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## EXPLORING THE INNATE IMMUNITY OF WHEAT GERMLASM AGAINST YELLOW RUST OF WHEAT

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### ABSTRACT

Wheat (*Triticum aestivum* L.) is a staple food for a significant portion of the world's population including Pakistan. Among all limiting biotic and abiotic factors, wheat rusts pose a substantial danger to production worldwide and can result in yield losses of over 90% in susceptible varieties or during epidemics. Yellow rust (*Puccinia striiformis* f. sp. *tritici*), one of several types of wheat rust, is particularly dangerous to wheat production in Pakistan and cause disease on 70% cultivated area of wheat. Keeping in view the devastating nature of yellow rust pathogen, two-year study 2020-2021 and 2021-2022 was conducted in order to screen out wheat breeding lines against yellow rust followed by its relationship with the environmental variables. In this study, one hundred wheat breeding lines were cultivated in the research area of MNS-University of Agriculture Multan and disease was observed in the months of January-April during 2020-21 and 2021-22. Very low level of disease severity (DS) and area under disease progress curve (AUDPC) recorded in all the breeding lines. Epidemiological variables play an important role in the disease progression. Hence, out of hundred breeding lines screened in 2020-2021, 88 breeding lines were supposed to be immune to all naturally occurring race(s) of yellow rust and 12 lines were categorized as moderately resistant to moderately susceptible. Similarly, 92 breeding lines were found immune and 8 showed moderately resistant to moderately susceptible response out of 100 breeding lines during 2021-2022. The findings showed that a high proportion of wheat breeding lines showed no response towards the disease and supposed to be immune. The results from the correlation and regression analysis showed that there was a positive correlation between the maximum, minimum temperature with yellow rust severity (%). While, there was a negative correlation of humidity during 2020-2021 and positive during 2021-2022. Sunshine hours showed the positive relationship with the yellow rust severity (%) during 2020-2021 and negative during 2021-2022. Current study revealed that breeding lines and pathogen were present, which was responsible for the disease in few breeding lines, while epidemiological factors were non-favorable for the disease to develop and progress at large scale. The findings of the current research clearly showed the importance of epidemiological factor which leads toward less disease development. The analysis of natural conditions with yellow rust helps to predict yellow rust outbreak and appropriate management strategies.

**Keywords:** Wheat; *Puccinia striiformis*; Yellow rust; Epidemiological factor.

### INTRODUCTION

Agriculture is unquestionably important around the globe because it is the foundation of all agricultural countries. More than 1000 million people across 60 countries consume wheat, which belongs to the genus

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*Triticum* and family Poacea, in a variety of ways. In Pakistan as well as other parts of the world, Wheat (*Triticum aestivum* L.) is grown as a substantial crop. One of the three major cereal crops is Wheat. It provides 20% of the calories consumed and is grown as a key food crop in 89 developing countries. It is the grain crop that is most widely grown worldwide. Two varieties of Wheat are used to produce the majority of the world's wheat. One of which includes *T. aestivum*, also known as bread wheat or common wheat (Goutam *et al.*, 2013). One of the top two primary foods for many people is

wheat, which is also reportedly the most widely grown as well as traded cereal in the world (Sendhil *et al.*, 2022). Stress is one of the foremost constraints on the growth, production as well as development of wheat. Unforeseen abiotic as well as biotic pressures resulting from unexpected environmental variations or pathogen translocation have put wheat production in risk (Figueroa *et al.*, 2018). Pakistan has experienced frequent environmental changes over the past two decades, including dryness in the winter and diseases brought on by the climate, irregular rainfall and floods in the summer, which resulted in significant losses in crop production (Ahmad *et al.*, 2019). The probability of increasing many natural disasters, including floods, droughts, storms, cyclones, and changes in precipitation patterns, is increased by climate change.

Variations in temperature, humidity, and rainfall have a negative impact on the production of crop plants because agriculture is heavily dependent on the environment and sensitive to agro-climatic conditions (Yohannes *et al.*, 2016; Aryal *et al.*, 2020).

