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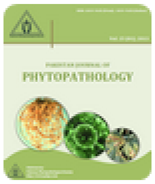

PURSUIT OF THE STRIPE RUST (*PUCCINIA STRIIFORMIS* F. SP. *TRITICI* (*PST*) DISEASE RESISTANCE AND ITS RELATION WITH MORPHOLOGICAL ATTRIBUTES IN WHEAT (*TRITICUM AESTIVUM* L.)

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ABSTRACT

Wheat, one of the most widely cultivated crops in the world is under continuous threat from rust diseases. Among different rusts, stripe (yellow) rust is a major cause of yield losses in wheat around the world. Identifying disease resistance against stripe rust in wheat crop is a key strategy in overcoming this menace. For this purpose, 31 genotypes were screened in field under artificial inoculation of stripe rust and diversity was assessed based on their morphological attributes. Stripe rust severity recorded on the basis of modified Cobb's scale showed that genotypes Anaj, 219, 220, 242, 245, 249, 278, 284, 293 (V45) were completely resistant while genotypes Morocco, Gandum, Sehar, TD 1 and Abdlstar were highly susceptible. Genotypes 92, 242, 245, 293(V45), Gandum, Abdlstar, TD 1 and Morocco were more diverse of all the genotypes on the basis of morphological traits as verified by biplot and dendrograms. Correlation and principal component analyses showed plant height (PH) had the positive relation with spike length (SL), peduncle length (PL) and number of spikelets per spike (NS). While NS had a positive relation with flag leaf area (FL). Genotypes assessed along with their morphological diversity could be studied further for being potential sources of resistance against stripe rust.

Keywords: Correlation, Diversity, Morphological traits, Stripe rust, Wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is generally regarded as king of cereals and is the most significant staple food in many countries of the world (Abd El-Mohsen *et al.,* 2015). Due to stagnant yields from the last few years, the gap between demand and supply has widened in Pakistan where wheat is a major staple diet, causing a threat to food security. There is a dire need of high yielding varieties to cope with increasing population. Climate change leading to fluctuations in temperature with unreliable weather pattern is another threat to wheat production in Pakistan.

Production of wheat worldwide is affected by numerous biotic and abiotic stresses. Among biotic stress the most common are fungal pathogen causing diseases like rusts, smuts, and bunts (Anwar *et al.* 2019). Three known wheat rusts are stripe (yellow) rust (*Puccinia striiformis tritici*), stem or black rust (*Puccinia graminis tritici*) and leaf or brown rust (*Puccinia triticina)*. These rust diseases had caused huge losses in wheat yield globally in recent years. Chemical control in the form of various fungicides is effective in suppressing these diseases but chemical control is not a cost-effective measure and threat to environment too. Breeding for disease resistant varieties is the most economical way in this regard reducing financial burden on farmers. However the approach is a continuous process as new races of pathogens evolve overcoming resistance.

Stripe rust of wheat is caused by *Puccinia striiformis tritici* decreases the yield, quality, and aesthetic value of grain. Disease results in seeds which are less vigorous and usually show poor emergence. Severity of the disease depends on the stage of the infection, if the plant is exposed at early stage and disease continues to spread during growing season,

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susceptible cultivars could end up with 100 percent yield loss while 10-70 percent loss in wheat yield is observed in majority of the wheat growing regions which exposed due to earliness of initial infection, susceptibility of cultivar, rate of disease development and duration of disease (Chen, 2005).

There are two forms of resistance depending on reaction of wheat cultivar to stripe rust i.e., adult plant resistance (APR) and seedling resistance. As seedling resistance is controlled by one gene, APR is more stable than seedling resistance (Chen *et al.,* 2014). Assessment of genetic diversity is the most effective tool in plant breeders' hands when selecting parents for a hybridization scheme. Narrow genetic diversity could be problematic in breeding for adaptation to biotic and abiotic stresses. Morphological characterization of wheat germplasm lines is necessary to have access to the diversity of commercially relevant morphological attributes. To put a material foundation for economically significant features in future breeding programs, it is pertinent to examine genetic diversity in wheat germplasm (Uddin *et al.,* 2008). Therefore present research was designed to study the morphological diversity in wheat genotypes and correlation between these morphological traits under artificial inoculation of stripe rust.

