## Quantification of Leaf Rust Resistance Source in Wheat Germplasm in Relation to Epidemiological Factors

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#### Abstract

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A comprehensive germplasm screening of 855 wheat advanced lines was conducted at Wheat Research Institute, Ayub Agricultural Research Institute, Faisalabad to identify new sources of leaf rust resistance during crop seasons 2015-2017. In a primary evaluation, 112 advanced lines were selected having high phenotypic uniformity for further testing at Wheat Research Institute, Faisalabad, a hotspot for leaf rust. The second round of evaluation included 54 lines having durable type resistance against leaf rust. Assessment of epidemiological factors indicated great influence on the progress of leaf rust disease development. Minimum and maximum temperatures, relative humidity, rainfall and wind speed of 11-18.2°C, 23-33°C, 44-67%, 2-8.5 mm and 1.6-3.1 km/h respectively proved most conducive for leaf rust disease development. Avirulence to virulence formula showed that 7 Lr genes namely Lr18, Lr19, Lr28, Lr32, Lr34, Lr36 and Lr23+ (GAZA) remained most effective at all four locations. It was concluded that resistant wheat advanced lines and Lr genes identified under natural environmental conditions can be an excellent source to be deployed in breeding for disease resistance by incorporating such resistance genes into the background of high yielding wheat cultivars through molecular or conventional breeding methodology, and are expected to contribute toward food security at national and global levels.

Keywords: AUDPC, Environmental factors, leaf rust, resistance source, virulence, wheat advanced lines

#### Introduction

Wheat ranks first most important cultivated cereal crop worldwide with annual production of 734.2 million tones (Shiferaw et al., 2013). In Pakistan, it is cultivated under an area of 9.204 million hectares with 25.482 million tons grain production (FAO, 2016). However, increasing production in the face of changing climate required protection against various biotic stresses that cause huge vield losses. Among biotic stresses, leaf rust caused by Puccinia recondita f. sp. tiritici is potential risk to wheat production all over the world (Kisana et al., 2003). Emergence of new rust races with increased virulence represent further risk to wheat production. Various races of this pathogen are known that cause severe yield losses (Hussain et al., 2016a; 2016b). In Asia, leaf and stripe rust could affect production on approximately 60 (63%) and 43 (46%) million hectares, respectively, if susceptible varieties are grown (Aquino et al., 2002).

Symptoms of leaf rust include reddish brown pustules that develop on leaves and sheaths. A single uredospore invade a leaf and produce a pustule with thousands of new spores within 7-10 days (Kolmer, 2013). Environmental factors such as temperature, humidity and rainfall played significant role in disease scattering and cause epidemic (Ali *et al.*, 2017). At the right time, wind blowing in the opposite direction bring vectors and spores far away from

the diseased plants. According to Khan *et al.* (2006), pathogen caused maximum infection at 15-16°C (minimum temperature), 30-35 °C (maximum temperature) with more than 80% relative humidity.

Ten to seventy percent yield losses on wheat are due to the vulnerability of the varieties, initial infection, duration of the disease and disease development rate (Chen, 2007). It results in lower kernel weight, reduction of kernels per head, degradation in grain quality and increased costs linked to chemical control (Bolton *et al.*, 2008; Huerta-Espino *et al.*, 2011). In North America, rusts cause an estimated average loss of 1 million tons (2%) annually, while in Australia it causes average annual losses \$913 million to the wheat industry (Murray & Brennan, 2009; Wiese, 1977). In Pakistan, estimated yield losses due to epidemics of stripe and leaf rusts are 2.2 million tons worth US\$ 330 million and while the stripe rust prevalence has never gone below 8% in the country's history since 1950 (Ahmad, 2004; Hafiz, 1986).

Due to high disease outbreaks, wheat breeders have developed several rust resistant varieties in collaboration with pathologist and International Wheat and Maize Improvement Centre (CIMMYT) by utilizing the advanced breeding material. In spite the development of rust resistant varieties, appearance of new types of virulent races under changing climatic conditions had led to a breakdown of resistance (Javaid *et al.*, 2018). Therefore, the effect of change races on commercial cultivars, genetic stocks, and new advanced lines should be monitored and new sources of resistance to new and predominant races should be sought continuously (Wan *et al.*, 2016). Keeping in view all

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above mentioned facts, the aim of study was to evaluate wheat advanced lines under natural environmental conditions and to monitor leaf rust virulence pattern by using avirulence/virulence formula.