The *Pucciniales*, or rust fungi, belongs to the varied group of fungal plant diseases, some of which have a serious negative influence on agricultural along with forestry production (Lorrain *et al.*, 2019). *Puccinia striiformis* f.sp. *tritici* also referred to as (*Pst*) responsible of causing yellow rust. This disease is found all over the world where wheat is grown (Line, 2002; Chen, 2005; Markell and Milus, 2008; Wellings, 2011). One of the top pathogens causing losses of more than 1% globally, according to a more recent assessment of diseases and pests in significant food crops, is yellow rust of wheat (Savary *et al.*, 2019). Production losses of wheat (*Triticum aestivum*) have been reported as a result of cereal rust diseases. In Asia (Pakistan & India), the chronic epidemics of the wheat rust was reported in early 1800. For the establishment of diseases on any crop, epidemiological variables are important. Therefore, the key to preventing the early start of disease is to understand how meteorological factors affect the development of yellow rust (Ali *et al.*, 2020). Temperature, humidity, wind speed, and rainfall in the field region all affect the development of yellow rust because they have a significant impact on the incidence and severity of the disease as well as the landing of urediniospores. For infection, urediniospores need six to eight hours of wetness (Hassan *et al.*, 2022). Temperature and moisture are likely significant factors

influencing the development of yellow rust epidemics, according to research conducted in other parts of the world. With temperatures between 5 and 10°C, yellow rust will begin to germinate after three hours of leaf wetness (Rapilly, 1979). Wheat crop is constantly at risk worldwide due to the rust diseases. The primary wheat disease in areas where wheat is grown, stripe rust, significantly reduced crop yields. The spread of the disease is also assisted by the favorable environmental factors and susceptible varieties.

Therefore, the appropriate strategies and tactics should be implemented to overcome these significant losses in wheat yield in order to maintain the income of the farmers and wheat productivity. To combat rust diseases, many management strategies are employed, including the adoption of resistant cultivars, the application of chemicals. The most efficient and sustainable method of protecting wheat crops from the rust outbreak is to cultivate resistant cultivars (Ali *et al.*, 2020). To escape the occurrence of yellow rust there is need to discover those varieties which are resistant towards yellow rust. The screening method is thought to be effective for future researches on finding sources of wheat that are resistant to yellow rust.

Due to the environmental conditions in the past few years, there is a need to screen out the available germplasm resource against yellow rust inoculum. Keeping in consideration the above mentioned significant characteristics of yellow rust in wheat, this current study was designed to screen out wheat breeding lines against yellow rust strain(s) and to correlate environmental factors with the disease.

#### **MATERIALS AND METHODS**

**Field Trial:** The experiment was conducted in the research area of MNS-University of Agriculture, Multan. For this purpose, set of 100 wheat breeding lines were grown, under augmented design (Robert *et al.*, 2022). Each genotype covered 1 m<sup>2</sup> field area with 18-inch row to row distance, using hand drill. An extremely susceptible wheat cultivar known as Morocco was sown near entrances to act as a spreader and as a check on the susceptibility of adult plants. Agronomic practices were adopted to achieve the health of crop to keep the crop in good condition.

**Data collection and analysis:** Observations were recorded on natural occurrence and at the first appearance of yellow rust infection on the susceptible check Morocco. The experimental field was observed

from February-April. Till February, there was no disease. After the initiation disease, it was recorded in five plants in each row (22<sup>nd</sup> March, 2021) while in 2022, (16<sup>th</sup> March) at 7 days' interval till the leaves were green. At the initiation of disease symptoms, rust severity % as well as plant response to disease were recorded for 3 consecutive observations after seven days' intervals. Severity of disease was recorded using Modified Cobb's Scale (Peterson *et al.*, 1948) at different intervals. The modified Cobb scale was used to assess yellow rust severity on the flag leaves of (five leaves per site). Data were recorded up to physical maturity of crop.

The infection types were recorded as 0 with no infection visible showed (immune) response. R; resistant (necrotic areas with or without uredia; resistant); MR; moderately resistant (necrotic areas with small uredia), MS; moderately susceptible (medium-sized uredia with no necrosis but some chlorosis) and S: susceptible (large-sized uredia with no necrosis and chlorosis). Visual observations were used to determine the severity. Below 5% severity the intervals were trace and usually 5% interval was used from 5 to 20%, and 10% interval between 20-100% severities. The response value of 0.0, 0.2, 0.4, 0.8 and 1.0 were assigned for 0, R, MR, MS and S, respectively.