MATERIALS AND METHODS

The research was conducted to access the effect of stripe rust on morphological traits of studied wheat genotypes. Experiment was conducted during the cropping season 2020-21 in field research area of Department of Plant Breeding and Genetics, Faculty of Agriculture and Environment, The Islamia university of Bahawalpur. Thirty-one genotypes used in this experiment were acquired from Regional Agriculture Research Institute Bahawalpur, Pakistan. Each entry had 2 lines and length of each line was 2m. Row to Row distance was 30cm. Morocco, a super susceptible wheat cultivar was used as check and spreader and was sown after every 10 genotypes. Recommended amount of Urea were applied to field in split doses. Usual culture practices like weeding and hoeing were carried out. Artificial inoculation was carried out throughout the field. Suspension was prepared in double distilled water with 300 mg spores per 1 litre of water. Inoculum was applied with help of knapsack sprayer as fine mist on plant and underside of leaf to preserve moisture 2-3 times before the sunset. Artificial moisture is maintained in the field with help of atomizer. Based on Modified Cobb's scale, the varieties were scored at 15-day interval starting from first appearance of symptoms till the disease ceased to progress. Severity of disease in the form of percentage was calculated (Roelf, 1992).

Ten plants in each line were tagged and data on morphological traits were recorded at different growth stages. Genotypes used in the study are given in Table 1.

Table 1. List of genotypes used in the experiment

Sr. No.	Genotypes	Sr. No.	Genotypes	Sr. No.	Genotypes
1	15B-1754-17	11	15B-1788-249	21	Ghazi
2	15B-1756-25	12	15B-1789-278	22	Sehar
3	15B-1763-26	13	15B-1790-284	23	Gandum
4	15B-1775-76	14	Gold	24	16005
5	15B-1779-92	15	Anaj	25	Aas11
6	15B-1780-207	16	Akbar	26	Johar
7	15B-1783-219	17	291(2190)	27	Bkharstar
8	15B-1784-220	18	293(V45)	28	Abdlstar
9	15B-1786-242	19	FakhraBkhar	29	TD1
10	15B-1787-245	20	296(2511)	30	Zincol
31 Morocco used as check and spreader					

Severity based on modified Cobb's scale was calculated as follows;

O (No Disease), T (below 5% severity), R or resistance (small uredinia surrounded by necrosis or chlorosis), MR or moderately resistance (medium sized uredinia surrounded by necrosis or chlorosis), RMR (Resistance to Moderately resistant), MS or moderately susceptible (medium large compatible uredinia without necrosis or chlorosis), M (Moderately resistance-moderately susceptible), MSS (Moderately susceptible to susceptible response), S or susceptible (large compatible uredinia without necrosis or chlorosis). Severity recorded in form of percentage 0 to 100 (Roelf, 1992).

Coefficient of infection (CI): The coefficient of infection (CI) was calculated by multiplying disease severity (DS) by infection type constant values (IT). R = 0.1, MR = 0.25, M = 0.5, MS = 0.75, S = 1 were the constant values for infection types (Pathan and Park, 2006).

Data of the following traits were collected: Data were recorded of the various morphological traits like plant height (cm) (PH), spike length (cm) (SL), number of spikelets per spike (NS), number of tillers (NT), peduncle length (PL), and thousand grain weight (g) (GW)

Leaf area (flag) was measured using the following formula (Aldesuquy *et al.,* 2014)

LA (cm^2) = Leaf Length × Leaf Width × 0.75

STATISTICAL ANALYSIS

Correlation analysis was carried on morphological data using Statistix 8.1 software. Principal component analysis (PCA) was carried out on morphological traits using software Past4project to get PC 1 and PC2 which in turn is used to construct a Biplot diagram. Two dendrograms were constructed using Software IBM SPSS 26. In this analysis Ward method with Euclidean distance was used for construction of tree diagrams. First tree diagram was constructed based on morphological traits while second tree diagram showed average linkage distance in 31 genotypes including susceptible check Morocco.

