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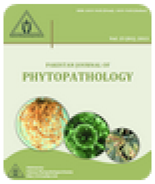

CHRACTERIZATION OF ENVIRONMENTAL CONDITIONS CONDUCIVE FOR LEAF RUST AND GENETIC DIVERSITY ON WHEAT CROSSES BASED UPON PHYSIOMORPHIC TRAITS

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ABSTRACT

The objective of present investigation was to investigate the thirty wheat advanced lines for characterization of environmental conditions conducive to leaf rust development. Moreover, eleven physio-morphological and yield attributing traits namely days to booting (DTB), days to heading (DTH), peduncle length (cm) plant height (cm), effective tillers, number of spikelet per spike, spike length, grains per spike, thousand-grain weight (g), grain yield (kg ha^{-1}) and protein percentage were assessed on these genotypes in a Randomized Complete Block Design (RCBD) at the Research Area of Plant Pathology University College of Agriculture, B.Z.U. Bahadar Campus Layyah, to evaluate the elite advanced lines after clustering them based on their genetic variation in performance. Epidemiological variables showed a great influence on the progress of disease development. Minimum temperature (11-18.2 °C), maximum temperature (25-33 ^{0}C), relative humidity (44-64%), rainfall (2.2-9.1 mm) and wind speed (1.7-2.8 km/h) proved the most conducive factors for the development of leaf rust epidemics. Multivariate analysis exhibited that thirty advanced lines formed 4 different clusters. Cluster 2 and 3 showed maximum values for spike length, effective tillers, number of spikelet per spike, number of grains per spike, protein percentage, thousand-grain weight and yield and lowest peduncle length, days to booting and days to heading. Cluster 1 demonstrated maximum values for days to heading, days to booting, peduncle length and plant height and lowest for effective tillers, protein percentage, number of grains per spike, grain yield and thousand grain weight. Considering cluster analysis and yield contributing traits, members of cluster 2 may be used as high yielding and rust-resistant wheat elite lines in prevailing environmental conditions of district Layyah.

Keywords: Cluster analysis, epidemiological variables, Genetic diversity, *Puccinia recondite,* PCA.

INTRODUCTION

Wheat (*T. aestivum*) is the most cultivated cereal crop of the world, covering 217 million hectares with a production of 653 million tons. It is staplefood crop for the people of South Asia, and in South Asia, it is cultivated on 38 million hectares with 139.88 million tons production (FAO, 2015). In Pakistan, wheat is cultivated on an area of 9.20 million hectares with 25.48 million tons of grain production (GOP, 2017-2018). It is considered a good source of dietary fibre, minerals, proteins and B group of vitamins (Adhikari *et al.,* 2016). Wheat flour is used to prepare noodles, biscuits, bread, confectionery products, and used in brewing industry. It is also used as animal feed. Nearly fifty-five percent carbohydrates consumed in the world are supplied by the wheat (Devindra *et al.,* 2017).

Changing environmental conditions require protection against several biotic and abiotic stresses which cause huge

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crop losses. Abiotic factors include wind, salinity and drought stress whereas biotic factors include attack of many plant pathogens, viz., fungi, bacteria viruses and nematodes. Among biotic factors, *P. recondita* is a potential risk to grain production all over the world (Kisana *et al.,* 2003). Several races of this rust are known which cause huge crop losses (Hussain *et al.,* 2014; Ali *et al.,* 2019). In Asia, stripe and leaf rusts caused 43 and 60% yield losses, respectively; if vulnerable cultivars are sown (Aquino *et al.,* 2002).

Symptoms of leaf rust include the development of reddish-brown pustules on the sheath, stem and leaves of infected plants. A single spore invades a leaf and produces thousands of new urediniospores within a week (Kolmer, 2013). Epidemiological variables, viz., temperature (minimum and maximum), rainfall and relative humidity played a vital role in disease scattering and caused severe epidemics (Javaid *et al.,* 2018). Similarly, wind speed and its direction favor the disease development by dispersing the spores away from infected plants. Khan *et al.,* (2006) indicated that *P. recondite* caused severe epidemics at 17-20 °C minimum temperature and 31-34 °C maximum temperature with more than 70 % relative humidity.

Genetic diversity and germplasm improvement are the key to sustainable production of wheat. For the utilization and evaluation of germplasm, measuring the extent of genetic variation is of paramount importance (Wang *et al.,* 2018; Islam *et al.,* 2021). Traits like 1000-grain weight, spike length, number of spikelet per spike, grain number per spike and optimum plant height contribute to wheat yield and genetic variation (Ahmed *et al.,* 2016). The use of multivariate statistical procedure is a significant method for analysis of the genetic relationship and sorting of germplasm among breeding materials (Wadood *et al.,* 2019).

In countries, breeding programs are mainly planned for the development of resistant varieties and higher grain yields. Identification and evaluation of elite lines from the germplasm is the basic step in a crop improvement program to get high crop production. Promising germplasm with desirable characteristics, good adaptation and higher grain yield, could reliably be used for commercial cultivation. Moreover, the use of elite germplasm for developing high yielding varieties with durable resistance against abiotic and biotic factors is necessary to obtain desired grain yield.

The present investigation was designed to characterize the most critical ranges of environmental conditions such as minimum and maximum temperature, relative humidity, rainfall and wind speed conducive for leaf rust development under natural prevailing environmental conditions and explore the extent of the relationship and genetic variation among wheat germplasm to generate further information for adaptation, better utilization and efficient selection using multivariate analysis. Elite lines indicating some improvement in crop production and other linked characteristics will be selected and used in further breeding programs. Moreover, elite lines of good characteristics could be helpful for future breeding programs or varietal development.

