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ADVANCED WINTER WHEAT (*TRITICUM AESTIVUM* L.) LINES REVEALED MORPHOLOGICAL TRAIT DIVERSITY AND RESISTANCE AGAINST YELLOW RUST

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A B S T R A C T

Wheat is important crop with two major categories as winter and spring wheat. Winter wheat was first time introduced in Rawalakot to check the adaptability and suitability hence winter wheat varieties had never been tested in the hilly areas of Azad Kashmir. For this purpose, 83 genotypes of wheat were used including six winter wheat check varieties and Pakistan-2013 as local check and intercropping of universal susceptible as spreader. Yellow rust data was taken by using Modified Cobb's scale to assess the disease severity and presence of resistance. Four winter wheat advanced lines 141137, 170915, 170196 and 170917 were found resistant. Analyses of variance have shown significant differences for all parameters apart from spike length, number of spikelets per spike and 1000-grain weight. Principal component analysis revealed 85.43% variability. The cluster analysis divided the genotypes in 5 clusters on the basis of genetic similarity and distance. Fourteen advanced lines were found more diverse and could be utilized in further breeding providing an opportunity for winter wheat to be adopted in this region. And among 14, four resistant genotypes can be used as source of yellow rust resistance in winter wheat and spring wheat breading.

Keywords: Spring wheat, Diversity, Stripe rust, Genotypes, Cluster analysis.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is an important cereal crop providing net approximate yield of 785.8 million tonnes comprising 27% of 2820.9 million tonnes of total cereal yield worldwide. The production area was recorded 215-220 million hectares with continuous increase in last few decades. The worldwide wheat production trend has been raised from 658.6 million tonnes during 2012-13 to 774.8 million tonnes during 2020-2021 (FAO, 2021). Winter wheats are cultivated on small areas in early times, since then it has been regularly increased. Meanwhile winter wheat production was increased to 34 million bushel during year 2016-17 as highest since 1987-88 (Jennifer and Olga, 2017). The growing conditions for winter wheat were suitable in Great Plains of USA. Adaptability of winter wheat to Great Plains turned into main growing region for hard winter wheat. The first hard winter wheat was named as "Turkey wheat" because it was originated from a little valley in Turkey and commenced by Mennonite settlers arrived from Kansas in 1874 (Paulsen and Shroyer, 2008; Quisenberry and Reitz, 1974). The Turkish wheat was important for the improvement of wheat in Great Plains, and it is believed that Turkish and US

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Great Plains wheats are genetically close. The genetic association among Turkish wheats and hard winter wheat varieties planted in Great Plains of USA were observed in term of genetic similarities and distance. The Genetic distance and similarity can estimate genetic variability among genotype populations (Stachel *et al*., 2000). Genetic diversity among wheat genotypes can be evaluated and explained by Principal component and cluster analysis showing relationship based on morphological traits. The genetic variability among individuals of the plant populations can be distinguished by screening based on morphological traits. Genetic diversity is imperative to improve the resistance against diseases and sustainable bread wheat yield by identifying the genotypes with respect to suitability and adaptability in a particular growing region (Akcura, 2011). Techniques explain the variation among the genotypes by calculating the complete variance of a population and contribution of different factors in total variance (Hailu *et al*., 2006; Skrbic and Onjia, 2007).

In wheat improvement programs the knowledge of the genetic variation and diversity of wheat genotypes can be very useful for the identification and development of more efficient crops with good adaptability to a specific region. Introduction of improved foreign germplasm is a good breeding approach. Genotypes should be evaluated on the basis of genetic similarity and genetic distance from the collection of wide range of germplasm. This evaluation helps to select the parental genotypes with wider range of diversity and genetic variability which is helpful in crop improvement programs (Korkut *et al*., 2001).