RESULTS

Disease severity score: Out of 31 genotypes, seven genotypes were completely resistant (22.58%), one genotype was resistant with 5R score (3.22%), one genotype with traces disease score less than 5R (3.22%), six genotypes were moderately resistant (19.35%), five genotypes were moderately resistance-moderately susceptible (16.12%), seven genotypes were susceptible (22.58%), two genotypes were moderately susceptible to susceptible (6.45%), one genotype was moderately susceptible (3.22%) and one genotype was resistant to moderately resistant (3.22%). About

twenty four genotypes (77.41%) had Cl value between 0-20 indicating high APR, four genotypes (12.90%) had CI value between 21-40 indicating moderate APR and 3 genotypes (9.6%) had CI value 41-80 indicating low APR (Table 2).

Table 2. Stripe rust severity data collected from genotypes

Genotypes	Severity %	CI	APR
15B-1754-17	20M	10	High
15B-1756-25	5M	2.5	High
15B-1763-26	30MS	22.5	Moderate
15B-1775-76	10MR	2.5	High
15B-1779-92	10MR	2.5	High
15B-1780-207	5MR	1.25	High
15B-1783-219	0	0	High
15B-1784-220	TRACES	0	High
15B-1786-242	0	0	High
15B-1787-245	0	0	High
15B-1788-249	0	0	High
15B-1789-278	0	0	High
15B-1790-284	0	0	High
Gold	20MSS	18	High
Anaj	5R	0.5	High
Akbar	30MSS	27	Moderate
291(2190)	5MR	1.25	High
293(V45)	0	0	High
FakhraBkhar	5MR	1.25	High
2511	5MR	1.25	High
Ghazi	20M	10	High
Sehar	50S	50	Low
Gandum	80S	80	Low
16005	10RMR	3	High
Aas11	20M	10	High
Johar	20S	20	High
Bkhrstar	20M	10	High
Abdlstar	40S	40	Moderate
TD 1	40S	40	Moderate
Zincol	20S	20	High
Morocco	80S	80	Low

Performance of genotypes on the basis of studied morphological traits: Mean values of morphological traits are given in the Table 3, PH varied from maximum in susceptible check Morocco (108.0 cm) to minimum in variety Gandum (70.6 cm). Maximum mean value of SL was observed in line 245 about (14.43 cm) while TD 1 had the shortest spike measured (9.13 cm). FL varied greatly from (38.9cm^2) in 293(V45) to (23.6cm^2) in TD 1. Thousand GW was observed maximum in Bkharstar (46.0g) and minimum in TD 1 at (27.0g). PL varied from (35.2 cm) in Line 92 to (25.4cm) in TD 1. Line 242 had the highest NS (22.33) while TD 1 had the lowest (12).

