

## **Abiotic Environmental Factors Conducive for Wheat Leaf Rust Disease: Finding the Source of Resistance for its Management**

Muhammad Iqbal<sup>1</sup>, Muhammad Asim<sup>1</sup> and Muhammad Ehetisham ul Haq<sup>\*2</sup>

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### **Abstract**

Wheat (*Triticum aestivum* L.) is the main source of food for the world population. The present study was aimed to find resistant germplasm against the leaf rust disease by screening the available varieties/lines and to study the relationship between abiotic environmental factors and leaf rust disease severity. Four hundred and eighty-seven wheat varieties/lines were screened against leaf rust disease under nursery conditions. No variety/line was found immune to the disease. Only four varieties/lines were resistant, six were moderately resistant, ninety-six varieties/lines were found moderately susceptible and 380 were found susceptible against the leaf rust infection. The relationship between maximum temperature, minimum temperature, relative humidity, rainfall and leaf rust disease severity on three susceptible wheat varieties (Hashim-08, SKD-1 and Khiram) was assessed using linear regression models. A strong negative linear relationship between leaf rust disease and maximum temperature was observed. Maximum disease severity was noted at 23-24 maximum temperature (°C). The relationship between leaf rust disease severity and minimum temperature was found positive and maximum disease severity was recorded at 15-16°C. Disease severity increased with the decrease of relative humidity. Maximum disease severity was recorded when relative humidity was between 84-85%. Maximum disease severity was recorded when rainfall was between 1-2mm.

**Keywords:** Screening, varieties, disease severity, humidity, temperature

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<sup>1</sup>Department of Plant Pathology, College of Agriculture, University of Sargodha, Sargodha, Pakistan, <sup>2</sup>Plant Pathology Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan. Corresponding author's email address: haq.uaf@gmail.com

## Introduction

Wheat (*Triticum aestivum* L.) is an important staple food around the globe. It is the most favorite diet of more than two billion people in the world. Taxonomically, it belongs to “Poaceae” family, Triticeae is the only tribe having 15 genera and 300 species both from wheat and barley (Breiman & Graur, 1995). Wheat cultivation is booming in a moderate region. More than 25000 types of wheat have been reported. Annually, it is cultivated on 215 million hectares, an area equal to Greenland, and is grown all over the world, from Scandinavia to South America and Asia, making it the most commonly grown staple food crop. Every year, nearly \$50 billion worth of wheat is traded around the world (CGIAR, 2019).

In Pakistan, wheat was produced 24.946 million tons in the area of 8825 million hectares and the average yield was recorded 2827 kg/ha. Wheat crop contribution is 3.1% in value-added in agriculture and it contributes 0.6% in GDP (GOP, 2020).

The wheat crop is afflicted by many diseases, of which rust is of considerable significance. Wheat is infected with three types of rusts that can be found all over the world. *Puccinia recondite* f.sp. *tritici* is the pathogen of leaf or brown rust disease. It is a serious production danger in wheat all over the world, and it is an airborne foliar disease that largely spares early maturing wheat cultivars from serious rust damage. Brick-red pustules (uredia) appear circular to oblong on leaves, stems, and later on heads. Black spores (telia) develop as the crop matures (Singh, 2018).

The most economical method to manage wheat rusts is the utilization of genetic resistance (Singh et al., 2005). It is the most efficient, cost-effective, and environmentally sustainable approach because it reduces the need for fungicides and lowers production costs (Kolmer, 1996). It is important to classify cultivars with resistant origins that can be recommended as the best for cultivation in diseased areas of the world, taking into account various ecological zones. Screening is believed to be the most effective and cost-effective means of distinguishing wheat cultivars that are resistant to leaf rust.

Optimal environmental conditions are needed for the pathogen multiplication and its virulence (Mekalanos, 1992). Similarly, the expression of R-genes also requires suitable abiotic environmental conditions for the final product (defense proteins) to hinder the pathogen’s infection (Hammond-Kosack & Jones, 1997). Hence, a disease may become endemic if the abiotic environmental condition becomes favorable to the pathogen virulence for a sufficient time. The results may be the total biological and economical yield loss of the crop.