Pakistan with varied agro-ecological climate includes the mountains and plains of Baluchistan, Panjab, Sindh, Khyber Pakhtunkhwa, Azad Jammu & Kashmir and Gilgit-Baltistan. The diverse environmental factors play a vital role in varietal improvement. Variability in germplasm is important for breeder in selecting the plants that could be utilized as parents in crop improvement programs (Akbar *et al*., 2003; Iqbal *et al*., 2003) and new emerging rust threat (Afzal *et al*., 2021). Therefore, the advanced lines of winter wheat were introduced for the first time in Rawalakot because of their winter hardiness and better adaptability to cooler regions than spring wheat. Among cereals, winter wheat was found very susceptible to fungal diseases like rusts, while synthetic hexaploid wheats due to their derivative nature from wild relatives and wheat landraces due to their diverse origin (Ghosh and Gulati, 2001; Tariq-Khan *et al*., 2020) were proved as useful genetic sources against yellow rust from CIMMYT (Tariq-Khan and Ul-Haque, 2011; Tariq-Khan *et al*., 2020; El-Orabey *et al*., 2020; Tariq-Khan. *et al*., 2012) for sustainable wheat production. Yellow rust causes heavy economic losses globally in the areas with cool humid temperature resulting in reduced yield. Considering current requirements for plant protection, especially the agro-technical methods should constitute the basis in reducing the development of pests (Thorne, 2016).

This study was carried out in Rawalakot conditions having temperate climate favoring frequent yellow rust infestation due abundant alternate host (Barberry), and short mild summer season favourable to winter wheat along with already grown spring wheat. It was imperative to evaluate winter wheat yield potential, adaptability and its resistance against yellow rust for durable wheat yield through winter wheat induction in agricultural economy of the region. Better performing lines with higher adaptability will be used in future breeding programs and can be released as new varieties.

MATERIALS AND METHODS

Study had been carried out in fields of Faculty of Agriculture, University of The Poonch Rawalakot, during Rabi 2016-17 and 2017-18. Facultative and winter wheat observation nursery was obtained from National Wheat Improvement Program of Turkey, CIMMYT, ICARDA and tested under Rawalakot conditions. Eighty three (83) advanced lines of winter wheat along with 6 control check lines were grown in Augmented Design (Table 1), to assess their performance including five winter wheat viz., Bezostaya, Seri, Sultan-95, Katya1 and Konya and Pakistan-2013. Universal susceptible check Morocco was sown all around the plot and within plot every 20th row as natural spreader. Row to row distance was 30 cm, while row length was 2.5m. Agronomic practices were adopted according to standard procedure recommended by the agricultural extension for the area.

Table 1. Winter wheat advanced lines reaction against yellow rust pathogen in field conditions

S. No	Entry No	Yellow rust severity	S. No	Entry No	Yellow rust severity	S. No	Entry No	Yellow rust severity
1	160185	50MS	29	160038	60MS	57	170910	5MR
2	160109	40S	30	160040	10MS	58	951027	90S
3	160275	60S	31	160041	50MS	59	170911	80S
4	171219	70S	32	160043	20MS	60	170912	80S
5	171220	60S	33	170898	70MS	61	170913	30S
6	171221	40MS	34	170899	90S	62	100953	30S
7	171222	40MS	35	170901	30S	63	150861	40MS
8	171224	50MSS	36	170902	40S	64	150866	90S
9	171225	20MR	37	170903	60MS	65	150867	60MS
10	171230	10MR	38	141053	90S	66	150869	70S
11	171231	40MSS	39	141055	60MSS	67	150870	60S
12	171237	60MSS	40	960105	80SS	68	170920	20S
13	171246	40MS	41	100719	30MSS	69	170921	30S
14	171247	20MRMS	42	100717	90S	70	170915	R
15	171248	10MRMS	43	141121	50MS	71	170916	R
16	171283	30MS	44	141128	20MS	72	170917	R
17	171343	20MR	45	141133	60MS	73	170918	5MS
18	171346	50MS	46	141136	10MS	74	160048	40MS
19	950189 (Check)	30MS	47	141137	R	75	160049	40MS
20	951027 (Check)	80S	48	150811	20MS	76	160051	40S
21	950129 (Check)	90S	49	150847	10MS	77	160052	90S
22	950590 (Check)	60S	50	150848	60S	78	160053	40MS
23	020986 (Check)	60S	51	150850	10MS	79	160054	40MS
24	Pakistan-2013	60MSS	52	150852	90S	80	160055	50MS
25	171350	70MSS	53	150854	10MS	81	160059	80S
26	171359	40MSS	54	150855	30MS	82	160062	60S
27	141107	90MS	55	170905	50MS	83	160063	40MSS
28	141110	90MS	56	170909	10MS			