Table 3. Mean value of Morphological traits

Genotypes	PH	SL	NS	PL	FL	NT	1000GW
17	90.2	13.1	19.0	27.2	33.21	12.0	40.9
25	80.2	11.73	16.1	29.4	35.7	13.0	34.3
26	75.3	10.23	15.46	31.1	27.47	9.0	34.2
76	73.5	10.5	15.0	28.3	32.5	11.0	35.4
92	103.4	12.13	18.0	35.2	29.14	12.0	38.5
207	92.2	10.1	16.0	29.5	28.7	9.0	37.4
219	83.3	11.21	18.0	28.5	31.2	15.0	31.4
220	95.6	13.5	19.2	28.7	37.09	11.0	39.7
242	86.4	13.5	22.33	29.7	38.74	13.0	34.8
245	95.2	14.43	20.33	30.4	36.54	12.0	35.6
249	74.7	10.75	19.0	28.3	33.75	10.0	29.8
278	90.6	11.26	18.0	29.3	31.56	9.0	31.9
284	78.2	10.86	17.33	26.5	30.72	7.0	38.4
Gold	90.2	13.13	21.0	29.4	31.15	13.0	28.2
Anaj	96.2	12.15	19.11	29.7	33.14	11.0	42.4
Akbar	87.5	11.87	16.0	30.4	34.2	10.0	40.9
291(2190)	91.4	13.36	19.0	29.2	27.53	10.0	37.5
293(V45)	93.5	13.12	20.33	26.4	38.9	8.0	42.3
FakhraBkhar	91.8	13.16	20.0	30.5	31.3	9.0	43.9
2511	87.4	12.22	17.0	26.7	35.14	7.0	36.9
Ghazi	96.4	11.56	18.0	31.6	34.18	8.0	42.2
Sehar	88.5	10.5	14.0	27.5	28.35	9.0	38.9
Gandum	70.6	10.4	15.0	26.3	36.75	8.0	35.7
16005	90.7	11.4	20.0	28.8	32.82	8.0	39.2
Aas11	91.2	13.25	20.5	27.2	37.56	8.0	36.6
Johar	95.7	12.66	20.33	28.9	31.5	13.0	33.9
Bkharstar	100.4	12.26	17.0	27.3	33.21	15.0	4.0
Abdlstar	74.1	9.34	13.0	26.3	32.31	9.0	39.2
TD 1	71.8	9.13	12.0	25.4	23.6	12.0	27.0
Zincol	91.1	10.0	16.0	29.4	28.55	9.0	33.2
Morocco	108.0	9.26	15.0	28.7	26.35	8.0	29.1

Correlation analysis: Correlation was computed to find relationship among different parameters (Table 4). PH correlated positively with the SL (0.4327), NS (0.4342) and PL (0.4481). While SL had a highly significant correlation with NS (0.8441) and FL (0.5921). A weak positive yet significant correlation was also found between FL and GW (0.3667). Based on these morphological traits we can access the yield potential of these lines and varieties. NT trait did not show any significant relation with any other morphological traits under observation.

Principal Component Analysis: Factor loading for morphological traits: Principal Component 1: Factor loading shown in Fig 1 gives us the maximum positive load of the trait SL, followed by NS, PH, FL, GW, PL and NT. This Principal can be effectively called for SL as justified by the correlation study showing the positive association of the SL with NS, PH and FL.

Table 4. Correlation analysis (Pearson)

	PH	SL	NS	PL	FL	NT
SL	0.4327**					
NS	0.4342**	0.8441**				
PL	0.4481**	0.2376	0.2857			
FL	0.0129	0.5921**	0.5343**	0.3106		
NT	0.0865	0.2986	0.1889	0.1738	0.013	
GW	0.3184	0.3635	0.1715	0.0922	0.3667*	-0.143

Where PH=Plant Height, SL=Spike Length, NS=Number of Spikelet, PL=Peduncle Length, FL=Flag Leaf Area, NT=Number of Tillers, GW=1000 Grain Weight

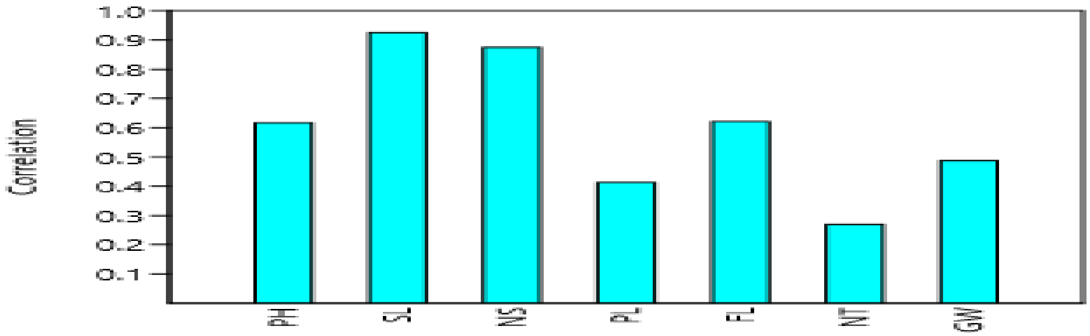

Figure 1. Factor loading for Principal Component (PC1)

