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Genetic Diversity and Traits Association in Tetraploid and Hexaploid Wheat Genotypes in Khyber Pakhtunkhwa Province of Pakistan

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Abstract | Information regarding the magnitude of variability as well as the correlation among agronomically important traits renders the basis for development of a successful crop improvement program. An experiment containing 16 wheat genotypes (8 durum and 8 spring wheat) was conducted in crop season of year 2015-2016, at The University of Agriculture, Peshawar. The experimental design used was a Randomized Complete Block Design (RCBD) with three replications. The parameters under study were days to heading, days to maturity, flag leaf area, plant height, tillers m^{-2}, spikes m^{-2}, spikelets spike^{-1}, 1000-grain weight, grain yield and harvest index. Among the genotypes for various study traits, statistically significant differences were observed. Except for the flag leaf area and days to maturity, durum vs. spring wheat contrast was significant for all other studied parameters. The flag leaf area of durum wheat was more than that of spring wheat and it took fewer days to initiate heading as well. In contrast, spring wheat genotypes had more average plant height, tillers m^{-2}, spikes m^{-2}, 1000-grain weight, grain yield and harvest index than durum wheat genotypes. Correlation analysis revealed that tillers m^{-2} and spikes m^{-2} had significantly positive association with grain yield while grain yield had significantly strong positive association with harvest index in tested germplasm. Durum genotypes *DWE3* and *DWE7* performed best for yield contributing traits while spring wheat verities, *Janbaz*, *Barsat* and *Shahkar* outperformed others in terms of 1000-grain weight, grain yield, and harvest index, respectively. These genotypes are recommended to be further tested at multi-locations to check for wider adaptability and a possible use in future wheat breeding programs in the area.

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Introduction

Wheat (*Triticum aestivum* L.) is one of the principal food crops of the world in general and of Pakistan, in particular. In Pakistan, nearly nine million hectares of land is utilized for the wheat cultivation and it has a production of approximately 25 million tonnes, whereas the average yield per hectare is 2700 kgs (Agricultural Statistics of Pakistan, 2003-04). While in Khyber Pakhtunkhwa province, it is planted on 0.7 million hectares and has a grain production and per hectare yield of 1 million tonnes and 1500 kgs, respectively (Crop Statistics Khyber Pakhtunkhwa, 2013-14). It has been reported that wheat yield in Pakistan lags behind the countries with comparable agroclimatic conditions (Dmitry and Oleksandr, 2013). An intensified improvement in wheat yield ensures a prosperous future of an ever-increasing human population with surged food demands. Plant breeding has always contributed to enhance the genetic potential of crop plants. A similar holistic approach is needed to improve the yield as well as quality parameters of wheat crop. Variability refers to the measure of differences among individuals of a plant population in terms of genetic constituents and growing environments (Veludandi *et al.*, 2017).

Existence of genetic variability provides a greater chance for population improvement by effective selection on traits of breeding interest. Efforts are needed to breed for suitable maturity groups in terms of early, intermediate and delayed planting. It is very often misleading to directly select for important traits such as grain yield due to complex genetic mechanisms controlling the trait (Arshad *et al.*, 2017). Therefore, information regarding genetic variability and association between morpho-agronomic traits provides a better understanding and confidence for selecting desirable genotypes (Dixet and Dubey, 1984; Uddin *et al.*, 2015). Correlation studies are also vital for plant breeders to understand the interrelationships among pairs of traits which leads to lay down a suitable selection criterion to run a successful breeding program (Aytaç and Kinaci, 2009).