Keeping in view the above facts, the present study was coined to find resistant germplasm against the leaf rust by screening the available varieties/lines. Furthermore, the research was aimed to study the relationship between abiotic environmental factors and leaf rust disease severity.

## Materials and methods

### Screening of germplasm

To find the resistance source, the field experimental trial was conducted at the experimental area of the Department of Plant Pathology, Agriculture College, University of Sargodha. Seeds of 487 wheat varieties/lines were taken from Crop Disease Research Institute,

National Agricultural Research Institute, Islamabad, Pakistan. Morocco was sown as a susceptible check after every ten lines of test genotypes, while four lines were sown around the nursery to increase the inoculum pressure. Each test genotype was sown in five-meter length row keeping row to row and plant to plant 30 cm distance. For artificial inoculum application, brown rust infected leaves were thoroughly mixed in talcum powder and then the mixture having leaf rust pustules were dusted twice a day (early morning and late evening) for three consecutive days. Maximum humid conditions were created by sprinkling the water on wheat leaves for increasing the infection chances. Disease data were recorded after appearing the symptoms on “Morocco” susceptible variety every week using Cobb’s scale (Peterson et al., 1948).

#### **Relationship between disease severity and environmental factors**

Certified seeds of Hashim-08, SKD-1 and Khiram wheat varieties were taken from Crop Disease Research Institute, National Agricultural Research Institute, Islamabad, Pakistan. The experiment was carried out in 2013 at experimental field of the Department of Plant Pathology, Agriculture College, University of Sargodha.

To deter soil-borne pathogen attack and eradicate seed-borne pathogens, each variety's seeds were treated with a systemic fungicide (Topsin-M). The wet towel procedure was used to determine seed germination efficacy. Seeds were sown in beds using a "dibbler" at a depth of 6 cm, with a plant to plant and row to row spacing of 30 cm. With three repeats, the experiment was performed in Randomized Complete Block Design (RCBD). Each agronomic activity was followed. Infection was accomplished by artificial inoculation, which involved dusting the pustules of leaf rust on healthy leaves. The abiotic environmental data (maximum and minimum air temperature, rainfall and relative humidity) were obtained from a conventional mini weather station that was already in operation at the In-Service Agricultural Training Institute (IATI), which was 3000 meters away from the experimental site. Dry and wet bulb thermometers were used to record the maximum (°C) and minimum (°C) temperatures. A hygrometer was used to measure relative humidity, and a rain gauge was used to record rainfall. On three wheat varieties, disease severity and abiotic variables (maximum and minimum air temperature, relative humidity, and rainfall) were used to establish leaf rust disease severity prediction models.

The intensity of leaf rust data recording was started after appearing the disease symptoms. The disease severity was recorded using the following formula at weekly intervals for consecutive four weeks.

$$\text{Disease Severity \%} = \frac{\text{Diseased leaf area}}{\text{Total Leaf area}} \times 100$$

#### **Statistical Analysis**

Environmental data were correlated with disease severity by “Pearson’s Correlation” method and relationships were expressed by simple linear regression models (Steel & Torrie, 1997). SAS (version 9.3) software was used for data analysis. Data was graphically represented through Microsoft Excel (version 2016) software.

#### **Results and Discussions**

##### **Screening of germplasm**

Out of 487 wheat varieties/lines, no one was found immune to the diseases. Only four varieties/lines (Faisalabad-2008, 15FJ03, 15FJ08, 94A 236-1 Marquis "B", Super Kauz) were resistant against the leaf rust infection. Six varieties/lines (DN-141, NW-21, NIFA-21,

PGB-17-20, Thatcher/CS (CI 14173), ER5155 S-203 (1995) Roelfs) exhibited moderately resistant response against the disease. Ninety-six varieties/lines were found moderately susceptible and 380 were found susceptible to the leaf rust disease in filed conditions (Table 1).