# **Materials and Methods**

Wheat germplasm of 855 advance lines (F6 generation) of 45 diverse crosses based on 8-10 year wheat rust history and high yield characteristics (Table 1) were selected from gene pool of Wheat Research Institute Faisalabad. The trial was sown during 2<sup>nd</sup> week of November, 2015-16 through hand drill following augmented design with single replication split with 9 blocks having 5 plots per block containing 19 genotypes with one check (Morocco). Each plot consists of 20 rows 2.5 m long and 25 cm apart. Morocco was inoculated using spraying, dusting and hypodermal needle injection methods twice during month of January and February to develop high rust inoculum pressure (Roelfs et al., 1988). Rust severity % age and filed response were recorded following modified Cobb's scale (Peterson et al., 1948) for five consecutive observations after every 7 days interval when morocco became 70-80% susceptible.

For further testing, during the year 2016-17 outstanding selected lines (F7 generation) were planted by power (Norvigion) in experimental area of wheat research institute (WRI), Faisalabad in Augmented design. Each test entry was planted in a plot (6 rows of 5 m length). To facilitate development of rust epidemic two rows of Morocco were planted across the experimental material i.e. along the paths on each side of experimental material. Artificial inoculation of experimental material was done with mixture of 1 stripe and 5 leaf rust races collected from Faisalabad, Murree and Kaghan by spraying uredospore suspension (30 gm of spore/16 L of water). The inoculation was done in the evening at regular intervals (4-5 times) from first week of January to first week of February using spraying, dusting and hypodermal needle injection method, twice a week, until a heavy inoculum develops (Roelfs et al., 1988). The applied inoculum consisted of stripe (80E85) and mixture of leaf rust (PHTTL, PGRTB, KSR/JS, TKTPR and TKTRN) races collected from Murree, Kaghan and Faisalabad. High humidity was maintained by frequent irrigations.

# Monitoring of leaf rust virulence pattern through avirulence/virulence formula

A set of rust trap nurseries consisting of 39 *Lr* near isogenic lines *Lr1*, *Lr2a*, *Lr2c*, *Lr3*, *Lr3ka*, *Lr3bg*, *Lr9*, *Lr10*, *Lr11*, *Lr12*, *Lr13*, *Lr14a*, *Lr14b*, *Lr15*, *Lr16*, *Lr17*, *Lr18*, *Lr19*, *Lr20*, *Lr21*, *Lr22a*, *Lr22b*, *Lr23*, *Lr24*, *Lr25*, *Lr26*, *Lr27+31*, *Lr28*, *Lr29*, *Lr30*, *Lr32*, *Lr33*, *Lr34*, *LR35*, *Lr36*, *Lr37*, *LrB*, *Lr13* (WL-711) and *L23*+ (Gaza) received from CIMMYT with known genes were planted in four different locations: three in Punjab including Bahawalpur, Khanewal, Faisalabad and one in Islamabad during cropping season of 2015-16 and 2016-17. The beds of rows were bordered by a line of Morocco, a highly susceptible rust spreader. The sowing was done at different times at different locations to encourage high rust pressure. In Khanewal, Bahawalpur it was time during mid-November, at Faisalabad two sowings were done each year around the first week of November and the end of December to provide long time succulent substrate for rust multiplication. In Islamabad the sowing was done at the end of November. Plots were inoculated with mixture of inoculum from different leaf rust samples using hypodermal needle injection, spraying and dusting methods to develop high rust pressure.

#### Data recording and statistical analysis

On the appearance of disease symptoms, field response and leaf rust reaction were recorded after every seven days interval through modified Cobb's scale (Peterson *et al.,* 1948). Area Under Disease Progress Curve (AUDPC) was calculated by using following method proposed by CIMMYT (Shaner & Finney, 1980).

$$AUDPC = \sum_{i=1}^{n-1} [xi + Xi1]/2](ti + 1 - t1)$$
  
where Xi = rust severity on date i

ti = time in days between i and date i + 1

n = number of dates on which disease was recorded.

Environmental data, which consist of maximum and minimum air temperature, relative humidity, rainfall and wind speed, were collected from Meteorological Station of Department of Crop Physiology, Wheat Research Institute, Ayub Agriculture Research Institute, Faisalabad for the year 2015-16 and 2016-17. The environmental factors (starting from the initiation of disease symptoms up to physical maturity of the crop) were correlated with leaf rust disease severity through linear regression analyses (Steel *et al.*, 1997).