Pathological studies: Field reaction of Winter Wheat Advanced Lines against Yellow rust in field conditions: Yellow rust was continuously monitored on the Morocco and data was taken while yellow rust covered the 80% of flag leaves. Disease on the tested lines along with testers varieties were taken according to modified Cobb's Scale on the basis of infection and genotypes were designated as Immune (zero Infection), Resistant (Necrotic lesions with or without minute uredia), MR (small uredia around necrotic lesions) and Moderately susceptible (medium uredia with some chlorosis) and S (large uredia and slight or no chlorosis) (Peterson *et al.,* 1948).

Morphological characters: Ten plants from each row were selected at random for morphological data collection, like booting stage, Days to flowering, flag leaf area, plant height, number of productive tillers per plant, spike length, number of spikelet per spike, 1000-grains weight and grain weight per plant.

STATISTICAL ANALYSIS

The mean values of all traits under study were used for estimating the variability and correlation among advanced lines of winter wheat. Analysis of variance was done by using the software Agri-STAT. Principal component and cluster analysis was carried out using the PAST 3.20 software.

RESULTS

Yellow Rust disease severity on the advanced winter wheat lines and its impact on principal components: Seventy-five advance winter wheat lines were found highly susceptible to moderately susceptible range when universal susceptible Morocco shown 80% disease coverage on flag leaves as spreader, while check lines were used for comparison of disease progress in natural conditions to avoid any probability of error. Four lines were found resistant and 4 moderately resistant (Table 1). Four advanced lines of winter wheat were found resistant against stripe rust and no infection was observed throughout the cropping season. These lines had the accession numbers as 141137, 170915, 170916 and 170917 respectively. These resistant lines could be utilized in breeding programs to improve stripe rust resistance in future varieties. All check varieties are found susceptible to yellow rust. The disease severity for all genotypes can be observed (Table 1). Among the resistant lines 1000-grain weight is most important yield feature of any bread wheat variety. Accession 141137 with 45.5 g having highest 1000 grain weight, followed by 170917 38 g, 170917 with 32 g. It was observed that the grain weight of resistant varieties against yellow rust has characteristics of early flowering as compared to other tested lines. It can be a linked character which is of agronomic and breeding importance. The improved grain weight in these lines was due to stripe rust resistant however their grain yield per plant was not on higher side because of late maturity and lesser grains per spike. The Analysis of variance at 5% and at 1% significance level showed significant differences in case of test entries for all traits accept 1000-grain weight, plant height, spike length, number of tillers per plant, spikelet per spike and while high significant variation was found among all blocks for all morphological traits. Non-significant differences for check varieties were observed for parameters as plant height, number of productive tillers per plant, spikelet per spike, grains yield per plant, flag leaf area and days to 50% headings. Out of all test entries 4 lines having the accession number 141137, 170915, 170916, 170917 were completely resistant. Principal component analysis showed a higher amount of total variability which was recorded as 85.2% the first two PCs were contributing the maximum amount of variability as 28.34 % and 23.65% respectively. The factor loading indicated that maximum positive load was attained by two parameters like days to 50% headings and flag leaf area and these two traits had the maximum contribution to total variation. The genotypes were grouped into five clusters; among them cluster 3 had 2 sub clusters (Figure 3). Eighteen genotypes within various clusters were outliers and were the genotypes with highest morphological agronomic diversity (Figure 2).