**Table 1:** Response of different wheat varieties/lines against leaf rust disease.

Sr. #	Name of varieties/lines	Response
1.	-	Immune
2.	Faisalabad-2008, 15FJ03, 15FJ08, 94A 236-1 Marquis "B", SUPER KAUZ	Resistant
3.	DN-141, NW-21, NIFA-21, PGB-17-20, Thatcher/CS (CI 14173), ER5155 S-203 (1995)Roelfs	Moderately Resistant
4.	Suleman 96, Tijban-10, NIA-Sunder, Ujala-15, Ihsan-16, NN-Gandum1, Shahid-17, Kohat-17, HYT-6057, PR-118, 14BT-022, V-14124, 15FJ01, 15FJ04, 17C092, 15BT001, TWS1578, TWS1581, CCRI-260, CCRI-269, CCRI-270, CCRI-271, SN-008, SN-009, KT-02, NS-78, Sak-5, V-15102, HYT-55-33, NW-5, NW-10, NW-22, NW-23, NW-29, NIFA-11, NIFA-13, NIFA-20, NIFA-26, NIFA-27, NIFA-29, V-9970, WV-2, NIA-18, NIA-19, NIA-23, NIA-25, PGB-17-2, PGB-17-7, PGB-17-10, PGB-17-11, PGB-17-16, PGB-17-18, PGB-17-23, PGB-17-24, RARI-1, RARI-2, RARI-7, RARI-12, RARI-17, RARI-19, RARI-24, RARI-27, RARI-29, WAL-5, WAL-10, WAL-11, WAL-12, WAL-15, WAL-18, TRB-4-84, WB-1, WB-3, WB-6, WRS-13, AUP-00514, WWC-2, WWC-5, WWC-7, WWC-9, Isr9a-Ra CI 14169, W2691*2/Khapstein, Prelude*2/Norka, Mq*6//Stewart*3/RL 5244, RL 6098 (1997) Dyck, Bt/Wld, Einkorn, Chamran = Attila, Cham 6, Cham 10 = Kauz//Kauz/star, Bacanora = Kauz's', RL6043, E84018, TONICHI 81, Yr2, Yr31, Cartens v	Moderately Susceptible
5.	Bakhtawar 92, Manthar-2003, Magalla-99, LU-26, Lasani-08, KT-2010, KT-2000, Kohsar 95, Kohistan 97, Kohinoor 83, Kirin 95, Khyber 87, Khirman, Kaghan 93, Jauhar-78, Janbaz, Iqbal 2000, Inqilab 91, Imdad-05, Hashim-08, Hamal-Faqir, Gomal-08, Galaxy-2013, GA-2002, Fareed-06, Fakhr-e-Sarhad, Faisalabad-08, Faisalabad 85, Faisalabad 83, Dharrabi-11, Dera-98, Darawar-97, Daman-98, Chakwal-97, Chakwal-50, Chakwal 86 Bhittai, Benazir-13, BARS-2009, Bahkhar-2002, Bahawalpur-97, Bahawalpur-2000, Auqab-2000, Atta Habib, AS-2002, Anmol-91, Amin-2000, Abadgar 93, Aas-2009, AARI-2010, Zincol, Boroloug-16, IBGE-Ghaneemat, Shalakot-13, NIFA-Insaf, Gold-16, Johar-16, Fatehjang-16, Umeed-2014, Wardan-17, Pasina17, Israr Shaheed, Local White, Khiram, Anaj-17, Barani-	Susceptible