### **Results**

#### Evaluation of wheat advanced lines against leaf rust

During 2015-16 out of 855 advance lines; one hundred and twelve lines were selected for rust resistance and high phenotypic uniformity (Table 1). Whereas, in the second round of evaluation, 14 lines showed immune response, whereas 22, 18, 14, 30, 10, and 4 advanced lines showed R, MR, MRMS, MS, MSS and S types of reactions against disease development with different AUDPC values (Table 2).

#### Characterization of environmental factors conducive for leaf rust development on five advanced lines during 2015-16 and 2016-17

Epidemiological factors played significant role in disease development. Five wheat advance lines i.e. TAM200/Tui/6/PVN/CRC422/ANA/5/BOW//CROW/BUC /PVN/3/YR/4/TRAP#1/7/\*21NO-91;

SH88/WEAVER/3/DWL5023/SNB//SNB;

SH88/2\*ATTILA/6/ACHTAR\*3//KANZ/KS8585/4/MILA N/KAUZ//PRINIA/3/BAV92/5/MILAN/KAUZ//PRINIA/3 /BAV92;

CNDO/R143//ENTE/MEXI\_2/3/AEGILOPSSQUARROS A(TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TACUPETO F2001/8/OASIS/SKAUZ//4\*BCN/3/2\*PASTOR; and ROLF07\*2/KIRITATI/3/SW8688//PBW343\*2/KUKUNA indicated by V60261, V60298, V60347, V60362 and V60854 during first crop season and V70055, V70058, V70059, V70061 and V70112 during second year were

employed to regression analysis for characterization of critical ranges of environmental factors i.e. maximum and minimum temperature, relative humidity, rainfall and wind speed conducive for leaf rust disease development.

Table 1. Selection of single head crosses from F7 generation of 45 crosses.