Principal Component 2: Factor loading shown in Fig 2 gives us the maximum positive load of trait like PL, followed by PH and NT while negative load was contributed by FL, GW and SL. The PC can be effectively called for PL as correlation analysis showed that PL had positive significant correlation with the PH.

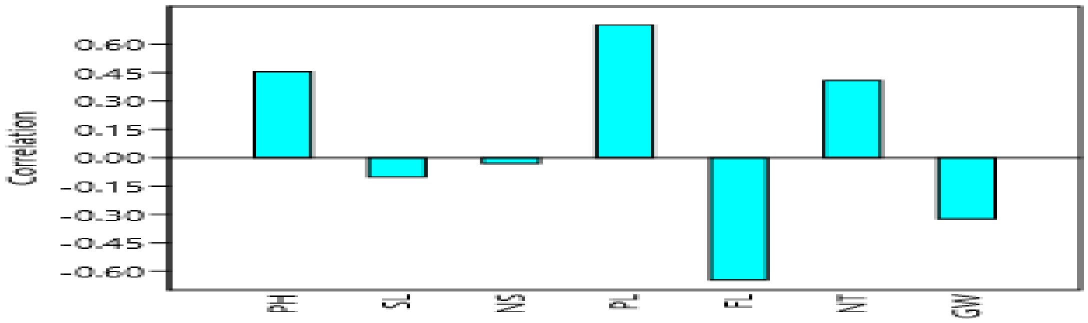

Figure 2. Factor loading for Principal Component (PC2)

Biplot diagram: These two PCs (Figure 1 and Figure 2) were used to construct a scatter biplot diagram (Fig.3) based on the morphological data of genotypes including Morocco super spreader and check. Lines representing PL, PH, GW and FL were far away from the origin indicating these morphological traits are responsible for most diversity that exist in between these genotypes. Genotypes 92, 242, 245, 293(V45) Gandum, Abdlstar, TD 1 and Morocco were far away from other lines in respect to origin showing that these were highly diverse in respect to morphological traits.