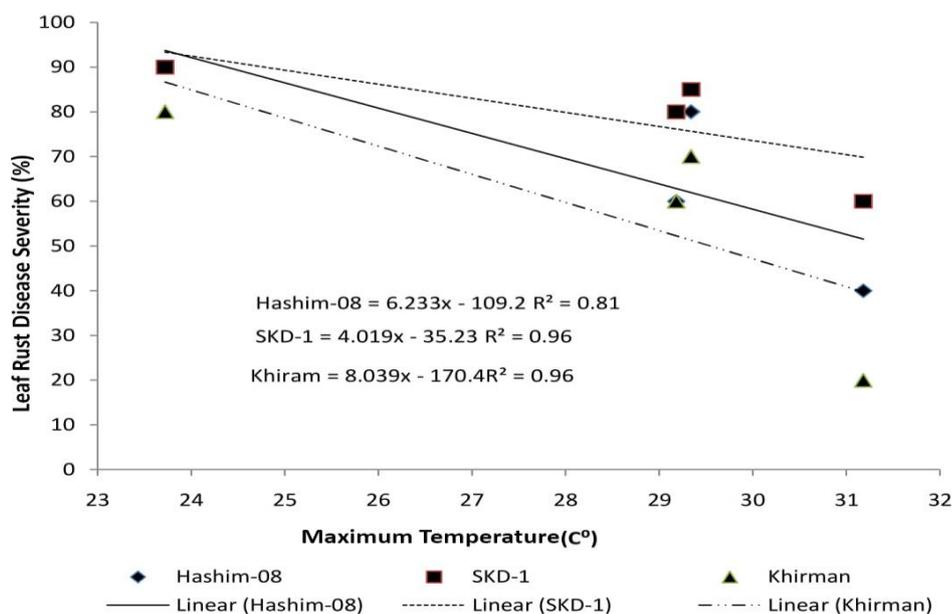
17, NIAB-317, NR-499, Gandum-4, NR-515, DN-123, PR-123, QA-4, 9515, PR-124, Gandum-5, V-14154, NR-488, 14C040, NR-505, HYT-80-34, CTG-1540-13, DN-117, Pakistan-2013, NR- 521, 15FJ02, 15FJ06, 15FJ11, 15FJ12, 15FJ13, 15FJ20, 15FJ23, 15FJ28, AZRC-DK-26, AZRC-DK-69, 17C084, 17C086, 17C088, 16C039, 15C042, 14BT004, 15BT018, TWS1334, TWS15109, TWS15118, TWS15129, TWS15137, TW1614, CCRI-262, CCRI-263, CCRI-264, CCRI-267, CCRI-268, CCRI-272, CCRI-273, CCRI-274, CCRI-279, CCRI-280, CCRI-288, SN-002, SN-010, KT-09, KT-12, KT-15, KT-22, KT-26, KT-28, NS-22, NS-23, BARDC-2-N-17, Seher-06, Local White, BARDC-13-N-17, BARDC-14-N-17, BARDC-19-N-17, Sak-8, Sak-9, Sak-12, V-15099, V-15100, V-15166, V-14116, V-15291, HYT-20-6, HYT-55-40, NW-3, NW-11, NW-12, NW-13, NW-14, NW-15, NW-16, NW-19, NW-20, NW-27, NW-28, Galaxy-13, NIFA-7, NIFA-10, NIFA-18, NIFA-19, NIFA-22, NIFA-23, NIFA-24, NIFA-25, ARI-4, V-9940, WV-1145, NIA-1, NIA-7, NIA-17, NIA-20, NIA-21, NIA-22, NIA-26, NIA-27, PGB-17-12, PGB-17-13, PGB-17-14, PGB-17-15, PGB-17-17, PGB-17-19, PGB-17-22, PGB-17-25, RARI-4, Seher-06, Galaxy-13, RARI-6, RARI-10, RARI-11, RARI-13, RARI-14, RARI-15, RARI-18, RARI-20, RARI-23, RARI-25, RARI-26, RARI-28, RARI-30, WAL-1, WAL-2, WAL-3, WAL-4, WAL-6, WAL-8, WAL-9, WAL-13, WAL-14, WAL-16, WAL-17, WAL-19, WAL-20, TRB-1-21, TRC-2-27, TRB-3-55, WB-2, WB-5, WB-7, WB-8, AUP-01014, AUP-01414, AUP-19314, WWC-4, WWC-8, WWC-10, Seher-06, Galaxy-13, Triticale, Barleta Benvenuto (CI 14196), Vernstein PI 442914, Chinese Spring\*7/Marquis 2B, W2691Sr10 CI 17388, Lee/6\*LMPG-6 DK37, Chinese Spring\*5/Thatcher 3B, Prelude/8\*Marquis\*2/2/Esp 518/9, Little Club/Sr18Mq Marquis "A", 94A 237-1 Marquis "C", McNair 701, T. monococtum/8\*LMPG-6, LcSr24Ag, DK16, Eagle Sr26 McIntosh, WRT 238-5 (1984) Roelfs, Kota RL471, Prelude/8\*Marquis/2/Etiol de Ch, Sr31 (Benno)/6\*LMPG-6 DK42, RL 5405 (1192) Kerber, RL 6099 (1995) Dyck, Prelude\*4/Line W (W3563), Trident Sr38, RL 6087 Dyck, Roughrider, Sisson, Chris, CsSSrTmp, Pavon 76, Seri 82, Kubsa = Attila, Cook, Altar, EL Nielain, Hidhab, Gemmeiza 9, Giza-168, Debeira, Aguilal, Thatcher, Triticale, THATCHER, RL6047, RL6002, RL6007, RL6004, W976, RL6011, RL6013, RL6006, RL6052, RL6005, RL6009, RL6044, RL6064, TRANSEC, RL6078, RL6049, RL5497, RL6058, RL6081, RL6051, WL711, Altar 84 D, Local Red D, Inia 66, Opata 85, Jupateco 73S, Jupateco 73R, Anahuac 75, Genaro 81, Babax#1, Babax#2, Parula, Pavon 76, Pastor, Attila, Amadina, Kukuna, Kakatsi, Yr7,