MATERIALS AND METHODS

The present study was carried out in RCBD with three replications in the Research Area of Plant Pathology, University College of Agriculture B.Z.U. Bahadar Campus, Layyah, during 3rd week of November, 2019-2020. The details of pedigree/parents of the wheat germplasm collected from gene pool of Wheat Research Institute, Ayub Agriculture Research Institute, Faisalabad is presented in Table 1. Each tested entry was sown in a plot size of 1.8 x 5 m^2 with 30 cm row to row distance by Power Norvigion. The farmyard manure at the rate of 5 ton/ha and fertilizer at the rate of 160:60:50 kg/ha were applied. To facilitate the rust epidemics, two rows of highly susceptible rust spreader variety Morocco were planted across the path of each side of experimental material. The artificial inoculation of genotypes were done with the mixture of 5 leaf rust races such as TKTRN, TKTPR, KSR/JS, PGRTB and PHTTL collected from Murree, Kaghan, Faisalabad and Layyah. The inoculum was applied by spraying urediniospores suspension @ 25gm of spores/15 litter of water. The inoculation was performed 5-6 times in late morning and early evening during the month of January. The other inoculation techniques including hypodermal needle injection and dusting methods were also used to develop high leaf rust disease pressure (Roelfs, 1998). Two weedings were done after 40 and 60 days of sowing. All recommended agronomic practices were used to keep crop in healthy conditions.

Data Recording:

Lear rust severity and epidemiological variables: On the appearance of leaf rust symptoms, data were recorded in the month of March (on 1, 11, 21 and 31 dates) after every ten days intervals by following modified Cobb's scale (Table 2). Area under disease progress curve (AUDPC) was also calculated by using CIMMYT Table proposed by Shaner and Finney (1977).

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Table 1. Details of wheat germplasm/lines used for the experiment

Treatments	Wheat Germplasm
1	AS-2002/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAL
2	CHENAB2000/INQ.91/5/WBLL1*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ
3	FSD.08/6/BABAX/3/FASAN/Y//KAUZ/4/BABAX/5/LU 26/HD2179
4	87094/ERA//PAK-81/2*V87094/3/SHAFAQ06/4/MAYA/PVN
5	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES/5/KAUZ//ALTAR
6	SH.88/PAK.81//MH.97//OTUS/TOBA97
7	TRM//MAYA 74'S'/MON'S'/3/INQ.91/4/PBW343
8	SH.88/PAK.81//MH.97//CUMHURIYET/NE
9	AS2002/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAL
10	SH88/WEAVER/6/LU26/HD2179/5/BABAX/3/MANGO/VEE#10//PRL /4/BABAX
11	PFAU/MILAN/5/CHEN/A.SQ(TAUS)//BCN/3/VEE#7/BOW/4/PASTOR/6/QINGHAIBRI/WBLLI//BRBT2
12	AUQAB 2000*2/LAKTA-1
13	SH88/WEAVER/3/DWL5023/SNB//SNB
14	KAUZ//ALTAR84/AOS/3/PASTOR/4/TILHI/7/CNO79//PF70354/MUS/3/PASTOR/4/BAV92/5/FRET2/KUKUNA//FRET2/6/MILAN/KAUZ//PRINIA/3/BAV92
15	OASIS/5*ANGRA//INQ.91///MILAN/S87230//BABAX
16	INQ/AUQAB/3/SH.88/90A204//MH.97
17	TRM//MAYA74'S'/MON'S'/3/INQ.91/4/PBW 343
18	TRM//MAYA74'S'/MON'S'/3/INQ.91/4/PBW 343
19	FSD.08/6/BABAX/3/FASAN/Y//KAUZ/4/BABAX/5/LU26/HD2179/7/PB.96/87094//MH.97
20	TAM200/Tui/6/PVN/CRC422/ANA/5/BOW//CROW/BUC/PVN/3/YR/YR/4/TRAP#1/7/*21NQ-91
21	CNDO/R143//ENTE/MEXI_2/3/AEGILOPSSQUARROSA(TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TACUPETOF2001/8/CROW'S'/NAC//BOW'S'
22	PFAU/SERI.1B//AMAD/3/INQALAB91*2/KUKUNA/4/WBLL1*2/KURUKU/5/PVN/YACO/3/KAUZ*2/TRAP// KAUZ
23	HUW234+LR34/PRINIA//PBW343*2/KUKUNA/3/ROLF07/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ
24	PRL/2*PASTOR//PBW343*2/KUKUNA/4/CAR422/ANA//TRAP#1/3/KAUZ*2/TRAP//KAUZ
25	INQALAB 91*2/KONK//INQALAB 91*2/KUKUNA/3/INQ-91*2/TUKURU
26	CNDO/R143//ENTE/MEXI_2/3/AEGILOPSSQUARROSA(TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TACUPETOF2001/8/OASIS/SKAUZ//4*BCN/3/2*PASTOR
27	INQALAB91*2/KUKUNA//KIRITATI///V-09014
28	WHEAR/KRONSTAD F2004/3/CROW'S'/NAC//BOW'S'
29	C80.1/3*BATAVIA//2*WBLL1/3/PBW343*2/KUKUNA/4/KAUZ / SITE

30	CNDO/R143//ENTE/MEXI_2/3/AEGILOPSSQUARROSA(TAUS)/4/WEAVER/5/IRENA/6/LERKE/7/TAN/PEW//SARA/3/CBRD

Table 2. Disease rating scale used for data recording of leaf rust severity (%) during crop season 2018-2019