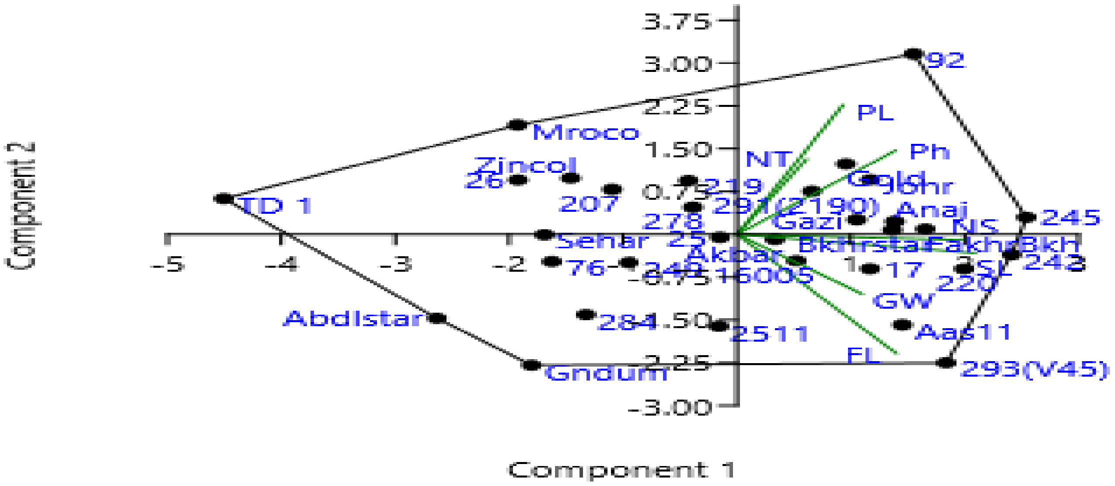

Figure 3. Biplot diagram

Cluster analysis: Dendrogram based on morphological traits: Based on these morphological traits a tree diagram is depicted in Fig. 4 which showed two clusters at linkage distance of 25. PH in the separate cluster which could be named as cluster 1 and rest of the traits were in the separate cluster named cluster 2. In cluster 2 we can clearly see that trait like SL and NS were present within same cluster showing positive relationship between traits. Similarly FL and GW were present in same cluster representing close relationship.