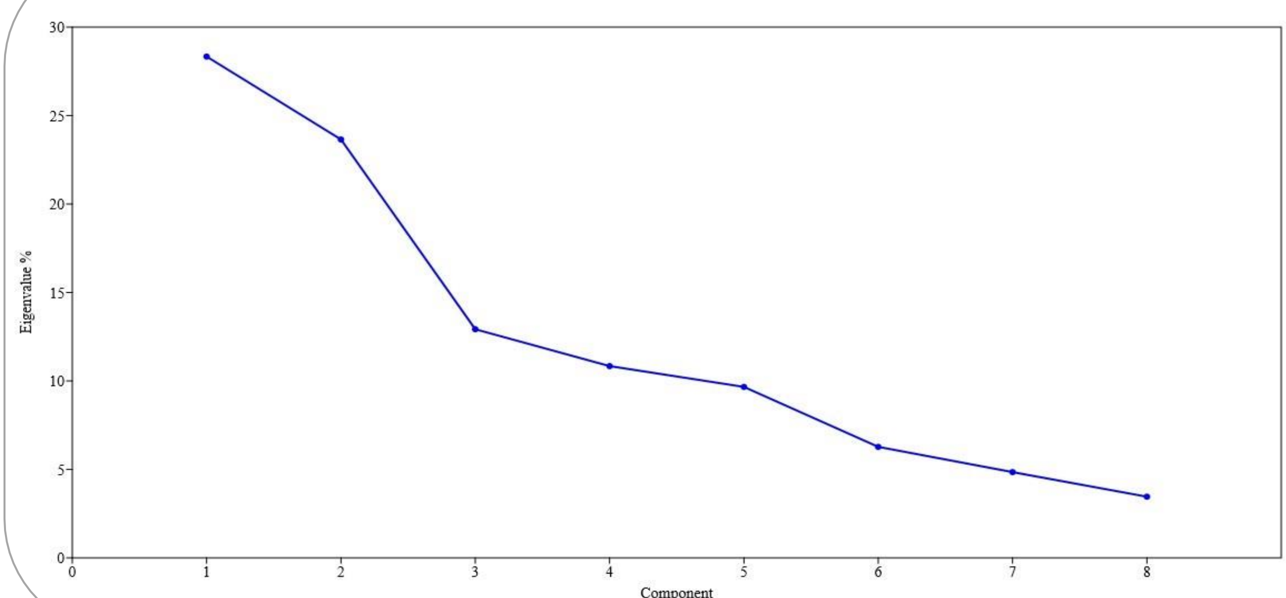

Figure 1. Scree plot diagram for 8 PCs in 83 winter wheat genotypes

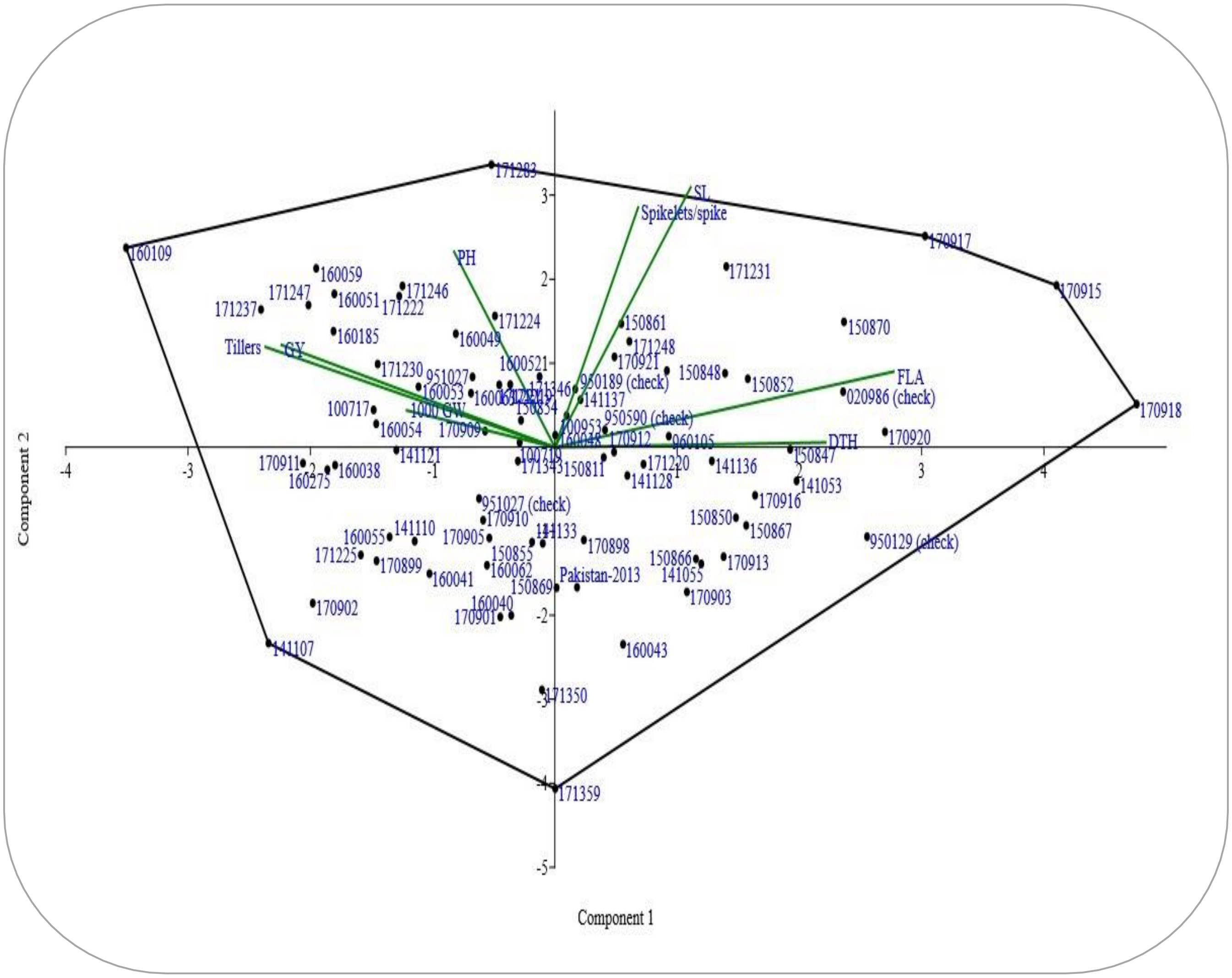

Figure 2. Biplot diagram for morphological traits in 83 winter wheat genotypes

Morphology of the agronomic traits: Analysis of variance for agronomic traits like days to 50% heading and flag leaf area and plant height showed high significant difference, while checks show non-significant differences for these traits. Highly significance differences for flag leaf area was also depicted in findings of Iqbal *et al*. (2017). Considerable variability was observed for these traits although the expression of such traits is greatly affected by the environmental factors. Days to 50% headings is an important trait that directly or indirectly contribute to grains yield and can successfully be used as selection criteria in selection of high yielding genotypes (Iqbal *et al*., 2017; Singh and Chaudhary, 2006; Jolánkai *et al.,* 2006). For flag leaf area our results were comparable with findings of Atkinson *et al.* (2008); Dodig *et al.* (2007) and Kenzevic *et al*. (2007). It was also found that our studies were contradictory to the Mursalova *et al.* (2015) whom denied the environmental impact on morphology.

Mean values were recorded from 60-120 cm for plant height. A 96.10% of advanced lines mean value for plant height was less than or equal to 89 cm, higher tillers (41.5%), spike length has high significant difference, contain more grains and results in increased grain yield and total grain mass. Total of 12.98% advanced lines were superior for this trait. Total superiority of the test entries was recorded as 23.73% over checks. Similar trend was cited in (Sabaghnia *et al*., 2014; Mecha *et al.,* 2017; Rameez *et al*., 2012; Bhushan *et al*., 2013). The 18 or more spikelets per spike may be kept under consideration as per selection benchmark for high yielding cultivars, such type of results previously were reported by (Mohibullah at el. 2011; Bhushan *et al.* 2013; Chachaiya *et al.* (2017).