Yr8, Yr9, Yr10, Yr15, BL 2217, Milan/Shan, PBW 343, PBW 373, HP 1633, HD 2204, WL 1562, Annapurna-1, Pasang Lamu, BL 1473, RR 21, Bhrikuti, BL 1813, BL 1887, Inqilab 91, Bakhtawar 93, Kohistan 97, Punjab85, Kanchan, Gaurab
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Genotype screening is the most common and efficient method of evaluating the morpho-genetic characterization of wheat genotypes for the protection of leaf rust disease. (Mutari et al., 2018). Resistance/susceptibility is largely determined by the genome's heredity (Biffen, 1905). The true resistance occurs when a plant resists against a pathogen under optimal environmental conditions. In the presence of a susceptible host and virulent pathogens, adverse environmental conditions can prevent infection establishment and progression. The genotypes that showed a resistant response can be susceptible in the field. The reason behind this is; under nursery conditions, inoculum pressure is always high compared to farmer field conditions. In previous studies, the varieties which were found resistant during screening trials were moderately resistant when cultivated on farmers' fields (Anwar et al., 2019; Gulnaz et al., 2019; Pooja et al., 2017; Rehman et al., 2019). The major reason behind the susceptibility of resistant cultivars on farmers' fields is mono-culturing for a longer period over large acreage. In Pakistan, some varieties were cultivated due to the high production rate on a large area for a longer time and have become susceptible to leaf rust disease due to the appearance of the new leaf rust races. Anwar et al. (2019) evaluated 37 wheat genotypes against leaf rust disease under field conditions during 2017-18. Out of thirty-seven genotypes, five wheat lines were immune. Seven lines were found to be resistant and ten lines were moderately resistant while five lines were categorized as moderately susceptible. Ten genotypes exhibited a susceptible response against leaf rust disease.