Sr. #	Nome of ouroges	Test	Selected
1	INTERIAD 2000/INC 01/5/INDEL 1*2///SNUTD AD#1/2/VAU7*2/TD AD//VAU7	10	
1	CHENAB2000/INQ.91/5/WBLL1*2/4/SNI/IRAP#/J5/KAUZ*2/IRAP//KAUZ AS 2000/5/JDET735/4/(SNI/TD AD#/2/WAUZ\$25/TD AD//Z I	19	5 1
2	AS-2002/3/FRE12 · 2/4/SNI/ IRAF#1/3/RAU2 · 2/ IRAF/RAL ESD 09 ( $\alpha$ /D AD V ( $\alpha$ /ES (AN/V)// A1/Y/D AD A V ( $\alpha$ // II ) 26/UD2170	19	1
3	FSD.06/0/DADAA/5/FASAN/1//KAUZ/4/DADAA/5/LU 20/FDZ1/9	19	4
4	KAUZ//ALTAK64/AUS/J/MILAN/KAUZ/4/IUTTES/J/KAUZ//ALTAK	19	0
5	SIL 69/PAK.61//MIL.9///ULS/10DA9/	19	3
07	SR.36/PAK.31//MR.9///CUMINUKIE1/NE 0ASIS/\$*ANDO A//NO 01///MILAN/\$7720//DADAY	19	5
0	UA31513 'ANURA/INQ.71///MILAN/367230//DADAA TDM/MAVA 7//S'/MONYSY2/INO 01/A/DW242	19	4
0	I NIW/MATATA 74 57 MION 577 MION 577 HIGH 545	19	7
9	0/07//ERA//FAR-01/2' V-0/07/9/30AFAQ-00/4/WIATA/FVN DEALI/MILAN/S/CHEN/A SO/TALIS//DEN/2/X/EP#7/DOW///DASTOD/6/OINCHAIDDI/WDLLU/DDDT3	19	3
10	FFAU/MILAN/3/CHEN/A.3Q(TAUS)//DCN/3/VEE#//DOW/4/FASTOR/0/QINOHAIDN/WBLLI//DKB12	19	3
12	INQALAD 91°2/IAUNA/NINTATI///v-09014	19	3
12	AUGAD 2000 22/2AK 1A-1 ESD 08/6/DA DA V/2/ESCAN/V//VAU7/4/DADAV/5/LU26/UD2170/7/DD 06/87004//MU 07	19	4
13	T3.000/0/DADAA/3/TA3AN/1//KA02/4/DADAA/3/DU20/HD21/3///ID.20/0/094//MH1.5/ T3.000/Twile(20VN/CDC202)/ANA/5/DOW//DUC/DVD/VD/VD/VD/VD/AD4/17/#21NO_01	19	2
14	1AM200/10/0/FV1/CRC422/ANA/3/DOW//CROW/DUC/FV1/3/1R/1R/1///1RAF#1///211Q-91	19	2
15	INQ/AUQAD/3/5II.00/70/AD04///MIL.7/	19	1
17	SH00/WEAVEN/JDWLJ023/SND//SND SH00/WEAVED/AII/10/5/DADAY/2/MANGO/VEE#10//DDI///DADAY	19	1
10	Shoo weaver $0/0/10/20/10/21/7/3/0/0000/00/00/000/000/0000/0000/000$	19	0
10	KIΝΔ//FRFT2/6/ΜΙΙΔΝ/ΚΔΙΙΖ//PRIΝΙΔ/3/ΒΔV02	19	0
19	SH88/2*ATTIL A/6/ACHTAR*3//KANZ/KS8585/4/MILAN/KAUZ//PRINIA/3/BAV92/5/MILAN/KAUZ//P	19	2
17	RINIA/3/BAV92	17	2
20	CNDO/R143//ENTE/MEXI_2/3/AEGILOPSSOUARROSA(TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TAC	19	3
	UPETO F2001/8/OASIS/SKAUZ//4*BCN/3/2*PASTOR		
21	CNDO/R143//ENTE/MEXI 2/3/AEGILOPSSOUARROSA(TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TAC	19	9
	UPETOF2001/8/CROW'S'/NAC//BOW'S'		
22	PFAU/SERL1B//AMAD/3/INOALAB91*2/KUKUNA/4/WBLL1*2/KURUKU/5/PVN/YACO/3/KAUZ*2/T	19	3
	RAP// KAUZ		
23	HUW234+LR34/PRINIA//PBW343*2/KUKUNA/3/ROLF07/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	19	0
24	PRL/2*PASTOR//PBW343*2/KUKUNA/4/CAR422/ANA//TRAP#1/3/KAUZ*2/TRAP//KAUZ	19	0
25	C80.1/3*BATAVIA//2*WBLL1/3/PBW343*2/KUKUNA/4/KAUZ / SITE	19	3
26	INQALAB 91*2/KONK//INQALAB 91*2/KUKUNA/3/INQ-91*2/TUKURU	19	1
27	WHEAR/KRONSTAD F2004/3/CROW'S'/NAC//BOW'S'	19	1
28	WHEAR/KRONSTADF2004/3/PB96/V87094//MH97	19	1
29	FRT/SA42/3/PB96/87094//MH-97	19	1
30	WHEAR/KRONSTAD F2004//KAUZ / SITE	19	7
31	PFAU/MILAN//PBW343*2/TUKURU/3/T.DICOCCON P194625/A.SQ (372)//TUI	19	1
32	PFAU/MILAN//PBW343*2/TUKURU/3/NR381	19	1
33	CROC_1/AE.SQUARROSA(205)//KAUZ/3/ATTILA/4/BOW/PRL//BUC/3/WH576/5/AMSEL/ATTILA/IN	19	3
	Q.91/PEW'S'		
34	CROC_1/AE.SQUARROSA (205)//KAUZ/3/PASTOR/4/THELIN/5/INQ/AUQAB	19	5
35	MINO/898.97/4/INIA66/7C//MAYA/3/PCI/TRM	19	1
36	CHONTE//PBW343*2/KUKUNA/3/CHENAB2000/INO.91	19	0
37	CHONTE//PBW343*2/KUKUNA/INQ.91*2/TUKURU/3/T.DICOCCOM/P194624/AE.SQ	19	1
	(409)//BCN/4/2*INQ.91/2*/		
38	PB96/87094/MH-97/3/AMSEL/ATTILA//INQ.91/PEW'S'	19	0
39	PB96/87094//MH-97/3/MILAN/S87230//BABAX	19	1
40	LU26/HD2179//TTR'S'/JUN'S'/3/HP1744//4/MILAN/S87230//BABAX	19	0
41	CNDO/R143//ENTE/MEXI_2/3/AEGILOPSSQUARROSA(TAUS)/4/WEAVER/5/IRENA/6/LERKE/7/TAN	19	5
	/PEW//SARA/3/CBRD		
42	PBW343*2/KUKUNA//KRONSTADF2004/3/PBW343*2/KUKUNA/4/CHENAB2000/INQ.91	19	1
43	PBW343*2/KUKUNA//KRONSTADF2004/3/PBW343*2/KUKUNA/4/CHENAB2000/INQ.91	19	1
44	ATTILA*2//CHIL/BUC*2/3/KUKUNA/4/WAXWING*2/TUKURU	19	1
45	ROLF07*2/KIRITATI/3/SW8688//PBW343*2/KUKUNA	19	2
	Total	855	112