Figure 1. Field experiment at MNS-University of Agriculture, Multan

**Coefficient of infection (CI) and Average coefficient of infection (ACI):** Coefficient of infection was calculated by means of data on severity of disease and host response by multiplying the severity value by a value of 0.10, 0.4, 0.8 or 1.00 for host response rating of R, MR, MS or S, respectively (Zerihun *et al.*, 2021). The total of each entry's CI values will be divided by the corresponding data recording years to obtain the Average Coefficient of Infection (ACI) (Kasa

and Negash, 2021).

**Calculation of AUDPC:** For each breeding line, area under the disease progress curve with 7 days interval was determined by using the following formula:

AUDPC = 
$$N1(X1 + X2) \div 2 + N2(X2 + X3) \div 2 + N3(X3 + X4) \div 2$$
 (Milus and Line, 1986). Where, X1, X2, X3 and X4 are rust intensities recorded on first, second, third and fourth recording date and N1 is interval day between X1 and X2.

Table 1. Rating scale of yellow rust infection at adult stage (Peterson *et al.*, 1948).

IT	Reaction	Field response	Response value
0	No disease	No sign of infection	0.0
R	Resistant	Necrotic area with/without minute uredia	0.2
MR	Moderately resistant	Necrotic regions with small uredia	0.4
MRMS	Moderately resistant, moderately susceptible	Necrotic regions with small uredia, no necrosis with medium uredia and possible distinct chlorosis	0.6
MS	Moderately susceptible	No necrosis with medium uredia and possible distinct chlorosis	0.8
MSS	Moderately susceptible-susceptible	No necrosis with medium uredia and possible distinct chlorosis along with large uredia with or without chlorosis	0.9
S	Susceptible	Large uredia with or without chlorosis	1.0

**Relationship of environmental factors conducive for yellow rusts:** Data on the weather, including wind speed, lowest and maximum temperatures, rainfall, and relative humidity, were retrieved from Automatic Weather Station, MNS-University of Agriculture, Multan, Regression as well as correlation analysis with the help of statistical software was used to study the association between environmental factors and disease severity. Environmental information was employed as an independent variable, and the severity of the disease was used as a dependent variable. By displaying the data visually, environmental factors that have a substantial impact on the development of yellow rust were thoroughly investigated.

**Statistical Analysis:** To evaluate the association between environmental variables with the disease

severity, the data of environmental factors as well as disease severity were subjected to correlation and regression analysis.

## RESULTS

**Screening of wheat breeding lines against yellow rust during year 2020-2021:** At the initiation of disease symptoms, rust severity % and plant response to disease were recorded for 3 consecutive observations after seven days intervals. During 2021, in the month of March and April, twelve breeding lines BL-1, BL-13, BL-20, BL-29, BL-50, BL-51, BL-55, BL-62, BL-75, BL-86, BL-94 and BL-97 showed moderately resistant to moderately susceptible reaction (MRMS) with AUDPC value 70, 87.5, 385, 52.5, 280, 70, 52.5, 52.5, 52.5, 70, 70 and 105, respectively (Table 2).

Table 2. Response of different wheat breeding lines during 2021 against Yellow Rust severity based on AUDPC during year 2020-2021.

Breeding line	22-03-21	29-03-21	5-04-21	ACI	AUDPC	Response value
BL-1	5	5	5	4.5	70	MRMS
BL-13	5	5	10	6	87.5	MRMS
BL-29	0	5	5	3	52.5	MRMS
BL-51	5	5	5	4.5	70	MRMS
BL-55	0	5	5	3	52.5	MRMS
BL-62	0	5	5	3	52.5	MRMS
BL-75	0	5	5	3	52.5	MRMS
BL-86	5	5	5	4.5	70	MRMS
BL-94	5	5	5	4.5	70	MRMS
Morocco-20	20	30	30	24	385	MRMS
Morocco-50	20	20	20	18	280	MRMS
Morocco-97	0	10	10	6	105	MRMS

**Screening of wheat breeding lines against yellow rust during year 2021-2022:**

At the initiation of disease symptoms, rust severity % and plant response to disease were recorded for 3 consecutive observations after seven days intervals. During 2022,

seven breeding lines BL-1, BL-13, BL-17, BL-20, BL-30, BL-50, BL-62 and BL-97 showed the moderately resistance to moderately susceptible reaction (MRMS) with AUDPC value 52.5, 52.5, 70, 17.5, 52.5, 52.5, 52.5, 17.5 respectively (Table 3).