Wheat yield consortium is an active project of CIMMYT that focuses on wheat crop improvement by conducting research in areas such as genetics and plant physiology. The targets of this consortium are to raise the potential yield of wheat crop by 2% per annum and by the same pace, reaching about 50% increase in next two decades around the globe (CGIAR, 2013). Pakistan lies among the first ten places in the list of countries that produce wheat as their main produce which clearly signifies its importance on global scale (Sen-Nag, 2019). On the other hand, climate change is a potential threat to agricultural economy of Pakistan which is the result of poor crop performance, less precipitation and increase in annual mean temperature (Ahmad, 2018). Crop varieties with high yield and better tolerance to environmental stresses can be bred by using genetic improvement tools to reach sustainability (Farshadfar *et al.*, 2013). Clearly, there is a huge responsibility on the shoulders of agriculture scientists to cope with the problem and breed for climate smart, resilient and better performing crop varieties. In such an alarming situation, genetic variability in crop plants seems to be a potential resource which needs to be exploited carefully. Evaluation of exotic as well as indigenous germplasm in current environmental conditions and usage of the best performers for breeding crops will not only provide the basis for fighting drastic climatic conditions but will also increase economic growth of the country.

Keeping in view the above-mentioned facts and their significance, this study has been conducted as an attempt to explore genetic variability in terms of important morphological traits in durum and spring wheat. The purpose of conducting study was to investigate the comparative performance of tetraploid and hexaploid wheat genotypes acquired through different sources and to reveal the correlation among different morphological traits of wheat crop.

Materials and Methods

Genetic resources and experimental site

The study was carried out at New Developmental Farms of the University of Agriculture, Peshawar with coordinates and the growing conditions (Table 1). A set of 16 wheat genotypes was considered in the study (Table 2) containing previously released spring wheat varieties in agro-climatic conditions of KPK Province of Pakistan and the durum advanced selection lines from CIMMYT, Mexico. The set contained eight durum and eight spring wheat genotypes and experiment was conducted during the cropping year 2015-16.

Table 1: *Climatic conditions and coordinates of the experimental site.*

Longitude	71.583° E
Latitude	34.017° N
Altitude	331m
Annual rainfall	384mm
Temperature range	Max 40 °C, min 12 °C
Relative humidity	32 – 90 %
Soil type	Alkaline (pH 7.2 – 9.1)

Experimental design and management practices

The germplasm was planted in a three-replicate

RCB Design on November 24th, 2015. Plots were maintained for each genotype in all the replications and border plots were left as well for minimizing the border effect. Each plot contained three rows of individual genotype whereas, the row length and distance among consecutive rows was 3m and 0.30 m, respectively. To minimize the environmental variance, all necessary crop management practices were applied. It includes the uniform application of fertilizers, pesticides and weedicides. Apart from that, land preparation and hoeing were done at proper intervals. Each plot was sickle harvested on May 21st, 2016.

Table 2: *List of Durum wheat and spring wheat genotypes evaluated during 2015-16.*

S. No	Durum wheat	S. No	Spring wheat
1.	Durum1	1.	FAKHRE SARHAD
2.	Durum2	2.	LASSANI-08
3.	Durum3	3.	JANBAZ
4.	Durum4	4.	BARSAT
5.	Durum5	5.	MARVI-2K
6.	Durum6	6.	ASS-11
7.	Durum7	7.	SHAHKAR
8.	Durum8	8.	LALMA

Data collection and measurements

Data were recorded on ten metric traits. Number of days to heading were counted from the appearance of first germinated plant till the first spike emergence in a block, and similarly days to maturity from germination to the visible physiological maturity per plot. For the calculation of flag leaf area, the leaf's width and length was measured and the obtained values were put into a formula *i.e.* width× length × 0.75 (Muller, 1991). A one-meter rod was used to measure the plant height starting from the plant base till the apex of the main stem excluding awns. A one-square meter quadrate was used to count productive tillers and respective spikes on per plot basis. Ten randomly selected spikes were collected to count number of spikelets and later those were further hand-threshed to count the number of grains as well. Harvesting of each plot was separately done by using sickle at proper maturity stage which then subjected to get dried under the sunlight. The biological yield (kg ha^{-1}) was estimated by further weighing the sun-dried bundles. After threshing the bundles, two hundred grains of each genotype were separated from each replication and converted to get 1000 grain-weight of each entry. Lastly, an electric balance was used to measure grain yield per plot and later extrapolated to per hectare grain yield for each genotype. A percentage ratio was computed from grain yield and biological yield to get the harvest index of each genotype per replication.