Reaction	Code	Symptoms
Immune	0	No visible infection
Resistant	R	Visible necrotic or chlorosis with or without uredia
Moderately Resistant	MR	Small uredia surrounded by necrotic areas
Mixed (Intermediate)	M	Small uredia present surrounded by necrotic areas as well as medium uredia with no necrosis but possible some distinct chlorosis
Moderately susceptible	MS	Medium uredia with no necrosis but possible some distinct chlorosis
Moderately susceptible-susceptible	MSS	Medium uredia with no necrosis but possible some distinct chlorosis as well as large uredia with little or chlorosis present
Susceptible	S	Large uredia are present with little or no chlorosis

Cobb's scale (Peterson *et al.,* 1948)

$$AUDPC = \sum_{i=1}^{n-1} [xi + Xi1]/2](ti + 1 - t1)$$

Where n = number of dates on which leaf rust severity was recorded; x*i* = disease severity on date *I;* t*i* = time in days between *i* and date *i* + 1

Data of epidemiological variables such as minimum and maximum air temperatures, wind speed, rainfall and relative humidity of the same month, in which data for leaf rust severity was recorded, collected from the Pakistan Meteorological Station, Karor Layyah for the year 2019-2020.

Physio-morphological and yield attributing traits: At crop maturity, data from 5 randomly selected and tagged plants per lines were recorded for four physio-morphological traits, viz**.,** days to booting (DTB), days to heading (DTH), peduncle length (cm), plant height (cm) and seven yield attributing traits, i.e., effective tillers, number of spikelet per spike, spike length, grains per spike, thousand-grain weight (g), grain yield (kg ha^{-1}) and protein percentage.

STATISTICAL ANALYSIS

The data of all epidemiological variables were correlated with disease severity through linear regression analyses (Steel *et al.,* 1997). Moreover, combined data of all Physio-morphological and yield attributing traits were subjected to multivariable analysis. During current research statistical software program, SPSS V-17 and Minitab V-20 were used (Poudel *et al.,* 2017).

RESULTS

Screening of wheat germplasm against leaf rust severity: Out of 30 wheat germplasm, 5 genotypes viz., 2, 11, 4, 26 and 19 showed resistance response to leaf rust severity with lower AUDPC values ranging from 45-180. Ten genotypes showed moderately resistant (MR) response, eight indicated moderately resistant to moderately susceptible response (MRMS) and seven exhibited moderately susceptible response to leaf rust severity with various values of AUDPC (Table 3).

Table 3. Evaluation of wheat germplasm against leaf rust severity based on AUDPC during crop season 2019-2020

Sr. No.	Treatments	Frequency	Range of AUDPC	Response to leaf rust severity
1	2, 11, 4, 26, 19	5	45-180	R
2	1, 3,8, 22, 28, 6, 29, 5, 24, 14	10	185-400	MR
3	7, 17, 30, 9, 27, 15, 18, 24	8	400-650	MRMS
4	10, 13, 16, 21, 23, 12, 20	7	655-1989	MS

R, Resistant; MR, Moderately Resistant; MRMS: Moderately Resistant to Moderately Susceptible; MS, Moderately Susceptible

Characterization of environmental conditions conducive for leaf rust severity: Five wheat genotypes, i.e., SH88/WEAVER/6/ LU26/HD2179/ 5/BABAX/3/ MANGO/VEE#10// PRL/4/ BABAX (10), SH88/WEAVER/3/ DWL5023/ SNB//SNB (13), INQ/AUQAB /3/SH.88/90A204//MH.97 (16), CNDO/R143//ENTE/MEXI_2/3/ AEGILOPSSQUARROSA(TAUS)/ 4/ WEAVER/ 5/ PICUS/6/TROST/ 7/TACUPETOF2001/ 8/CROW'S'/NAC//BOW'S (21), HUW234+ LR34/ PRINIA//PBW343*2/KUKUNA/3/ROLF07/4/SNI/TRAP#1 /3/KAUZ*2/TRAP//KAUZ (23), indicated by V-274, V-293, V-365, V-381, and V-898 were employed to regression analysis to determine the critical ranges of epidemiological variables such as minimum and maximum temperature, relative humidity, rainfall and wind speed conducive for leaf rust severity. A significant relationship was determined between disease severity and all epidemiological variables. With one unit increase in minimum temperature, maximum temperature, relative humidity, rainfall and wind speed ranging from, 11-18.5 ^{0}C, 25-33 ^{0}C, 44-64%, 2.2-9.1 mm and 1.7-2.8 km/h disease severity also increased as indicated by their r values (Figure 1-3).

Physio-morphological and yield attributing traits:

Cluster analysis: Cluster analysis sequestrated all the 30 genotypes of wheat into 4 clusters (Table 4). The dendrogram of 30 genotypes was created in average linkage and Euclidean distance (Figure 4). The distance between various clusters centroids of wheat advanced lines is demonstrated in Table 5.