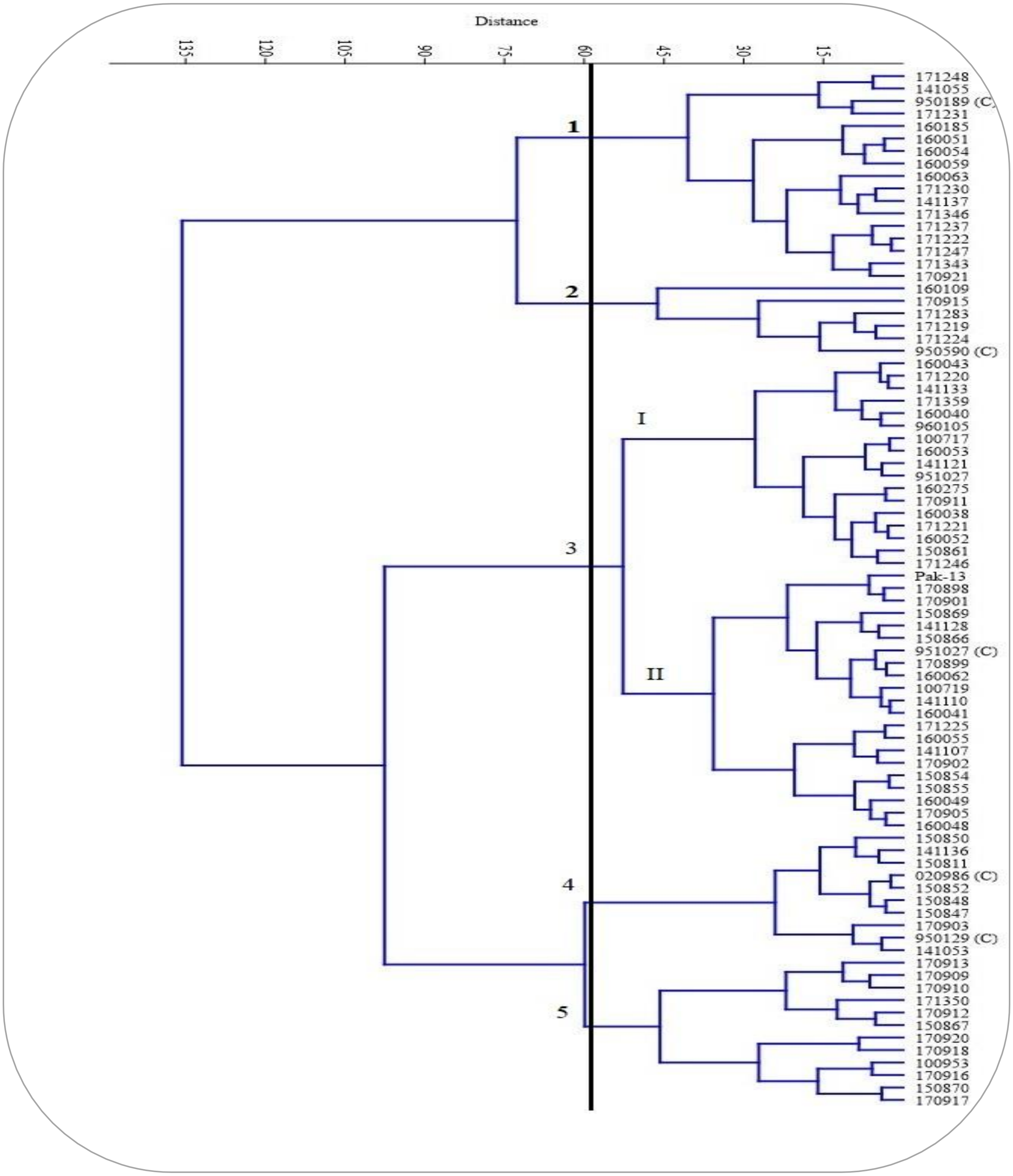

Figure 3. Morphological based diversity and Clustering of 83 winter wheat genotypes

Principle component analysis in case of morphological traits for 83 winter wheat genotypes is presented (Table 2). The total principal components were 8 and 5 were found significant as Joliff's value was 0.75 and this value was used as criteria of significance in selection of significant components. The Scree plot diagram for 8 PCs in 83 winter wheat genotypes were shown (Figure 1). The highest eigenvalue was estimated in the PC 1 was

2.27 and the lowest eigenvalue was observed in PC 5 as 0.77 (Table 3; Figure 1; 2). The variability was high among 83 advance lines of winter wheat as five significant principal components contributed total variation of 85.42%. The PC1 with the maximum variance of 28.34 %, PC2 (23.65%), PC3 (12.92%), PC4 (10.84%) and PC5 added 9.67% of variation to the total variance (Figure 2).