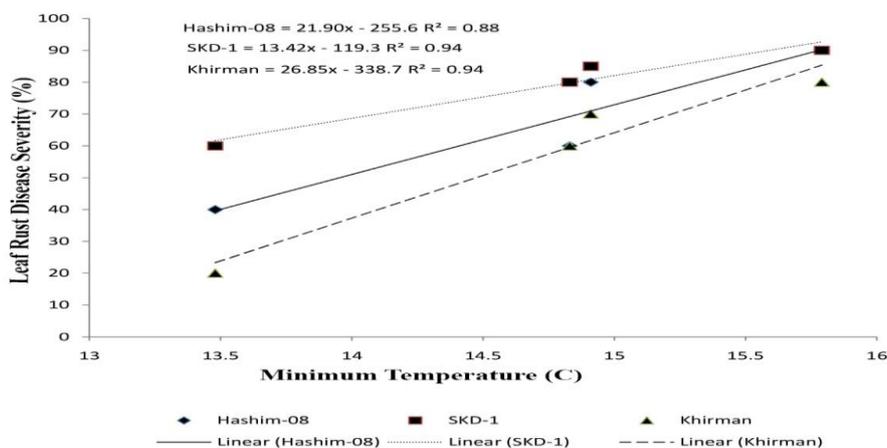
#### **Relationship of environmental factors with leaf rust severity**

The strong negative linear relationship between leaf rust disease and maximum temperature observed on three wheat varieties. With the increase of maximum temperature, leaf rust severity decreased. Maximum disease severity was recorded when the maximum temperature was between 23-24°C (Figure 1).



**Figure 1:** Relationship between leaf rust disease severity and maximum temperature

The relationship between minimum temperature and leaf severity was linear and positive. With the increase of minimum temperature, leaf rust severity increased. Maximum BR severity was recorded when the minimum temperature was between 15-16°C. Strong impact of minimum temperature on disease severity ( $R^2 = 0.88, 0.94, 0.94$ ) of three wheat varieties i.e. Hashim-08 (.88), SKD-1 (0.94) and Khirman (0.94) was observed (Figure 2).