Table 3. Response of different wheat breeding lines during 2022 against Yellow Rust severity based on AUDPC

Breeding line	16-03-22	23-03-22	29-03-22	ACI	AUDPC	Response value
BL-1	0	5	5	3	52.5	MRMS
BL-13	0	5	5	3	52.5	MRMS
BL-17	5	5	5	4.5	70	MRMS
BL-30	0	5	5	3	52.5	MRMS
BL-62	0	5	5	3	52.5	MRMS
Morocco-20	0	0	5	1.5	17.5	MRMS
Morocco-50	0	5	5	1.5	52.5	MRMS
Morocco-97	0	0	5	1.5	17.5	MRMS

**Correlation of environmental factors with yellow rust during year 2020-2021:**

All environmental factors i.e. maximum and minimum temperature, rainfall,

relative humidity and sunshine hours had different non-significant correlation with yellow rust of wheat on eight breeding lines during 2020-2021 (Table 4).

Correlation of environmental factors with disease severity of yellow rust during 2020-2021

Breeding line (BL)	Maximum temperature(°C)	Minimum temperature(°C)	Relative humidity (%)	Wind speed (km/hr.)	Sunshine hours(h)
BL-13	R <sup>2</sup> = 0.145% 0.38(0.75)	R <sup>2</sup> = 0.39 % 0.63(0.56)	R <sup>2</sup> = 0.56% -0.75(0.45)	R <sup>2</sup> = 0.82% 0.08(0.94)	R <sup>2</sup> = 0.48% 0.69(0.51)
BL-29	R <sup>2</sup> = 0.98 % 0.99(0.08)	R <sup>2</sup> = 0.97 % 0.98(0.10)	R <sup>2</sup> = 0.89% -0.94(0.20)	R <sup>2</sup> = 0.82% 0.90(0.27)	R <sup>2</sup> = 0.94 % 0.97(0.15)
BL-55	R <sup>2</sup> = 0.98 % 0.99(0.08)	R <sup>2</sup> = 0.97 % 0.98(0.10)	R <sup>2</sup> = 0.89 % -0.94(0.20)	R <sup>2</sup> = 0.82% 0.90(0.27)	R <sup>2</sup> = 0.94 % 0.97(0.15)
BL-62	R <sup>2</sup> = 0.98 % 0.99(0.08)	R <sup>2</sup> = 0.97 % 0.98(0.10)	R <sup>2</sup> = 0.89 % -0.94(0.20)	R <sup>2</sup> = 0.82% 0.90(0.27)	R <sup>2</sup> = 0.94 % 0.97(0.15)
BL-75	R <sup>2</sup> = 0.98% 0.99(0.08)	R <sup>2</sup> = 0.97 % 0.98(0.10)	R <sup>2</sup> = 0.89 % -0.94(0.20)	R <sup>2</sup> = 0.82 % 0.90(0.27)	R <sup>2</sup> = 0.94 % 0.97(0.15)
Morocco	R <sup>2</sup> = 0.98 % 0.99(0.08)	R <sup>2</sup> = 0.97% 0.98(0.10)	R <sup>2</sup> = 0.89 % -0.94(0.20)	R <sup>2</sup> = 0.82% 0.90(0.27)	R <sup>2</sup> = 0.94% 0.97(0.15)
Morocco-2	R <sup>2</sup> = 0.98 % 0.99(0.08)	R <sup>2</sup> = 0.97 % 0.98(0.10)	R <sup>2</sup> = 0.89 % -0.94(0.20)	R <sup>2</sup> = 0.82% 0.90(0.27)	R <sup>2</sup> = 0.94% 0.97(0.15)

The positive association of maximum temperature with yellow rust severity % on most breeding lines was best elucidated by the linear regression model especially in case of breeding lines, BL-29 and BL-13 as indicated by 0.98, 0.145 r values respectively (Table 4). With increase in maximum temperature 29°C to 35°C yellow rust severity increased gradually. Five breeding lines, BL-86, BL-1, BL-51, BL-94 and Morocco-1 did not exhibit any relationship against maximum temperature.