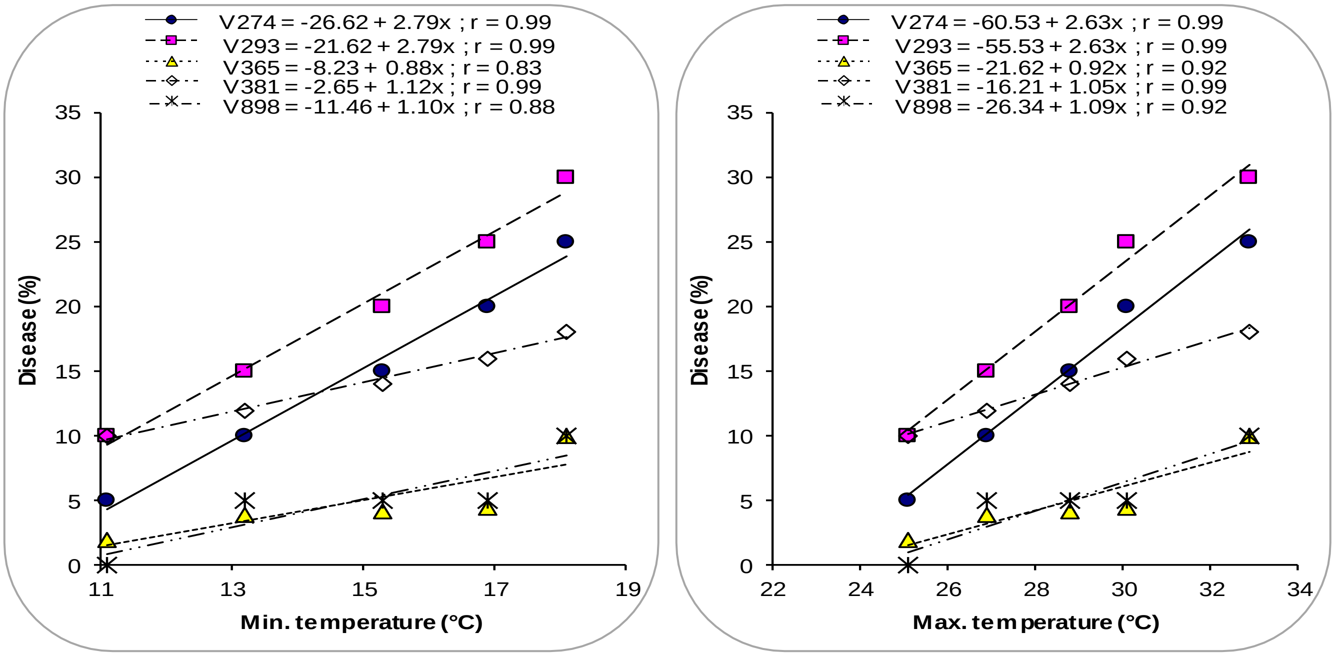

Figure 1. Relationship between temperature (minimum and maximum) and disease severity recorded on five wheat genotypes during 2019-2020 crop season

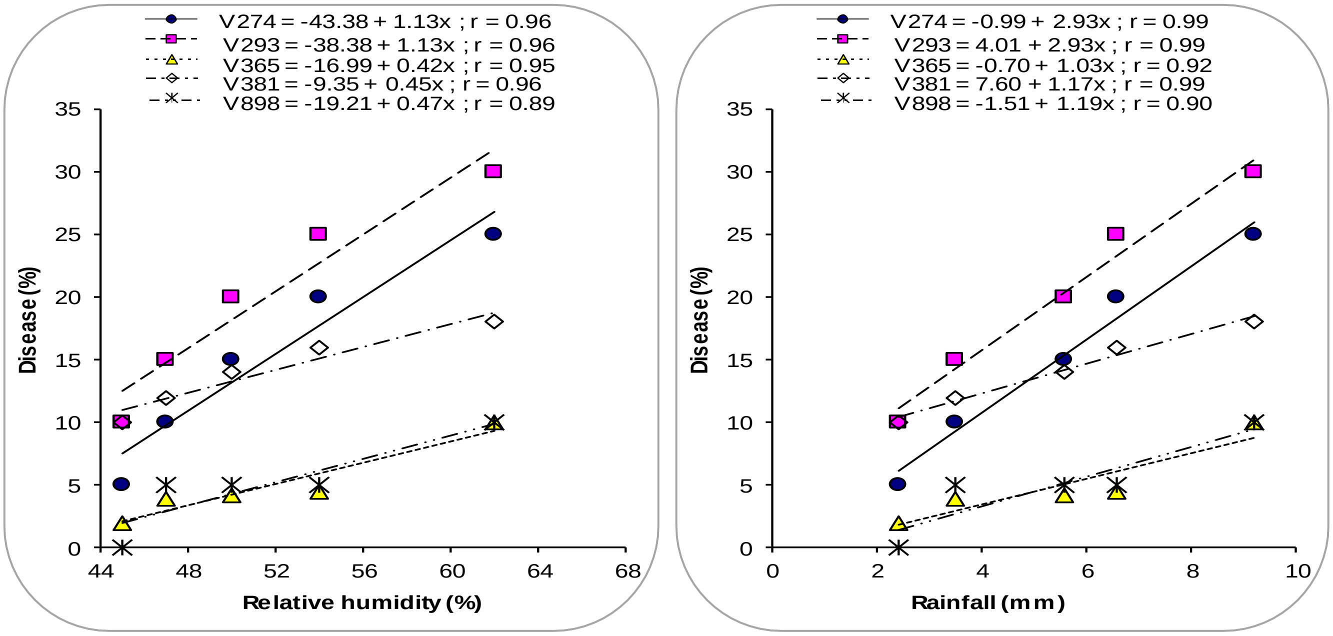

Figure 2. Relationship between relative humidity (%), rainfall (mm) and disease severity recorded on five wheat genotypes during 2019-2020 crop season