A significant correlation was observed with all environmental factors and leaf rust disease development on all wheat advanced during 2015-16 and 2016-17. During first season maximum disease severity was observed at 11-18.2°C minimum temperature, while during second crop season 2016-2017, maximum disease severity was recorded at minimum temperature ranging from 12-17.5°C (Figure 1). In 2015-16, linear regression model best explained the relationship between leaf rust disease percentage and maximum temperature. All advanced lines showed different response to maximum temperature recorded after disease initiation. Leaf rust disease percentage was maximum at 30°C maximum temperature during 2016-17 (Figure 2). There was positive relationship between relative humidity and leaf rust severities. With an increased in relative humidity ranging from 44-63 and 49-67%, values of leaf rust severity also increased during both disease rating seasons 2015-16 and 2016-17 respectively. This relationship was best explained by linear regression model as indicated by their r values (Figure 3.) Rainfall was positively correlated with disease severity on most of the new advanced lines which was exhibited by linear regression model and maximum disease was noted at 8-8.5 (mm) during both crop seasons; it demonstrated that disease severity increased with increased rainfall (Figure 4). A positive relationship was recorded between wind speed and leaf rust disease severity. During both crop seasons, leaf rust severity increased with an increased in wind speed ranging from 1.6-3.1 (km/h) (Figure 5).

Table 2. Evaluation of wheat germplasm against leaf rust.

Sr. No.	Frequency of genotypes	Ranges of AUDPC	Reaction*
1	14	0	Ι
2	22	35-170	R
3	18	140-300	MR
4	14	300-560	MRMS
5	30	700-1050	MS
6	10	1200-1600	MSS
7	4	1820-1905	S

\* I= immune, R= resistant, MR= moderately resistant, MRMS= moderately resistant to moderately susceptible, MS= moderately susceptible, MSS= moderately susceptible to susceptible, S= susceptible.

# Virulence analysis of leaf rust pattern through avirulence/virulence formula

The results of both years of study indicating avirulence/virulence pattern of leaf rust tester lines at all locations is presented in Table 3. During 2015/16 very high virulence against *Lr* genes i.e. *Lr1*, *Lr2c*, *Lr3*, *Lr3ka*, *Lr3bg*, *Lr10*, *Lr11*, *Lr13*, *Lr16*, *Lr17*, *Lr20*, *Lr22b*, *Lr23*, *Lr24*, *Lr25*, *Lr26*, *Lr2+31*, *Lr30*, *LrB*, and *Lr13* (*WL-711*) were found common at three locations Faisalabad, Khanewal, and Bahawalpur. The virulence against *Lr21* and *Lr22b* was recorded only in Faisalabad, while the virulence against *Lr35* and *Lr14b* was observed both in Faisalabad and as well as at Bahawalpur. On the other hand *Lr* genes i.e. *Lr1*, *Lr2c*, *Lr3*, *a Lr3ka*, *Lr3bg*, *Lr10*, *Lr11*, *Lr12*, *Lr26*, *Lr30*, *LrB*, *Lr13*(*WL 711*) showed the common virulence pattern in Islamabad (Table 3).



**Figure 1.** Relationship between minimum temperature and disease percentage recorded on whaet advanced lines V60261, V60298, V60347, V60362, V60854 during 2015/16 (A) and observed on V70055, V70058, V70059, V70061 and V70112 during 2016/17 (B).



**Figure 2.** Relationship between maximum temperature and disease percentage recorded on whaet advanced lines V60261, V60298, V60347, V60362, V60854 during 2015/16 (A) and observed on V70055, V70058, V70059, V70061 and V70112 during 2016/17 (B).



**Figure 3.** Relationship between relative hmidity and disease percentage recorded on what advanced lines V60261, V60298, V60347, V60362, V60854 during 2015/16 (A) and observed on V70055, V70058, V70059, V70061 and V70112 during 2016/17 (B).



