate multicollinearity. Also, several lack-of-fit tests proved to be non-significant ($P = 0.228$-0.914) for all regression models, which indicating of good models that were fitted to the data. In conclusion, the analysis affirms that both temperature and humidity are significant environmental factors for

stripe rust in Punjab. Among examined cultivars, Akbar-19 had the highest response to environmental change followed by MH-21, with Fareed-06 being less responsive than others thus being relatively more stable in the existing environmental conditions (Figure 6; Table 6).

Table 6. Summary of regression models for stripe rust among wheat cultivars

Cultivar	Disease parameter	β (Temperature)	β (Humidity)	Model P-value	R^2 (%)
Akbar-19	**Incidence**	9.46	7.66	<0.001	88.69
Akbar-19	**Severity**	4.86	4.02	<0.001	80.17
MH-21	**Incidence**	7.71	6.52	<0.001	84.86
MH-21	**Severity**	3.58	3.06	<0.001	74.09
Fareed-06	**Incidence**	4.36	3.76	<0.001	77.01
Fareed-06	**Severity**	1.55	1.37	<0.001	69.98

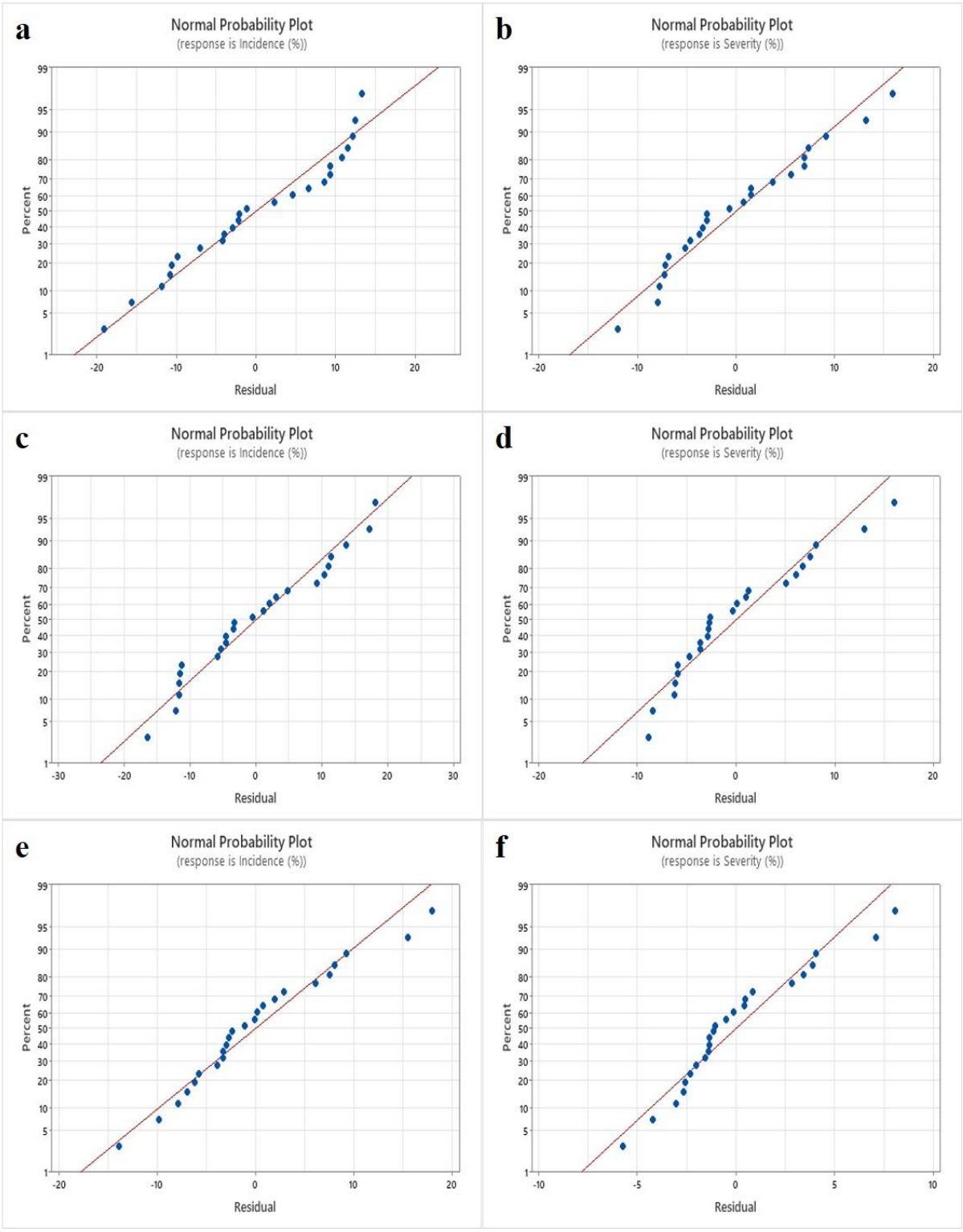


Figure 6. Relationships between temperature, relative humidity, and stripe rust development in three wheat cultivars across Punjab. Panels: (a) Akbar-19 incidence, (b) Akbar-19 severity, (c) MH-21 incidence, (d) MH-21 severity, (e) Fareed-06 incidence, and (f) Fareed-06 severity.