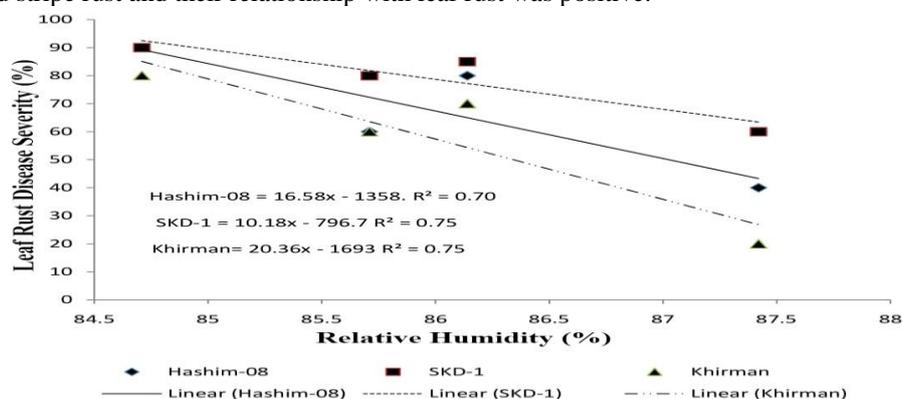


**Figure 2:** Relationship between leaf rust disease severity and minimum temperature

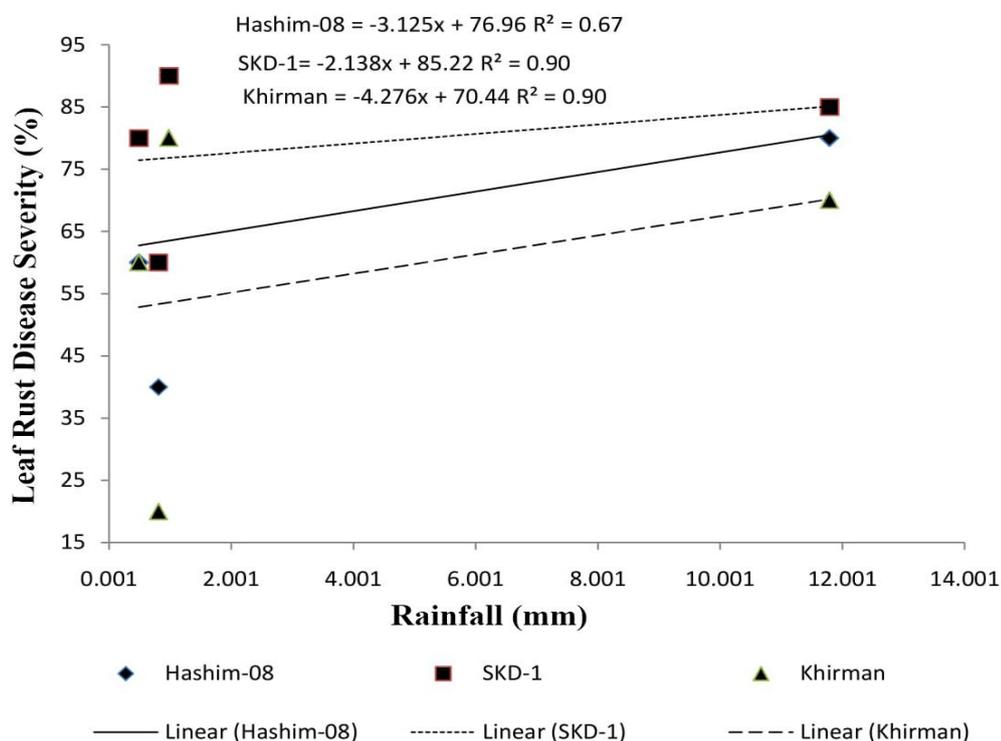
The relationship between relative humidity and leaf rust severity was found linear and negative. Disease severity increased with the decrease of relative humidity. Maximum disease severity was recorded when relative humidity was between 84-85% (Figure 3).

The linear positive relationship was observed between rainfall and leaf rust disease severity. Disease severity increased with the increase in rainfall. Maximum disease severity was recorded when rainfall was between 1-2mm. SKD-1 and Khirman varieties expressed a similar response i.e.  $R^2 = 0.90$  and  $0.90$  respectively except Hashim-08 where weak dependence ( $R^2 = 0.60$ ) of disease severity on rainfall was noted (Figure 4).

In disease progression, the environment plays an important role. For an infection and disease development, the suitable environment is necessary. The contact time between the host and the pathogen is lengthened when the environment is reliably favorable. Pathogens require the optimal temperature to multiply, produce, and activate their lethal weapons (enzymes and toxins). Plants with R genes develop resistance to intruders, which necessitates ideal conditions. Polymerase 1, polymerase 2, and polymerase 3 are enzymes involved in the transcriptional and translational processes of gene un-coding. Their normal activity requires ideal environmental conditions. However, if these conditions do not persist for a set period of time, the R-gene is unable to infer resistance, and the resistant germplasm may become susceptible to a pathogen. Relative humidity and rainfall, particularly for rust fungi, are important factors in fungal infection. The leaf wetness provides the anchorage support to the fungal spore. The urediospore of leaf rust only needs adequate moisture for its infection peg development. Furthermore, the rainfall splashes facilitate the urediospore dispersal to other healthy leaves. Grabow et al. (2016) studied the relationship of environmental conditions with the epidemic of leaf and stripe rust of wheat. Relative humidity (87 %), soil moisture and temperature (7 to 12 °C) played a significant role in disease development. Gulnaz et al. (2019) studied the impact of environmental variables on leaf rust disease severity and found that the temperature plays a significant role in disease development. A linear positive relationship was found between air temperature and leaf rust disease severity. Rehman et al. (2019) found that minimum temperature and rainfall were significantly correlated with leaf and stripe rust and their relationship with leaf rust was positive.



**Figure 3:** Relationship between leaf rust disease severity and relative humidity



**Figure 4:** Relationship between leaf rust disease severity and rainfall

#### Conclusion

The varieties/lines (Faisalabad-2008, 15FJ03, 15FJ08, 94A 236-1 Marquis "B", Super Kauz) which were found to be resistant against the disease may be used for the future breeding program to create resistance against the leaf rust disease. Furthermore, the relationship between disease severity and abiotic environmental factors revealed that by predicting the disease severity, we may take prophylactic measures to minimize the yield losses of the crop.

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