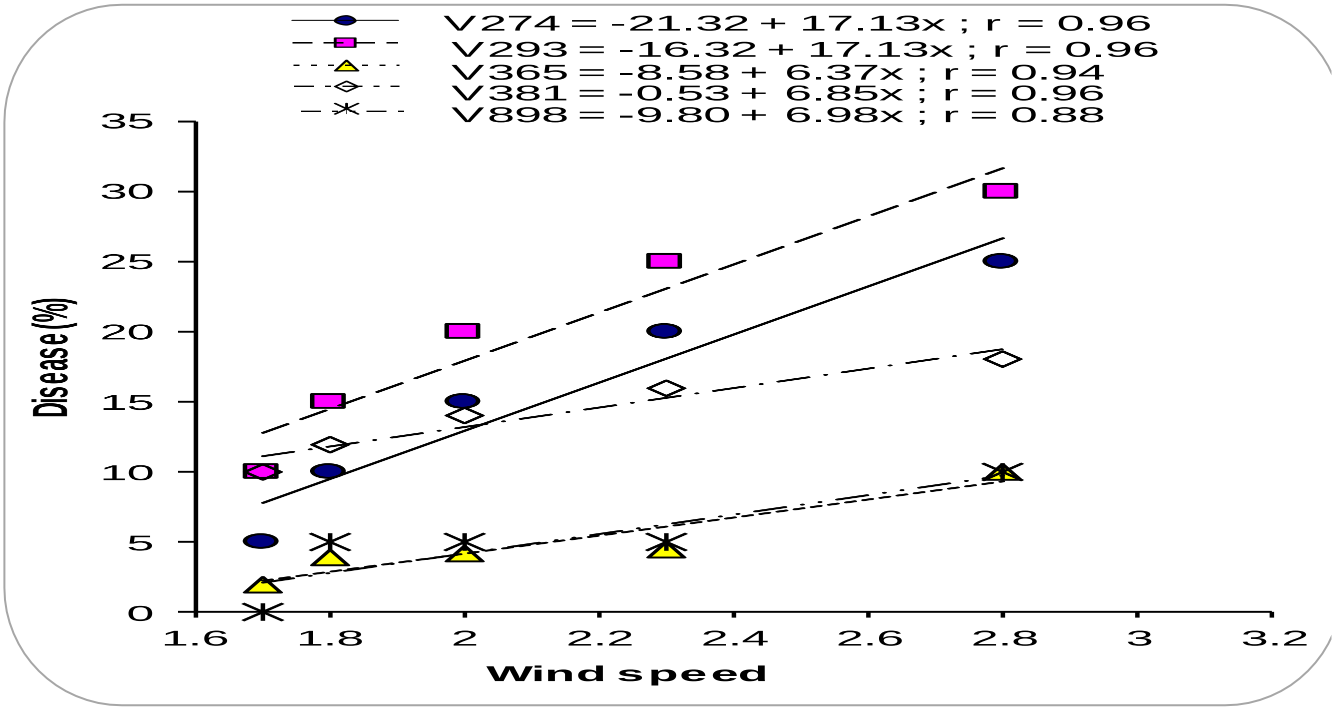

Figure 3. Relationship between wind speed (km/h) and disease severity recorded on five wheat genotypes during 2019-2020 crop season

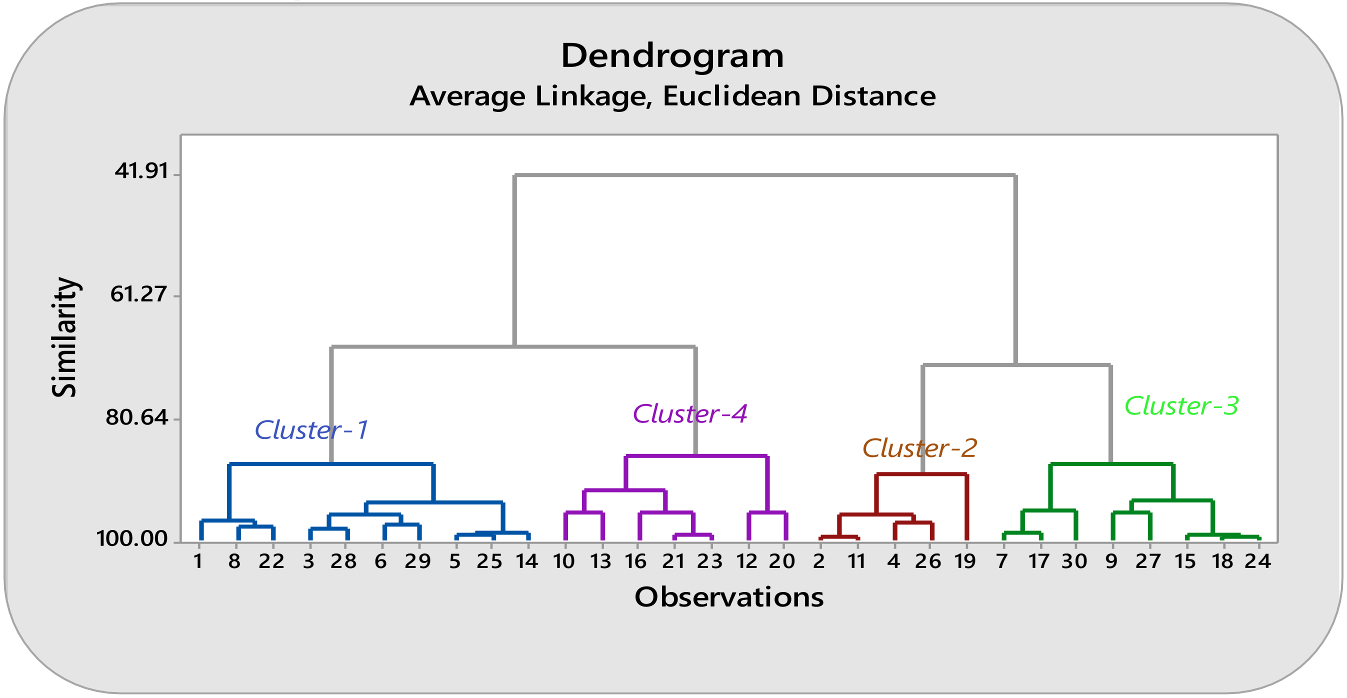

Figure 4. Cluster diagram of 30 genotypes of wheat based on traits under investigation