4. Discussion

The present study demonstrated that temperature was the primary climatic factor influencing the incidence and severity of leaf rust and stripe rust across the major wheat-growing districts of Punjab, Pakistan. Regression analysis showed a positive correlation between the temperature and disease appearance, with some regions showing a rise in the diseases with every 1 ^{o}C increase. These findings are consistent with previous studies showing that moderate temperatures enhance pathogen development, sporulation, and infection cycles [25,26,18].

On the other hand, Relative humidity showed little or no statistically significant effect on disease incidence or severity across most districts. Although moisture is essential for spore germination and successful infection, temperature appeared to be the dominant environmental driver under the semi-arid conditions of Punjab. These observations correlate with the findings of previous investigations showing that temperature is the most important predictor of wheat rusts outbreaks, while humidity was not very useful by itself [27,28].

The difference in the observed regression coefficients and performance of the models among the districts shows that the influence of climatic variables on disease development varied among agroecological regions. For example, performance of models in Toba Tek Singh, Kasur, and Mandi Bahauddin was better (with higher R^2) as compared to Rahim Yar Khan. These regional disparities could be attributed to the differences in microclimatic peculiarities, cultivar resistance to disease, and the agronomic practices that are not included in the formulation of the regression models. The regional variation is widely reported in the studies on the epidemiology of wheat rust under different conditions [29,30].

The cultivar-based assessment proves that wheat varieties react differently to the climatic conditions. For example, Akbar-19 was the most responsive to changes in temperature and humidity and exhibited the highest disease incidence and severity. In contrast, Fareed-06 showed comparatively lower disease levels, suggesting greater tolerance to prevailing environmental conditions.

The findings of this study have major consequences for disease control related to wheat in changing climates because temperature was identified as the key driving factor for rust outbreaks. These findings support the integration of temperature-based forecasting models into routine wheat disease surveillance systems to improve early warning, optimize fungicide application, and guide the deployment of resistant

cultivars. Projected increases in temperature associated with climate change are expected to increase the frequency and severity of wheat rust epidemics [31,32].

A limitation of the present study is that only temperature and relative humidity were included in the regression models. Other environmental and biological factors like leaf wetness duration, rain, and wind spore dispersal, soil conditions, and resistance of host contribute to disease development as well. Future studies should incorporate additional environmental variables, including rainfall, leaf wetness duration, wind-mediated spore dispersal, soil conditions, and host resistance, to develop more comprehensive predictive models of wheat rust epidemics [33].

Overall, the findings demonstrate that temperature is the principal climatic determinant of wheat rust development across Punjab and highlight the importance of integrating climate-based forecasting with resistant cultivars for sustainable wheat disease management under changing climatic conditions.

5. Conclusion

This study demonstrated that temperature is the most important climatic factor influencing the incidence and severity of *Puccinia triticina* and *Puccinia striiformis* f. sp. *tritici* in the major wheat-growing districts of Punjab, Pakistan. The results of multiple linear regression analyses showed that higher temperatures were associated with increased leaf rust and stripe rust incidence while relative humidity had no significant independent effect under the prevailing environmental conditions. District-level differences reflected the influence of regional agro-climatic conditions while cultivar comparisons showed that Akbar-19 is the most susceptible to rust disease and Fareed-06 is relatively tolerant. The findings of the study provide important data for developing the temperature-based disease forecasting systems and deployment of resistant cultivars as a component of integrated wheat rust management strategies under changing climatic conditions.

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Ethics approval and consent to participate

This study involved field surveys of cultivated wheat (*Triticum aestivum* L.) grown in farmers' fields across major wheat-growing districts of Punjab, Pakistan. The collection of disease observations from cultivated plants complied with the relevant institutional and national guidelines for plant research. No endangered, protected, or wild plant species were collected or used in this study. Ethical approval for research involving human participants or animals was not required.

Permissions for plant survey and sample collection

Permission to access wheat fields and record disease observations was obtained from the respective landowners/farmers before conducting field surveys. The study did not involve the collection of protected plant species or material from protected areas, and no additional governmental permits or licenses were required under the applicable regulations.

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Author Contributions:

A.Z., M.U., and H.A. conceived and designed the study. A.Z. conducted the field surveys and collected the experimental data. M.U. performed the statistical analyses, interpreted the data, prepared the figures and tables, and wrote the original draft of the manuscript. S.A., M.K., S.A.K., M.E.-U.-H., U.A., and S.R. contributed to methodology development, data validation, interpretation of results, and critical revision of the manuscript. H.A. supervised the research, acquired resources, reviewed and edited the manuscript, and administered the project. All authors read and approved the final version of the manuscript.

Consent for publication

Not applicable.

Conflict of interest

The authors declare that they have no competing interests.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Abbreviations

ANOVA – Analysis of Variance

AWS – Automatic Weather Station

CIMMYT – International Maize and Wheat Improvement Center

PCA – Principal Component Analysis

PMD – Pakistan Meteorological Department

RH – Relative Humidity

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