With one unit increase in the minimum temperature yellow rust severity also increases to 1.976 in case of breeding line 13 which shows the R<sup>2</sup> value of 0.3%. While with one degree in increase of the minimum

temperature, the yellow rust severity also increased to 3.105 with R<sup>2</sup> value of 0.97% in case of breeding line 62. The values of correlation explained the non-significant positive relationship of all the breeding lines with minimum temperature.

All the assessed breeding lines responded differently to relative humidity. The association of relative humidity with disease was best explained by linear regression model as indicated by 0.56% and 0.89% R<sup>2</sup> values for BL-13 and BL-29. With one unit increase in relative humidity from 66% to 70% yellow rust severity also decreased gradually (Figure 6). Five breeding lines, BL-86, BL-1, BL-51, BL-94, and Morocco-1 did not exhibit any relationship against relative humidity. The values

of correlation explained the non-significant negative relationship of all the breeding lines with relative humidity.

All the breeding lines showed positive relationship with sunshine hours. Increase in the sunshine hours increases the disease to 9.4 in case of breeding line 62. Breeding line 62 shows the highest R<sup>2</sup> value of 0.94%. The values of correlation explained the non-significant positive relationship of all the breeding lines with sunshine hours.

The association of wind speed with the yellow rust was positive for all the breeding lines. The breeding line 17 showed the non-significant response. Five breeding lines, BL-86, BL-1, BL-51, BL-94, and Morocco-1 did not exhibit any relationship. As the wind speed increased from 5.50 to 6.50 km/h the yellow rust also progressed. At 5.50Km/h the disease severity was the maximum for the breeding line 13 with the r value of 0.82%. The values of correlation explained the non-significant positive relationship of all the breeding lines with wind speed.

**Correlation of environmental factors with yellow rust during 2021-2022:** The association between maximum temperature and yellow rust severity was best explained by linear regression model as indicated by the r value of 0.73% and 0.77 % for Morocco-97 and BL-62 (Table 5). While, BL-1, BL-13, BL-30, BL-62, Morocco-20 exhibited 0.73% R<sup>2</sup> value. BL-17 showed the non-significant results by not altering the disease severity % with the increase of maximum temperature. The values of correlation explained the non-significant positive relationship of all the breeding lines with

maximum temperature.

The association of the minimum temperature with yellow rust was negative within five breeding lines. Morocco-20 and Morocco-50 showed the positive response. While the BL-17 did not show any response. The r values of BL-62 and Morocco-97 was 17.3% and 33.6% (Table 5). All the breeding showed negative relationship with minimum temperature along with non-significant disease progression except Morocco-20 and Morocco-97.

The association between relative humidity and yellow rust disease was best explained by linear regression model as indicated by 96.8% and 41.8% r values for BL-62 and Morocco-97 (Table 15). BL-17 showed no response towards relative humidity. The values of correlation explained the non-significant but positive relationship of all the breeding lines with humidity. As the wind speed increase from 3.8 to 4.3 km/h, the yellow rust progression also increased. At 4.3 Km/h the severity of disease was maximum for the breeding line 62 with the r value of 0.64%. In case of Morocco 97, the regression value showed the negative impact on the progression of disease. The values of correlation explained the non-significant but positive relationship with all the breeding lines except Morocco-50 and Morocco-97 with wind speed.

One-hour increase in sunshine hour negatively affect the yellow rust disease severity (%). The values of correlation explained the non-significant negative relationship of all the breeding lines with sunshine hours except Morocco-20 and Morocco-97 (Table 4).