**Figure 4.** Relationship between rainfall and disease incidence (%) recorded on what advanced lines V60261, V60298, V60347, V60362, V60854 during 2015/16 (A) and observed on V70055, V70058, V70059, V70061 and V70112 during 2016/17 (B).



Figure 5. Relationship between Windspeed and disease percentage recorded on wheat advanced lines V60261, V60298, V60347, V60362, V60854 during 2015/16 (A) and observed on V70055, V70058, V70059, V70061 and V70112 during 2016/17 (B).

But the virulence pattern changed during the year 2016-17 which indicated that virulence against *Lr1*, *Lr2c*, *Lr3*, *Lr3ka*, *Lr3bg*, *Lr10*, *Lr11*,*Lr12*, *Lr13*, *Lr14b*, *Lr16*, *Lr17*, *Lr20*, *Lr22b*, *Lr23*, *Lr24*, *Lr25*, *Lr26*, *Lr2+31*, *Lr30*, *LrB*, and *Lr13* (WL-711) were found common at three locations Bahawalpur, Khanewal and Faisalabad. The virulence against *Lr15* was found at Faisalabad and

Khanewal. Isogenic lines *Lr29* and *Lr22b* showed virulence in Bahawalpur as well as in Faisalabad. However, the virulence against *Lr29* was common only in Faisalabad. A similar virulence pattern of *Lr1*, *Lr2c*, *Lr3*, *Lr3ka*, *Lr3bg*, *Lr10*, *Lr11*, *Lr12*, *Lr26*, *Lr30*, *LrB*, *Lr13* (*WL* 711) was present in Islamabad (Table 3). Table 3. Avirulence/virulence formula for leaf rust near isogenic lines at different locations during 2015/16 and 2016/17.

	2015-16	2016-17
Location	Avirulence/virulence formula	Avirulence/virulence formula
Faisalabad	18, 19, 22a, 28, 29, 32, 33, 34, 36, 37, 23+(GAZA) / 1, 2a, 2c, 3, 3ka, 3bg, 9, 10, 11, 12, 13, 14 (a, b), 15, 16, 17, 20, 21, 22b, 23, 24, 25, 26, 27+31, 30, 35, LrB, 13(WL 711)	9, 18, 19, 22a, 28, 29, 32, 33, 34, 35, 36, 37, 23+(GAZA) / 1, 2a, 2c, 3, 3ka, 3bg, 10, 11 12, 13, 14 (a,b), 15, 16, 17, 20, 21, 22b, 23, 24, 25, 26, 27+31, 30, LrB, 13(WL 711)
Khanewal	9, 14 (a, b), 18, 19, 21, 22 (a, b), 28, 29, 32, 34, 35, 36, 37, 23+(GAZA) / 1, 2a, 2c, 3, 3ka, 3bg, 10, 11, 12, 13, 15, 16, 17, 20, 23, 24, 25, 26, 27+31, 30, 33, LrB, 13(WL 711)	2a, 9, 14a, 18, 19, 21, 22a, 28, 29, 32, 34, 35, 36, 37, 23+(GAZA) / 1, 2c, 3, 3ka, 3bg, 10, 11, 12, 13, 14b, 15, 16, 17, 20, 22b, 23, 24, 25, 26, 27+31, 30, 33, LrB, 13(WL 711)
Bahawalpur	3ka, 9, 15, 18, 19, 21, 22b, 28, 29, 32, 34, 36, 23+(GAZA) / 1, 2a, 2c, 3, 3bg, 10, 11 12, 13, 14 (a, b), 16, 17, 20, 22a, 23, 24, 25, 26, 27+31, 30, 33, 35, 37, LrB, 13(WL 711)	3ka, 15, 18, 19, 28, 29, 32, 34, 35, 36, 37, 23+(GAZA) / 1, 2a, 2c, 3, 3bg, Lr9, 10, 11 12, 13, 14 (a, b), 16, 17, 20, 21,22(a, b), 23, 24, 25, 26, 27+31, 30, 33, LrB, 13(WL 711)
Islamabad	2a, 9, 13, 14 (a,b), 15, 16, 17, 18, 19, 20, 21, 22 (a, b), 23, 24, 25, 27+31, 28, 29, 32, 33, 34, 35, 36, 37, 23+(GAZA) / 1, 2c, 3, 3ka, 3bg, 10, 11, 12, 26, 30, LrB, 13(WL 711)	2a, 9, 13, 14 (a, b), 15, 16, 17, 18, 19, 20, 21, 22 (a, b), 23, 24, 25, 27+31, 28, 29, 32, 33, 34, 35, 36, 37, 23+(GAZA) / 1, 2c, 3, 3ka, 3bg, 10, 11, 12, 26, 30, LrB, 13(WL 711)