Statistical analysis

Data obtained on all 16 wheat genotypes was statistically analysed with the help of Statistix 8.1 for an RCB Design. To ascertain the differences among wheat genotypes, the analysis of variance (ANOVA) was conducted by following the method explained by Gomez and Gomez (1984). The total sum of squares partitioning to genotypes were further partitioned into two groups *i.e.* durum and spring wheat. A single degree of freedom contrast of durum vs. spring wheat was also computed. Means of the genotypes for all the traits were compared by conducting protected least significant difference (LSD) test (P=0.05). Pearson's correlation (P=0.05, 0.01) among all the traits was also computed for all the genotypes.

Results and Discussion

The analysed results for different crop parameters of 16 wheat genotypes (durum and spring wheat inclusive) are presented as follows:

Days to heading (DH) and maturity (DM)

Days to heading as well as maturity are important traits in wheat breeding programs. ANOVA for both the traits demonstrated significant differences among wheat genotypes for (P= 0.01) and (P=0.05), respectively (Table 1). Among durum as well as spring wheat genotypes, prominent genetic differences existed for days to heading and maturity at 1% probability. Whereas, a single degree of freedom contrast among both wheat types for days to heading was found out to be highly significant (P=0.01) but in the evaluation of days to maturity, the differences computed were non-significant (Table 1). The coefficient of determination (R^2) and coefficient of variation (CV%) for days to heading was 0.88 and 0.94% and in case of days to maturity it was 2.43% and 0.51, respectively (Table 1). The findings of the present study are in an agreement to those found by Anwar *et al.* (2009). Mean performance (Table 2) revealed that on average durum genotypes took two more days to start heading while the mean performance in terms of days to maturity for both spring and durum type wheat was same. For cultivar *Shahkar*, minimum days to heading were recorded *i.e.* 114 days, while, the maximum was achieved by cultivar *Fakhre Sarhad* and *Lassani-08 i.e.* 121 days. On the other hand, same genotype *Shahkar* took least number of days to mature (147 days) in contrast to *Marvi-2k* which took maximum days to maturity (158 days) (Table 4). Plant breeders often strive to breed early heading and late maturing varieties to expand the grain filling time, hence obtaining maximum grain yields. On contrary, in areas of higher pest infestations and climatic constraints, plant breeders prefer early maturing varieties to promote escape strategy.

Flag leaf area (FLA) and plant height (PH)

Flag leaf area is believed to be a significant contributor to the total photosynthetic ability of a plant (Faisal and Tahir, 2014) hence, larger and broader leaves are desirable to get better yields. On the other hand, plant height is also an important trait that is under selection for decades. ANOVA depicted prominently significant genetic variation (P= 0.01) for both plant height and flag leaf area, among wheat genotypes (Table 3). In case of durum genotypes, genetic differences were found to be non-significant in case of plant height but were significant in terms of flag leaf area. On the other hand, the spring wheat genotypes have shown highly significant differences for both the traits. A single degree of freedom contrast has shown significant differences for plant height, but no such differences were detected for flag leaf area. Coefficient of variation (CV%) and coefficient of determination (R^2) for plant height was 5.93% and 0.82 whereas for flag leaf area 10.85% and 0.68, respectively (Table 3). Abinasa *et al.* (2011) have reported similar findings for spring wheat. Mean performance shows that the spring wheat genotypes were 10cm longer than the durum wheat genotypes (Table 4). *Marvi-2k* was the most heighted genotype (108.8 cm) while *Barsat* was the genotype with largest Flag leaf area (40.84 cm^2). Interestingly on the other hand, durum genotype *DWE2* has least average plant height (74.93 cm) while durum genotype *DWE1* had the least flag leaf area (24.22 cm^2). A semi-dwarf genotype is of more breeding interest as it tends to resist lodging in standing wheat crop. On the other hand, a broader flag leaf is an indicator of better yield performance as well (Faisal and Tahir, 2014).