Correlation of environmental factors with disease severity of yellow rust during year 2021-2022

Breeding line (BL)	Maximum temperature(°C)	Minimum temperature(°C)	Relative humidity (%)	Wind speed (km/hr.)	Sunshine hours(h)
BL-1	R <sup>2</sup> = 0.73% 0.85 (0.34)	R <sup>2</sup> = 0.17 % -0.41 (0.72)	R <sup>2</sup> = 96.8% 0.98 (0.11)	R <sup>2</sup> = 0.64 % 0.80(0.40)	R <sup>2</sup> = 0.57 % -0.75 (0.45)
BL-13	R <sup>2</sup> = 0.73% 0.85 (0.34)	R <sup>2</sup> = 0.17 % -0.41 (0.72)	R <sup>2</sup> = 96.8% 0.98 (0.11)	R <sup>2</sup> = 0.64 % 0.80(0.40)	R <sup>2</sup> = 0.57 % -0.75 (0.45)
BL-30	R <sup>2</sup> = 0.73% 0.85 (0.34)	R <sup>2</sup> = 0.17 % -0.41 (0.72)	R <sup>2</sup> = 96.8% 0.98 (0.11)	R <sup>2</sup> = 0.64 % 0.80(0.40)	R <sup>2</sup> = 0.57 % -0.75 (0.45)
BL-62	R <sup>2</sup> = 0.73% 0.85 (0.34)	R <sup>2</sup> = 0.17 % -0.41 (0.72)	R <sup>2</sup> = 96.8% 0.98 (0.11)	R <sup>2</sup> = 0.64 % 0.80(0.40)	R <sup>2</sup> = 0.57 % -0.75 (0.45)
Morocco-20	R <sup>2</sup> = 0.73% 0.87 (0.34)	R <sup>2</sup> = 0.17 % -0.41 (0.72)	R <sup>2</sup> = 96.8% 0.98 (0.11)	R <sup>2</sup> = 0.64 % 0.80(0.40)	R <sup>2</sup> = 0.57 % -0.75 (0.45)
Morocco-50	R <sup>2</sup> = 0.77% 0.87 (0.31)	R <sup>2</sup> = 0.33. % 0.57 (0.60)	R <sup>2</sup> = 41.8% 0.64 (0.55)	R <sup>2</sup> = 0.013% -0.11 (0.92)	R <sup>2</sup> = 0.036% 0.18 (0.87)
Morocco-97	R <sup>2</sup> = 0.77% 0.85 (0.31)	R <sup>2</sup> = 0.33. % 0.57 (0.60)	R <sup>2</sup> = 41.8% 0.64 (0.55)	R <sup>2</sup> = 0.013% -0.11 (0.92)	R <sup>2</sup> = 0.036% 0.18 (0.87)

## DISCUSSION

Wheat is one of the three primary cereal crops (Rahman *et al.*, 2021). In the majority of Asian countries, wheat is crucial in ensuring both food security and nutrition. Throughout 2.5 billion people around the world use wheat as a staple food, and it accounts for around 20% of an average person's daily caloric intake. Fungal pathogens cause severe damage to the successful cultivation of wheat, particularly in Pakistan. Wheat is impacted not only by biotic factors but also by a number of harmful fungal pathogens. The main wheat disease that significantly reduces wheat yield is wheat yellow rust (*Puccinia striiformis* f. sp. *tritici*), which affects all wheat-growing regions worldwide. The emergence of fungal diseases is also influenced by susceptible cultivars as well as favorable environmental factors. The disease reaction/response of varieties, a phenotypic resulting from wheat-Pst interaction, is typically used to evaluate their resistance level at the field level. However, this interaction is impacted by the varying pathogen population as well as the local climatic variables, which may cause variations in the way the same types of hosts respond. The main objectives of the current study were to identify resistant germplasm against the race(s) of wheat yellow rust pathogen and to determine how these resistances related to environmental conditions.