Table 2. Analysis of variance (ANOVA) for morphological traits diversity in 83 winter wheat genotypes

Source	DF	50% Heading	Flag leaf area	Plant Height	Number of productive tillers per plant	Spike length	Number of spikelets per spike	1000-grain weight	Grain yield per plant
Block	3	115.5826**	233.8746**	503.7673**	12.0938**	4.6667**	27.7744 **	252.3147*	8.6861**
Genotypes	82	42.7462*	42.1514**	60.3521 NS	2.6646 NS	0.7474 NS	3.4352 NS	37.1352 NS	6.5745**
Checks	5	17.3000 NS	9.7967 NS	9.2304 NS	1.4427 NS	0.2282 NS	0.3667 NS	49.5169 NS	0.4592 NS
Test Entries	76	49.4378*	37.7942**	83.2570 NS	3.0858 NS	0.8038 NS	4.7522 NS	38.8454 NS	6.0129**
Error	15	14.0556	8.5832	82.1193	1.3887	0.5209	2.4556	58.5129	0.5127

Table 3. Principal component analysis for morphological traits in 83 winter wheat genotypes

PC	Eigen value	Variance (%)	Total Variance %
1	2.27	28.34	28.34
2	1.89	23.65	51.99
3	1.03	12.92	64.91
4	0.87	10.84	75.75
5	0.77	9.67	85.42

Joliff's cut off = 0.75

The genotypes in Biplot diagram accession number 160109, 171283, 170917, 170915, 170918, 171359 and 141107 were found highly diverse and distant so can be selected for future breeding programs. The 1000-grain weight was less variable as it was spotted nearest to the origin point. A negative relationship was also observed for flag leaf area, days to 50% heading, spike length and spikelet per spike with the traits like grain yield per plant, number of tillers per plant, plant height and 1000-grain weight as they were present in opposite quadrates. Another trend visible in this Figure was that the lines having higher days to 50% heading tend to have more flag leaf area, lower grains yield per plant, number of tillers and 1000-grain weight (Figure 2). Grain yield per plant was also positively correlated with plant height and 1000-grain weight. These genotypes included 020986 (Check), 170920, 150847 and 159870. The advanced lines with the high number of productive tillers per plant were recorded as high yielding lines. The genotypes plotted below the X-axis and extending towards the negative Y-axis were lower in performance for almost all the traits as they could not be plotted near any of the parameters. Genotypes spotted morphologically near are higher in performance in that particular trait like 171237, 171247, 160059, 160051 and 160185.

Dendrogram based on genetic expressions of 8 morphological traits in relationship among genotypes displayed as cluster among 83 winter wheat genotypes for (Figure 3) as 60 genotypes are divided into 5 clusters. The genotypes having the accession number 160185, 160063, 171346 and 171237 are outlier, hence were most diverse genotypes within this cluster. These genotypes could be used in crossing programs to make the possible combination of desirable genes. The cluster 4 had 10 genotypes and among them the genotypes with the accession numbers 150850 and 170903 were outliers and considered most diverse. Only 12 genotypes were grouped in cluster 5 and two were outliers and considered diverse (advanced lines 170913 and 171350). The checks were found randomly distributed among all clusters showing diversity. Outliers displayed in various clusters could be used as a source of breeding material for future. Overall no distinction could be made whether which cluster is high yielding and which one is low, same is the case for rest of the traits. The genotypes seemed to be randomly distributed throughout the clusters.

DISCUSSION

Yellow rust is caused by *Puccinia striiformis* f. sp. *tritici* is a common threat to wheat production all over the world especially cool temperate areas. Handling of its epidemics are real challenge to the plant pathologist, agronomists and breeders to safe production for food security. In the year 2019-20 the disease targeted even tropical areas of Pakistan, spreading its pathogenic potential in all tropical and temperate areas from coastal line to Himalayan foothills and Hindukush range. Yellow rust alternate known host (*Barberry*) is abundant in the area. This host facilitate the fungus to mutate and come up with very much virulent strains targeting yellow rust yield. Himalayan range serve as source of natural mutation in rust pathogen so continuously emerging new yellow rust races breaking the major gene resistance in bread wheat cultivars supported by narrow genetic base. This trend is continuous and observed in the region and in Pakistan (Afzal *et al.*, 2008; Ali *et al.*, 2008) using modified Cobb's scale. The disease incidence was severe as observed that dry season was followed by heavy rains. In this field based study disease attack was severe on all the test entries and checks because the moist season favored the disease infection which was followed by warm and dry weather such type of results were also described by Fayyaz *et al.* (2017).