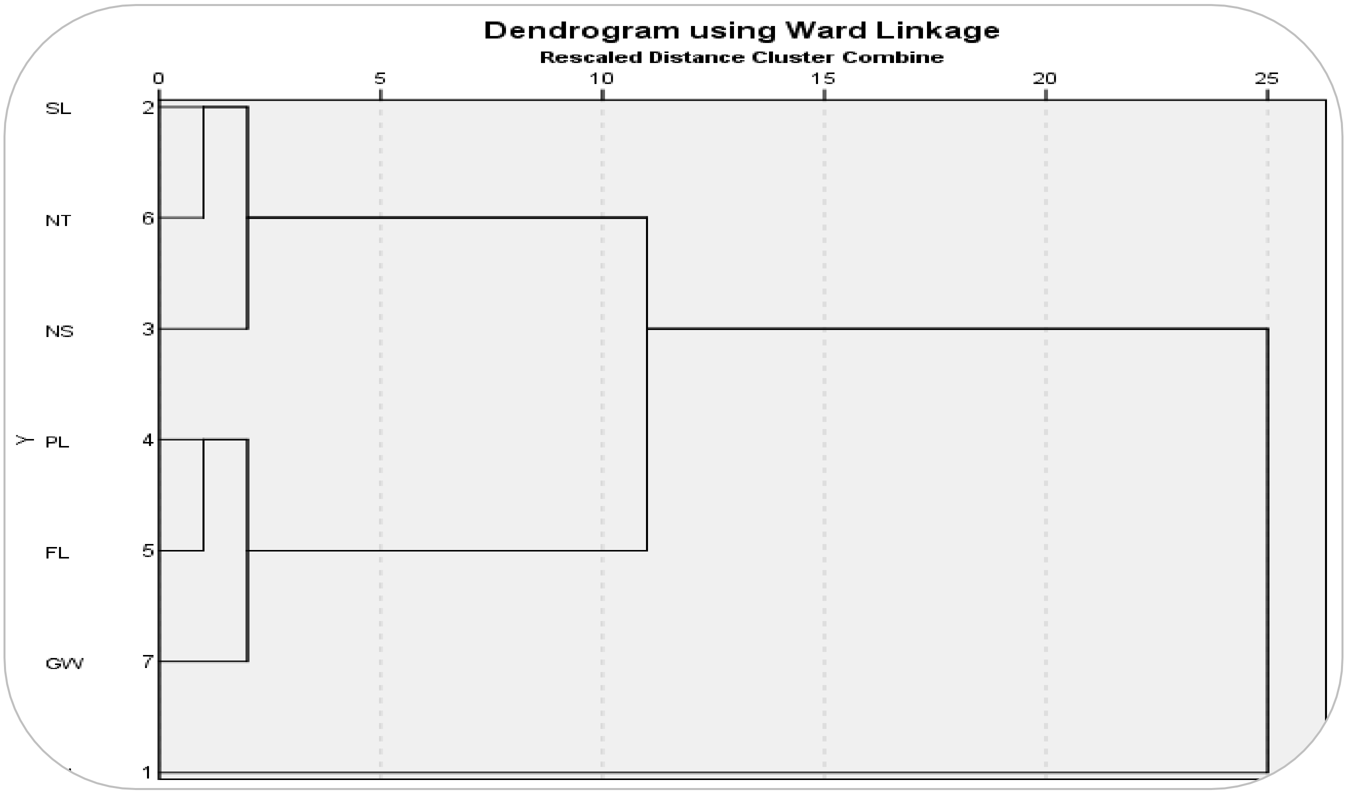

Figure 4. Tree diagram based on morphological traits

Data of 31 genotypes including Morocco susceptible check were further used to construct another tree diagram (Figure 5) which also depicts two clusters namely cluster 1 and cluster 2. Cluster number 1 was further sub-divided into A and B, division A contained TD 1 while B is further divided into B1 and B2. In B1 line 249 was the outlier while line 25 and line 219 were closely related. In B2 Gandum was outlier and line 76 and Abdlstar in same cluster and lines 26 and 284 were in same cluster. Likewise cluster 2 was further sub-divided into C which further divided into C1 and C2. C1 contained Morocco while in C2 line 92 was outlier and Bkharstar, Anaj and Ghazi were in same cluster. D was further divided into D1 and D2. D1 contained Johar, line 220, Line 245 line 245, 2511, Aas 11 and line 293 (V45). D2 contained Line 278, Zincol, Gold, Akbar, FakhrBkhr, line 17, 16005, Sehar, line 207 and line 291(2190).