In UPGMA clustering, the dendrogram exhibited four clusters with a 41.91% similarity level. Cluster 1 consisted of 10 genotypes including 1, 3, 8, 22, 28, 6, 29, 5, 24, and 14 as indicated in Table 4. Members of this cluster showed highest days to heading, and plant height and lowest in grain yield, thousand-grain weight, number of spikelet per spike, number of grains per spike, effective tillers and protein percentage. These genotypes had almost low quantitative traits as demonstrated in Table 6.

Table 4. Grouping of 30 genotypes into different clusters

Cluster	Frequency	Treatments	Germplasm	Proportion
1	10	1, 3,8, 22, 28, 6, 29, 5, 24, 14	AS2002/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAL,SH.88/PAK.81//MH.97//CUMHURIYET/NE, PFAU/SERI.1B//AMAD/3/INQALAB91*2/KUKUNA/4/WBLL1*2/KURUKU/5/PVN/YACO/3/KAUZ*2/TRAP//KAUZ, FSD.08/6/BABAX/3/FASAN/Y//KAUZ/4/BABAX/5/LU26/HD2179, WHEAR/KRONSTADF2004/3/CROW'S'/NAC//BOW'S', SH.88/PAK.81//MH.97//OTUS/TOBA97, C80.1/3*BATAVIA//2*WBLL1/3/PBW343*2/KUKUNA/4/KAUZ/SITE, KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES/5/KAUZ//ALTAR, PRL/2*PASTOR//PBW343*2/KUKUNA/4/CAR422/ANA//TRAP#1/3/KAUZ*2/TRAP//KAUZ, KAUZ//ALTAR84/AOS/3/PASTOR/4/TILHI/7/CNO79//PF70354/MUS/3/PASTOR/4/BAV92/5/FRET2/KUKUNA//FRET2/6/MILAN/KAUZ//PRINIA/3/BAV92	33.34
2	5	2, 11, 4, 26, 19	CHENAB2000/INQ.91/5/WBLL1*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ, PFAU/MILAN/5/CHEN/A.SQ(TAUS)//BCN/3/VEE#7/BOW/4/PASTOR/6/QINGHAIBRI/WBLLI//BRBT2, 87094/ERA//PAK-81/2*V87094/3/SHAFAQ06/4/MAYA/PVN, CNDO/R143//ENTE/MEXI_2/3/AEGILOPSSQUARROSA(TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TACUPETOF2001/8/OASIS/SKAUZ//4*BCN/3/2*PASTOR, FSD.08/6/BABAX/3/FASAN/Y//KAUZ/4/BABAX/5/LU26/HD2179/7/PB.96/87094//MH.97	16.67
3	8	7, 17, 30, 9, 27, 15, 18, 24	TRM//MAYA 74’S’/MON’S’/3/INQ.91/4/PBW343, TRM//MAYA74’S’/MON’S’/3/INQ.91/4/PBW 343, CNDO/R143//ENTE/MEXI_2/3/AEGILOPSSQUARROSA(TAUS)/4/WEAVER/5/IRENA/6/LERKE/7/TAN/PEW//SARA/3/CBRD, AS2002/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAL, INQALAB91*2/KUKUNA//KIRITATI///V-09014, OASIS/5*ANGRA//INQ.91///MILAN/S87230//BABAX, TRM//MAYA74’S’/MON’S’/3/INQ.91/4/PBW 343, PRL/2*PASTOR//PBW343*2/KUKUNA/4/CAR422/ANA//TRAP#1/3/KAUZ*2/TRAP//KAUZ	26.66
4	7	10, 13, 16, 21, 23, 12, 20	SH88/WEAVER/6/LU26/HD2179/5/BABAX/3/MANGO/VEE#10//PRL /4/BABAX, SH88/WEAVER/3/DWL5023/SNB//SNB, INQ/AUQAB/3/SH.88/90A204//MH.97, INQ/AUQAB/3/SH.88/90A204//MH.97, CNDO/R143//ENTE/MEXI_2/3/AEGILOPSSQUARROSA(TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TACUPETOF2001/8/CROW'S'/NAC//BOW'S', HUW234+LR34/PRINIA//PBW343*2/KUKUNA/3/ROLF07/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ, AUQAB 2000*2/LAKTA-1, TAM200/Tui/6/PVN/CRC422/ANA/5/BOW//CROW/BUC/PVN/3/YR/YR/4/TRAP#1/7/*21NQ-91	23.34

Table 5. Distances between various cluster centroids of wheat advanced lines.