Tillers m^{-2} (T/m^2) and spikes m^{-2} (S/m^2)

Total number of the productive tillers and grain bearing spikes are two principal yield components in wheat and a high count of both the traits is desirable to achieve maximum yield. The analysis of variance (ANOVA) for tillers as well as spikes m^{-2} for the different genotypes revealed highly significant differences (P= 0.01) as shown in Table 3. For tillers and spikes m^{-2} genetic differences among durum wheat genotypes were significant at 1% and 5% probability, respectively. The genetic differences for tillers m^{-2} among spring wheat genotypes were highly significant but no prominent differences have been observed for spikes m^{-2}. Single degree of freedom contrast among durum and spring for tillers and spikes m^{-2} were highly significant (Table 3). The coefficient of variation (CV%) and coefficient of determination (R^2) for tillers m^{-2} was 8.42% and 0.95 while for spikes m^{-2} is 16.49% and 0.80, respectively (Table 3). Mean performance revealed that *Janbaz* had the highest number of tillers as well as spikes per square metre (122.22 and 101.1, respectively). On contrary, durum genotype *DWE 1* had poor performance regarding both the yield contributing traits (44.81 and 40.37, respectively) (Table 4). The findings of the study are in accordance with Fellahi *et al.* (2013) who reported a wider genetic variability in tested spring wheat germplasm for yield contributing traits. A higher yield is expected for those genotypes that have a prominently greater number of productive tillers per plant.

Spikelets per spike (Sp/S) and grain yield (GY)

Total crop yield is influenced by number of spikelets per spike. ANOVA depicted prominently significant differences among the genotypes of wheat for spikelets per spike as well as for grain yield (Table 3). In reference to durum genotypes, the differences were not significant in terms of spikelets per spike, but for grain yield they were significant. Whereas, differences were noted down among spring as well as durum wheat (P=0.05, P=0.01) for both the traits. A similar trend was observed for single degree of freedom contrast among durum and spring wheat genotypes (Table 3). The coefficient of variation (CV%) and coefficient of determination (R^2) for spikelets per spike was 6.71% and 0.63 while for grain yield it was 9.04% and 0.98, respectively (Table 3). Coefficient of variation (CV) gives a measure of population variability and is commonly used to ensure the validity of a field experiment. For yield performance in plant breeding experiments, a CV value of 10 to 15% meets the researcher's expectation while a higher CV value generally disqualifies on trusting the trait values (Daryl, 2001). On the other hand, the co-efficient of determination (R^2) is a more robust index of

Table 3: *Mean squares for 10 metric traits of durum and spring wheat genotypes.*

SOURCE	df	DH	DM	FLA	PH	T/m²	S/m²	Sp/S	TGW	GY	HI
Replication	2	5.02	4.08	10.30	65.37	238.13**	151.39	7.00**	44.54	7146	0.004
Genotype	15	17.77**	28.89*	45.28**	244.98**	1399.74**	930.09**	5.81**	74.49**	2182000**	0.04**
Durum	7	16.23**	20.36**	20.03*	66.73	1077.95**	827.16*	6.86	69.96*	3480000**	0.05**
Spring	7	14.10**	41.12**	70.63**	291.06**	1092.87**	704.81	4.53*	63.27	596132**	0.02
Durum vs Spring	1	54.19**	3.00	2.59	1170.19**	5800.32**	3227.55**	530.68**	199.72*	4364514**	0.04*
Error	30	1.20	13.71	10.56	27.21	37.26	114.32	1.90	29.32	13906.43	0.01
CV%	-	0.94	2.43	10.85	5.93	8.42	16.49	6.71	14.86	9.04	18.92
R^2	-	0.88	0.51	0.68	0.82	0.95	0.80	0.63	0.58	0.98	0.77