Based on the comparison of avirulence/virulence formula from 2015-16 and 2016-17, *Lr18*, *Lr19*, *Lr28*, *Lr32*, *Lr34*, *Lr36* and *Lr23*+(GAZA) were found effective indicating low terminal rust intensity with either 0; R and MR type of reactions, while virulence to *Lr1*, *Lr2c*, *Lr3*, *Lr3bg*, *Lr10*, *Lr11*, *Lr12*, *Lr26*, *Lr30*, *Lr13* (WL 711), *LrB* remained stable with high terminal rust severity with either S, and MSS type of reactions in all four locations (Table 3). As the leaf rust races were avirulent to these genes during these studies, it may be used for hybridization work to develop resistant wheat germplasm.

#### Discussion

In all wheat growing areas of the world leaf rust caused by *P. recondita* f. sp. *tritici* is the major wheat disease causes enormous yield losses resulting in socio-economic instability (Rehman *et al.*, 2013). Susceptible cultivars and favorable environmental conditions also give rise the development of this disease. To avoid infection of leaf rust sowing of disease resistant cultivars is the only effective and sustainable solution.

The current study was conducted to observe the response of different wheat advanced lines against leaf rust and their relationship to environmental factors. Out of 855 advanced lines, 54 lines indicated high to moderate level of rust resistance with lower area under disease progress curve (AUDPC) values. Our findings agreed with those of Pretorious (1983) and Prabhu *et al.* (1993) who identified sources of resistance against *P. recondita* f. sp. *tritici* infection on the basis of AUDPC. Results obtained by Singh and Tewari (2001), Khan *et al.* (2002a), Sohail *et al.* (2013) and Mateen *et al.* (2015) on assessment of leaf rust resistance were also similar to the infection data presented here. Reis *et al.* (2000) used same method for evaluation of 200 hundred genotypes against *P. recondita* infection. Among them 66 lines were immune, 48 indicated

moderately resistant to moderately susceptible response, 66 susceptible, and 79 lines demonstrated highly susceptible response against leaf rust severity.

Considering characterization of environmental conditions all epidemiological factors including minimum and maximum temperature, relative humidity, rainfall and wind speed remained highly significant against leaf rust development. Maximum leaf rust severity was recorded at 18.2 and 30°C minimum and maximum temperatures respectively, during both rating years. With one unit increase in temperature disease severity also increased. At both minimum and maximum temperatures ranging from 7-15 and 5-22°C intensity of urediospores per unit area of infected leaves increased rapidly and fungus produced more spore lesions per day (Ali et al., 2017; Milus et al., 2009). Relative humidity nearby to wetness provides optimum conditions for germ tubes growth and spore germination. In the presence of humidity appressoria germination increased between 4-25°C minimum and maximum temperatures.

Rainfall and wind speed ranging from 8-8.5 mm and 1.6-3.1 km/h were positively correlated with disease development on most of the genotypes during both rating seasons. Raindrops released fungal spores by splashing with rains or by direct impact of 5-10 mm per hour and uredia are emptied within about one hour. Similarly, uredospore dispersal increased with increase in wind blast and wind speed. Hence the results of different models indicated that with respect to the canopy roughness uredospore dispersal is nil for wind speed under 0.25 m/sec while, uredospores must be considered as a gas for speed over 2.5 m/sec (Ali *et al.*, 2017; Rapilly *et al.*, 1970). Singh and Tewari (2001), Sajjid *et al.* (2010) also observed the response of different varieties in relation to environmental conditions of leaf rust and summarized the same results.