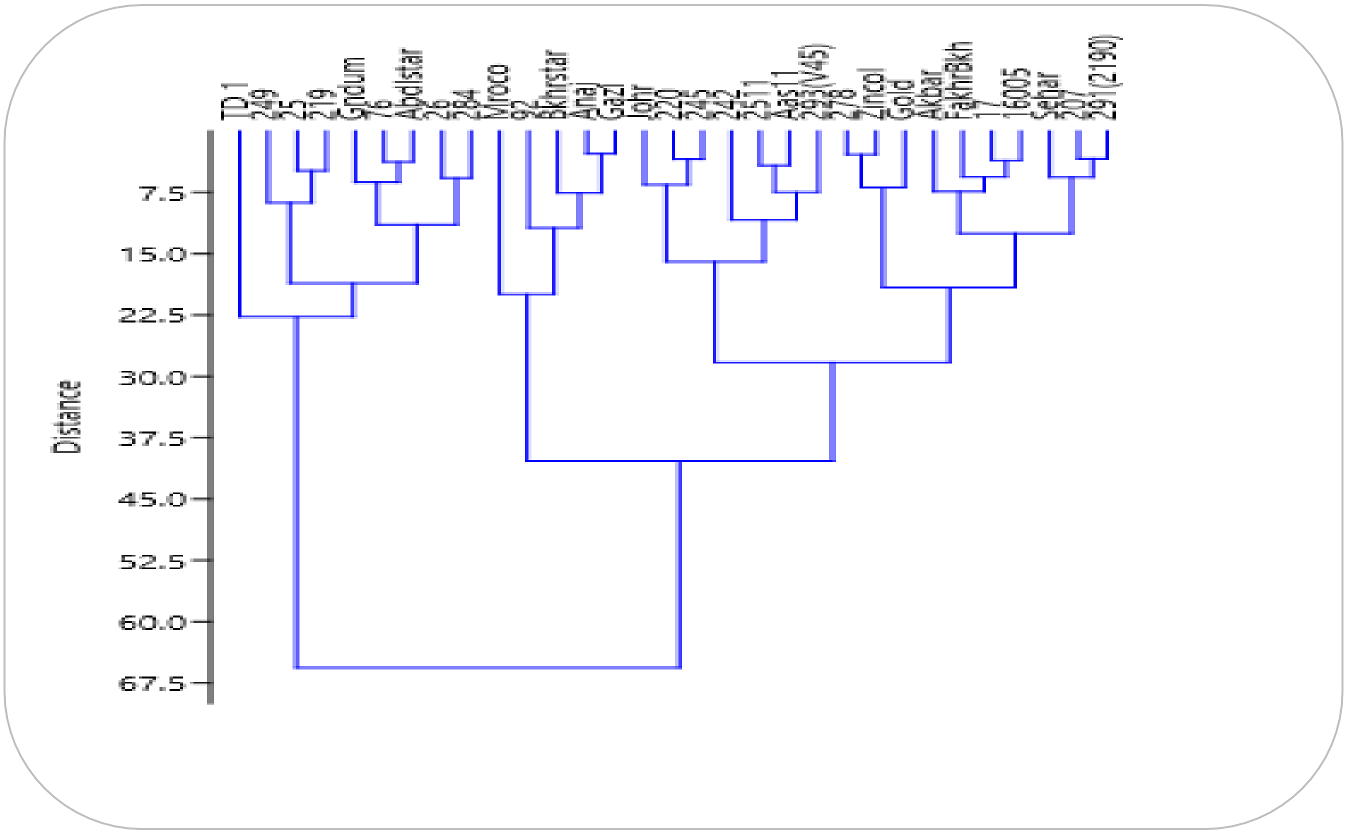

Figure 5. Average linkage distance among 31 wheat genotypes

DISCUSSION

Stripe rust, one of the devastating diseases of wheat crop, is responsible for major losses in yield worldwide. Development of disease resistant varieties is environmentally sound way to combat the disease decreasing our dependency on fungicides. As new races of stripe rust evolve breeding for resistant genotypes is an ongoing process. Screening against disease in new lines and present cultivars is the first step to identify the potential candidates for use in future breeding. Understanding genetic diversity in agricultural species is essential for crop improvement in changing conditions (Sajjad *et al.,* 2011). In our research plant height, peduncle length, 1000 grain weight and flag leaf area were the morphological traits responsible for most of the diversity that existed between genotypes. Genotypes 92, 242, 245, 293(V45) Gandum, Abdlstar, TD 1 and Morocco were highly diverse in terms of morphological traits from the rest of genotypes. Alsaleh *et al.* (2016) investigated diversity in 'Kunduru landraces' of Durum wheat (*Triticum turgidum*) based on 35 genotypes. Few morphological characteristics, as well as a yellow rust score compared among landraces to assess resistance. Ali *et al.* (2008) and Singh and Dwivedi (2002) claimed that cluster analysis can be effective for identifying high yielding wheat genotypes, findings of their study further revealed a considerable genetic divergence among wheat genotypes. In correlation analysis we found significant relationship between different morphological traits. These finding were supported by Khodadadi *et al.* (2011) who also reported that traits like grain yield, number of spikelets and spike length had positive association. Raza *et al.* (2018) also performed screening of diverse wheat germplasm and found 60 lines/varieties with complete immunity to all the races of yellow rust. Quite recently, Mukhtar *et al.* (2021) studied 83 genotypes for their morphological trait diversity and resistance to yellow rust. They also performed cluster analysis and identified 14 most diverse genotypes.

CONCLUSION

It is concluded that majority of genotypes evaluated in current study were resistant with high APR. However the susceptible genotypes like TD-1, Gandum, Abdlstar and Morocco with moderate to low APR were highly diverse in term of morphological traits. The identified resistant genotypes could further be exploited in future breeding programs against stripe rust.

CONFLICTS OF INTEREST

The authors declared that they have no conflict of interest

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Contribution of Authors:

Abdul Rehman	:	Designed the layout and performed experiments
Muhammad S. Naeem	:	Performed experiments, statistical analysis and wrote the manuscript
Muhammad F. Naseer	:	Wrote the initial and revised draft of the manuscript
Mueen A. Khan	:	Designed and supervised the research