	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Cluster1	0.00	2043.82	1392.64	717.57
Cluster2		0.00	651.33	1326.39
Cluster3			0.00	675.13
Cluster4				0.00

Table 6. Mean values of average linkage method of cluster analysis of wheat germplasm

Variable	Cluster1	Cluster2	Cluster3	Cluster4	Grand centroid
DTB	71.93	58.86	73.34	74.93	70.83
DTH	75.75	69.90	69.90	70.81	70.81
ET	3.24	5.24	4.04	4.04	3.84
PL	17.33	14.82	16.43	17.37	16.68
PH	111.90	109.60	107.63	103.71	108.47
SL	9.49	10.50	9.52	9.63	9.70
NSS	45.55	45.12	45.48	43.94	45.08
NGS	44.40	69.80	61.88	54.71	55.70
TGW	35.40	42.22	38.90	35.74	37.55
Y	2554.00	4597.60	3946.50	3271.43	3433.33
P	10.74	12.20	11.55	11.04	11.27

Cluster 2 consisted of 2, 4, 11, 19, and 26 advanced lines and this clusters exhibited the highest value of yield, thousand-grain weight, number of spikelet per spike, number of grain per spike, spike length, effective tillers and protein percentage and lowest in days to heading, days to booting and peduncle length. Members of these genotypes can be used in breeding programs to develop high yielding varieties. Moreover, genotypes of this cluster are best for commercial cultivation due to its high yield and yield attributes.

Cluster 3 consisted of 8 advanced lines and the members of this cluster consisted of inferior traits values for yield attributing characteristics under investigation. Cluster 4 consisted of 7 advanced lines and had the highest values in peduncle length, and day to booting. The representative of this cluster can be used for induction of peduncle length and early booting.

Principal component analysis: The principal component analysis had grouped the eleven quantitative traits into five main components (Table 7). The first two principle component analyses were plotted on PC axis 1 and 2 which exhibited high variability in the wheat genotypes (Figure 5). The first four components with more than 1 Eigen-values accounted for more than 73.8% of total variations for yield traits. Similarly, 5, 3, 2, and 1 component indicated 82.4, 63.4, 52.1 and 36.8 % of the total cumulative variance, respectively. The first principal component elucidated phonological traits with positive loading of effective tillers, number of grains per spike, 1000-grain weight and protein percentage.

Table 7. Eigen values, proportion and percentage of the cumulative variance of physio-morphological and yield attributing traits of wheat germplasm

Variable	PC1	PC2	PC3	PC4	PC5
DTB	-0.306	-0.232	0.198	0.467	-0.162
DTH	-0.213	-0.406	0.270	0.003	-0.603
ET	0.381	-0.046	-0.060	0.036	-0.526
PL	-0.234	0.283	0.373	0.216	0.202
PH	-0.021	-0.422	0.381	-0.598	0.202
SL	0.162	-0.465	-0.158	0.358	0.372
NSS	0.052	-0.524	-0.397	0.021	0.158
NGS	0.388	-0.079	0.304	0.385	-0.031
TGW	0.409	0.095	-0.172	-0.221	-0.216
Y	0.441	0.091	0.115	0.196	0.103
P	0.347	-0.082	0.531	-0.107	0.174
Eigen value	4.0462	1.6837	1.2448	1.1476	0.9395
Proportion	0.368	0.153	0.113	0.104	0.085