To monitor the leaf rust virulence pattern, the present study was conducted on the basis of reaction types in a host pathogen system at different locations of Pakistan. In this study we found that during 2015-16, *Lr18*, *Lr19*, *Lr23*+ (GAZA), *Lr28*, *Lr29*, *Lr32*, *Lr34* and *Lr36* were found effective against leaf rust pathogen populations under field conditions. While during 2016-17, *Lr18*, *Lr19*, *Lr23*+ (GAZA), *Lr28*, *Lr29*, *Lr32*, *Lr34*, *Lr35*, *Lr36* and *Lr37* were found effective against leaf rust prevalent virulent races. Similarly, Hussain *et al.* (2016a) studied leaf rust virulence pattern results showed that *Lr9*, *Lr18*, *Lr19*, *Lr25*, *Lr34*, *Lr36* and *Lr37* genes were found resistant under field conditions.

The comparison of field response of tested lines during both the years of study find out the distribution of rust virulences, their relative intensities and avirulent/virulent pattern were under field conditions as *Lr2a, Lr9, Lr12, Lr14a, Lr18, Lr19, Lr23+(GAZA), Lr28, Lr29, Lr32, Lr34, Lr35, Lr36, Lr3711/Lr1, Lr2c, Lr3,*  Lr3ka, Lr3bg, Lr10, Lr11, Lr13, Lr13(WL711), Lr14b, Lr15, Lr16, Lr17, Lr20, Lr21, Lr22a, Lr22b, Lr23, Lr24, Lr25, Lr26, Lr27+31, Lr30, Lr33, Lr35, and LrB (Chaudhry et al., 1996; Hussain et al., 2016b). However, the virulence against Lr35 was present only in Faisalabad and the virulence for gene Lr26 was dominant during 1991-1994 and was present in Pak 81, widely cultivated in the country (Chaudhry et al., 1995). Virulence studies in the country showed that novel leaf rust race for gene Lr24 was identified for the first time at numerous places in Peshawar and Punjab in 2001 (Khan et al., 2002b). The virulence surveys confirmed that in Pakistan changing virulence pattern is a constant feature, evidently proved by monogenic rust differential lines planted in multilocations (Bux et al., 2011; Khan et al., 2002b).

## الملخص

علي، ياسر، محمد اسلم خان، حافظ محمد عاطف، محمد اعجاز، محمد عتيق، محمد بشير، محمد زيشان منشا، أزهر عباس خان ومحمد حسين. 2020. تكميم مصدر المقاومة لصدأ الورقة في الأصول الوراثية للقمح بارتباط مع العوامل الوبائية. مجلة وقاية النبات العربية، 38(4): 344-353.

أجريت غربلة واسعة لـ 855 سلالة متقدمة من القمح في معهد بحوث القمح، ومعهد أيوب للبحوث الزراعية، فيصل أباد لتحديد مصادر مقاومة جديدة لصدأ الورقة خلال المواسم الزراعية 2015–2017. تم في تقويم أولي انتخاب 112 سلالة متقدمة تمتلك تجانساً مظهرياً لمزيد من الاختبار في معهد بحوث القمح، فيصل أباد ويعد هذا الموقع بؤرة ساخنة لصدأ الورقة. شملت الجولة الثانية من التقويم 54 سلالة متقدمة تمتلك تجانساً مظهرياً لمزيد من الاختبار في معهد بحوث القمح، فيصل أباد ويعد هذا الموقع بؤرة ساخنة لصدأ الورقة. شملت الجولة الثانية من التقويم 54 سلالة تمتلك مقاومة مستديمة إزاء صدأ الورقة. أظهر تقدير العوامل الوبائية تأثيراً قوياً على تقدم تطور صدأ الورقة. كانت درجات الحرارة العظمى والدنيا، الرطوبة النسبية، الهطل المطري وسرعة الرياح: 11–12. °س، 20–33 °س، 44.6%، 20–33 °س، 6.44%، قوياً على تقدم تطور صدأ الورقة. كانت درجات الحرارة العظمى والدنيا، الرطوبة النسبية، الهطل المطري وسرعة الرياح: 11–12. °س، 20–34%، 20%، 44.6%، 20%، 46.64%، 20%، 46.64%، 20%، 46.64%، 20%، 46.64%، 20%، 46.64%، 20%، 46.64%، 20%، 46.64%، 20%، 46.64%، 20%، 46.64%، 20%، 46.64%، 46.74%، وموريات 12 حديدها في ظل الطروف البيئية الجريئية أو التقليدية وبتوقع أن تسهم في الأمن الغذائي على المرضى عن طريق والعالمي. تلك في خلفية أصناف القمح المخلية مالمرض الطري اليبيئية، مصدر أمعادية وبتوقع أن تسهم في الأمن الغذائي على المري و

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