Genotypes with resistance are found diverse and can be used successfully in crossing programs for developing varieties with desirable combinations of genes (Table 1). Days to 50% headings is an important trait that directly or indirectly contribute to grains yield and can successfully be used as selection criteria in selection of high yielding genotypes (Iqbal *et al*., 2017). Alternatively, winter wheat lines which take more days for maturity are generally considered not suitable for cultivation (Table 2; 3). The results of our studies matched with the conclusions of Singh and Chaudhary, (2006) and Jolánkai *et al.* (2006). For flag leaf area our results were comparable with findings of Atkinson *et al.* (2008); Dodig *et al.* (2007) and Kenzevic *et al*. (2007). It was also found that our studies were contradictory to the Mursalova *et al.* (2015). They concluded that no genotype by environmental interaction was noted for this trait along with other morphological traits. The height of plant is correlated by many other traits as an average plant height improve the performance of a genotypes in specific environment especially biomass yield increased with increase in plant height (Table 2), such findings are in support with previous finding of Rameez *et al*. (2012) and Bhushan *et al*. (2013).

The genotype with the highest value for this trait confirmed complete resistance against stripe rust and no disease infection was observed during the whole cropping season. The genotype with the highest grain yield per plant was found 80% susceptible for stripe rust. While other two genotypes with high yield of grain per were also identified as 40 to 70% susceptible to yellow rust and genotype with lowest grain yield per plant found completely resistant against stripe rust the reason is stated as resistant genes need more energy to coup with pathogen (Tariq-Khan *et al*., 2012; Tariq-Khan and Ul-Haque, 2011). The environmental factors also influence the performance of a genotype, hence it is very important to understand the adoptability and suitability of genotype to specific environmental conditions (Singh and Choudary, 2006). Biplot diagram presented the performance and variability among the morphological traits of 83 advanced winter wheat lines under study (Figure 2). The biplot depicted those traits like flag leaf area, number of tillers and grain yield per plant were highly variable as they were plotted outermost from their origin point. Biplot diagram showed flag leaf area, number of spikelet per spike and spike length were correlated negatively with grain yield per plant, plant height and 1000-grain weight. Same results were also previously explained by Bhushan *et al.* (2013); Sabaghnia *et al*. (2014) and Iqbal *et al*. (2017). However, the results of Rameez *et al*. (2012) did not match with our findings as his conclusion was that the grain yield is in positive correlation with spike length and this trait can be use as criteria for selecting high yielding genotypes. Total of 12.98% advanced lines were superior for this trait. Total superiority of the test entries was recorded as 23.73% over checks. Similar trend was cited in (Sabaghnia *et al*., 2014; Mecha *et al.,* 2017; Rameez *et al*., 2012; Bhushan *et al*., 2013). The 18 or more spikelets per spike may be kept under consideration as per selection benchmark for high yielding cultivars, such type of results previously were reported by (Mohibullah at el. 2011; Bhushan *et al.* 2013; Chachaiya, (2017).

The checks were randomly distributed in all clusters showing diversity. Outliers displayed in various clusters could be used as a source of breeding material for future. Overall no distinction could be made whether which cluster is high yielding and which one is low, same is the case for rest of the traits. The genotypes seemed to be randomly distributed throughout the clusters.

CONCLUSION

Advanced lines of winter wheat showed high adoptability for this region the overall performance was superior over checks as well as over local check variety (Pakistan-2013). The four genotypes having the accession numbers 141137, 170915, 170916, 170917 were marked as resistant against stripe rust. These genotypes could be used in crossing programs to incorporate stripe rust resistance in high yielding genotypes. These lines can possibly be used for general cultivation in this region after approval due to superior winter hardiness and better adoptability as compare.

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