% of Cumulative variance	36.8	52.1	63.4	73.8	82.4

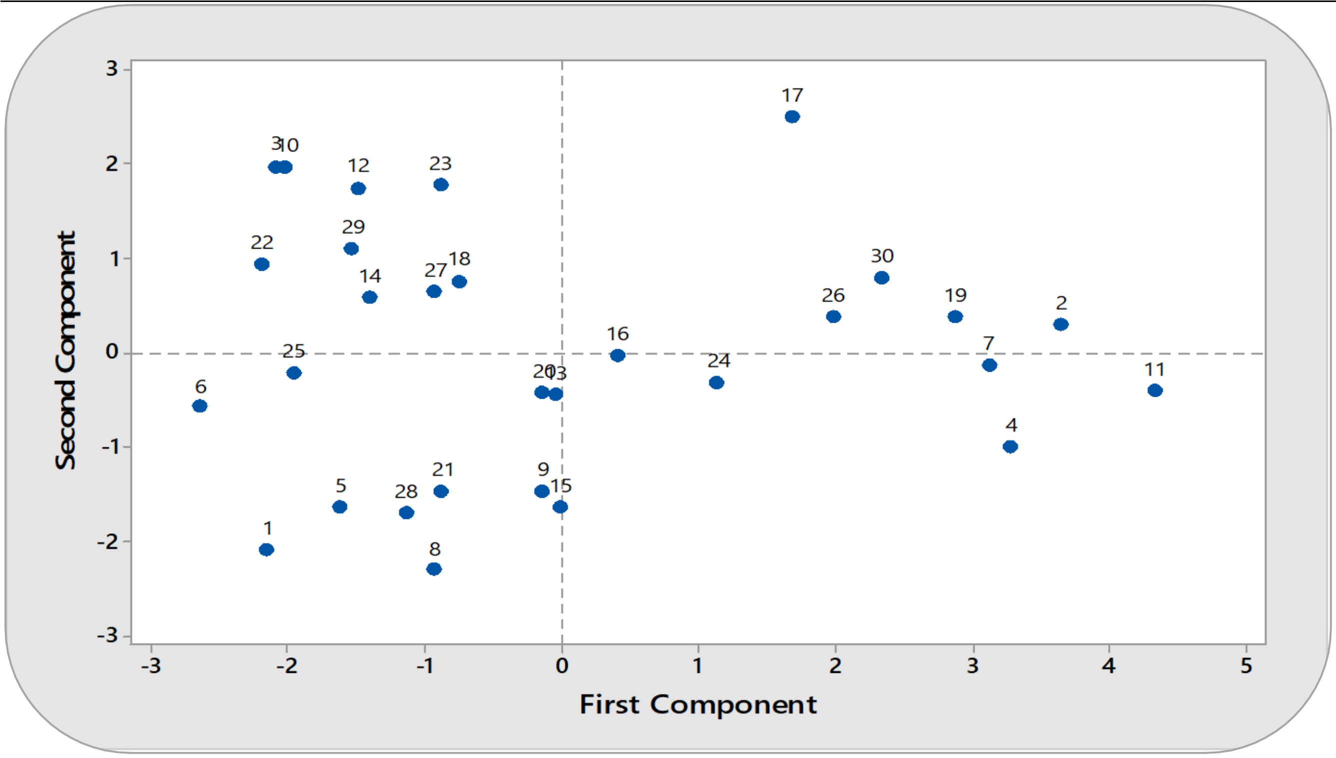

Figure 5. Scattered diagram of first two principle component analysis of 30 wheat genotypes

The second principle component contributed positively to yield, thousand-grain weight and peduncle length and negatively to days to heading, days to booting, spike length, and the number of spikelet per spike. The third component had positive loading with peduncle length, plant height, protein percentage and the number of grains per spike and negative loading with spike length and number of spikelet per spike.

The fourth principle component exhibited positive linkage with days to booting, spike length, peduncle length, and number of grains per spike and negative linkage with plant height and thousand-grain weight. The fifth principle component demonstrated positive loading with spike length, number of spikelet per spike and negative loading with days to heading, days to booting, effective tillers, number of grains per spike and thousand grains weight.

DISCUSSION

In all wheat growing regions of the world leaf rust is a major threat to crop production resulting in severe yield losses (Rehman *et al*., 2013). Conducive environmental conditions and vulnerable cultivars favor disease development. To avoid disease epidemics, cultivation of resistant varieties is the only sustainable and effective strategy. In present investigation, genotypes showed a high to moderate level of resistance against leaf rust with a lower AUDPC values. These results were in line with the findings of Prabhu *et al*., (1993) and Pretorious (1983) who identified the resistant sources against leaf rust epidemics on the basis of AUDPC. Similarly, during assessment of leaf rust resistance, Ali *et al.,* (2020a), Ali *et al.,* (2020b) and Khan *et al.,* (2002) reported the same infection data as presented here.

Characterization of epidemiological variables showed that all environmental conditions remained significant against disease development. Temperature (both minimum and maximum) rapidly enhanced the urediniospores intensity at per unit area of infected leaves leading to the production of more spores per day (Milus *et al.,* 2009; Ali *et al.,* 2017). Under high relative humidity, appressoria germination of fungus increased between 4-25 ^{0}C maximum and minimum temperature, respectively. Wind speed and rainfall were also positively associated with the leaf rust development on most of the treatments. The raindrops favor the release of fungal spores with the direct impact of 5-10 mm per hour or by splashing with rains. Similarly, spores dispersal increased with the increase in wind speed and wind blast (Rapilly *et al.,* 1970).

In the present investigation, sustainable genetic variations were observed in yield attributing and physio-morphological traits of wheat advanced lines. Cluster analysis showed that all genotypes formed 4 different clusters. The low yielding and high yielding advanced lines formed various clusters. The advanced lines of the second cluster were observed most appropriate to Layyah conditions for general cultivation. Ali *et al.,* (2019) also reported that cluster analysis helps in the selection of genotypes more easily. The lines which exhibited more yield and yield attributing traits are grouped in one cluster from present investigation, the advanced lines of the second cluster were recorded most suitable for Layyah conditions of Pakistan which had highest values for yield and its attributes. Iqbal *et al.,* (2017) showed that cluster analysis is a powerful tool for the selection of high yielding wheat advanced lines.

Aharizad *et al.,* (2012) applied principal component analysis by using the Wards method and groped 94 wheat advanced lines into three different clusters. The level of genetic variation was high for the grain yield, number of spikes, flag leaf area, and peduncle length. Fufa *et al.,* (2005) clustered winter wheat genotypes based on molecular markers, end-use quality traits, morphological characters and pedigree information to evaluate the extent of genetic diversity. Similarly, Leilah & Al-Khateeb (2005); Ahmad *et al.,* (2008); Ali *et al.,* (2008); and Soleymanfard *et al.,* (2012) used the principal component analysis to assess the genetic variations in wheat genotypes based on grain yield and its components.

In current investigation, the principal component analysis used to reduce the eleven traits of wheat advanced lines into six component and information about each trait supports the results of cluster analysis. Wolde *et al.,* (2016) also used principal component analysis to classify traits of wheat advanced lines which contributed to most of the variation in data. Similar investigation about the presence of a high genetic divergence in wheat advanced lines reported by Ali *et al.,* (2019). PCA is mainly used in breeding programs for reduction of yield attributing traits and grouping of wheat germplasm. Hence, PCA can be a useful tool to get parental lines for effective hybridization programs.

CONCLUSION

In conclusion, all environmental conditions proved most conducive for disease development on all wheat genotypes. All advanced lines grouped into 4 clusters and 1 and 2 clusters were best due to their desirable physio-morphological and yield attributing traits and leaf rust resistance. The presence of high level of genetic variations among tested wheat advanced lines grouped into different clusters and supported by PCA exhibited their suitability for further investigation by selecting superior wheat advanced lines.

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