For the current study hundred breeding were assessed in order to examine the resistant sources as well as the characterization of environmental variables. Two year study (2020-2021) and (2021-2022) was conducted in order to assess the yellow rust severity as well as correlation of the diseases with environmental variables. Previous studies showed how spring wheat lines responded to slow rusting. In comparison to MqPd and Thather, all knott lines displayed much lower AUDPC values. In comparison to MqPd and Thatcher, four K lines—K-12469, K-12481, K-12485, and K-12569 were found to rust more slowly (Yadav, 1985). Our results were in line with these findings as our results showed that the value of AUDPC in both the study years were low. Depending on the pattern of the climate and geography, function of each environmental variable as well as its significance might change under field circumstances. The main factor of yellow rust progression is mainly temperature. The development of yellow rust also depends heavily on highly specific climatic conditions in the presence of inoculum and

susceptible hosts, much like other diseases where these three elements are crucial in the disease triangle. Wind, moisture and temperature are the three key weather elements that influence outbreaks of yellow rust. The spores of stripe rust that form pustules on afflicted leaves must be transported or dispersed by wind. When expelled from a leaf, spores can survive for a few days before they can mature and infect a leaf if they drop on some other living wheat plant, providing the right circumstances are present. High humidity is necessary for infection for 4 to 6 hours at 10 to 15°C, with longer times needed at lower and higher temperatures. Below roughly 2°C, infection rarely happens, and over 23°C, it stops. The high temperature in the disease recording year did not favor the disease. According to recent studies it was confirmed that the most convincing factor for the progression of disease was the temperature in January and February (Elshamy *et al.*, 2022). Another finding suggested that progression of yellow rust was slowed down by temperatures above 23°C (Chakraborty *et al.*, 2011). Another study showed that that the success of Pst infection depends significantly on whether the mean summer temperature falls below the threshold, which is roughly 22–23°C, as this fungus is sensitive to high temperatures. Our findings were in line with this study as it showed that during January 2021 and 2022, maximum temperature was 20°C and 25°C respectively. While, during February, the maximum temperature was 25°C and 26°C respectively and there was a huge fluctuation in maximum temperature during the consecutive two years that resulted in the slow progression of disease. There was no disease when the temperature was higher than 23 °C (Fig 14). Recent studies explained the impact of humidity on the development of yellow rust under natural conditions (Sandhu *et al.*, 2017). Another study showed that the influence of humidity on disease severity (Khan, 1994). These results were in line with our findings of regression analysis that showed the humidity is negatively correlated with the development of disease as shown in (Table 9). Another study showed the impact of humidity with the disease (Mateen and Khan, 2014). This result was not in line with our findings where the relative humidity was highly correlated with the disease. During the study year 2022, the results from the

regression analysis showed that there was a positive relationship of humidity with disease progression.

Present study revealed that the disease which caused the huge destruction in the previous years is now significantly lower in the study years. There are many reasons to explain the fact that this disastrous disease is now of lower impact. One of the main reason is the huge fluctuation in the climatic conditions. Previous years exhibited the optimum climatic conditions that favored the disease and its progression. Fungus requires the optimum conditions to RQive and infect the particular host. When these conditions are not available, the pathogen fails to infect and ultimately the host escaped the disease and termed as immune. The results from the present study showed the facts that temperature is much higher than the optimum temperature which caused the disease to escape. Current study provided the facts that there many reasons for the disease to escape. One of the main reason is the fluctuation in the environmental conditions from the previous years. According to the disease triangle, these conditions must be fulfilled for the disease to occur. Disease is only possible when there is susceptible host, suitable environmental conditions as well as virulent pathogen. Our findings clearly provided the reasons of disease to slow down. The breeding lines were present in addition to virulent pathogen but the only factor that missing was favorable climatic conditions crucial for the disease to occur. Our results showed that there was a huge change in the environmental conditions which doesn't favors the disease and ultimately the disease escape. May be the other factor is also responsible such as adult plant resistance. But mainly the main reason of the disease escape was the fluctuating environmental conditions.

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**Contribution of Authors:**

Areeba Rauf	: Performed experiments, recorded and analyzed data
Mirza A. Mehmood	: Conceived idea, designed experiments, supervised the research, wrote manuscript
Muhammad Ashfaq	: Conceived idea, prepared figures and tables, wrote manuscript
Zulfiqar Ali	: Provided breeding material, designed experiments and wrote manuscript
Rana Binyamin	: Analyzed data and proofread the manuscript
Iqra Mushtaq	: Recorded and analyzed data