,= Significant at 5 and 1% probability level, respectively. CV refers to the percentage of co-efficient of variance. Whereas DH: days to heading; DM: days to maturity; FLA: flag leaf area; PH: plant height; T/m2: tillers/m2; S/m2: spikes/m2; Sp/S: spikelets/spike; TGW: 1000-grains weight; GY: Grain Yield; HI: harvest index.*

Table 4: *Mean performance and least significant differences (0.05) for all 16 genotypes of wheat.*

	Genotype	DH (days)	DM (days)	FLA (cm²)	PH (cm)	T/m² (No.)	S/m² (No.)	Sp/s (No.)	TGW (g)	GY (kg ha⁻¹)	HI (%)
Spring wheat	Fakhre Sarhad	121	151	30.36	87.87	78.78	76.78	21.33	31.82	2337.0	0.39
	Lassani-08	121	153	28.83	82.07	87.00	66.66	19.40	37.09	1885.2	0.39
	Janbaz	116	155	27.92	94.53	122.22	101.10	22.33	47.25	3141.2	0.41
	Barsat	118	154	40.84	96.27	93.33	81.11	20.86	37.90	3203.3	0.53
	Marvi-2k	119	158	27.77	108.80	70.40	68.70	21.53	34.61	2733.9	0.45
	Ass-11	116	155	25.24	104.27	83.96	80.40	18.73	40.94	2987.9	0.50
	Shahkar	114	147	30.52	82.27	62.36	55.93	21.60	37.96	2932.8	0.64
	Lalma	118	148	26.22	87.67	82.07	53.77	21.60	40.16	2911.1	0.52
Mean		117.87	152.62	29.71	92.96	83.49	73.05	20.92	38.47	2766.5	0.48
Durum wheat	DWE1	116	151	24.22	79.93	44.81	40.37	19.80	35.07	1314.8	0.39
	DWE2	119	153	30.21	74.93	56.45	39.63	20.20	32.64	1244.4	0.39
	DWE3	115	154	31.43	82.27	57.04	51.11	17.40	42.44	1566.9	0.41
	DWE4	115	155	30.38	80.93	48.08	46.33	20.20	29.28	1163.9	0.53
	DWE5	117	151	32.52	85.67	60.67	59.60	21.86	34.47	2409.7	0.45
	DWE6	118	148	31.53	87.13	67.78	64.82	19.93	33.50	3007.0	0.50
	DWE7	113	155	27.73	90.20	103.74	90.48	19.40	39.69	4248.7	0.64
	DWE8	112	149	33.38	83.67	62.51	60.93	22.33	28.06	2448.5	0.52
Mean		115.62	152.00	30.17	83.09	62.63	56.65	20.14	34.39	2175.4	0.42
LSD		1.82	6.17	5.41	8.69	10.17	17.82	2.29	9.02	196.64	0.12

DH: days to heading, DM: days to maturity, FLA: flag leaf area, PH: plant height, T/m2: tillers/m2, S/m2: spikes/m2, Sp/S: spikelets/spike, G/S: grains/spike, TGW: 1000-grains weight, GY: Grain Yield, HI harvest index.

Table 5: *Pearson's correlation among 10 different agro-morphological traits of 16 wheat genotypes.*

TRAIT	DM	FLA	PH	T/m²	S/m²	Sp/S	TGW	GY	HI
DH	-0.05	0.07	0.05	0.05	-0.08	-0.05	-0.46	-0.24	-0.34
DM	-	-0.13	0.25	0.16	0.20	-0.18	0.25	0.01	0.01
FLA		-	-0.04	0.05	0.06	0.20	-0.25	0.02	-0.12
PH			-	0.44	0.63**	0.23	0.14	0.54*	0.16
T/m²				-	0.75**	0.14	0.44	0.67**	0.03
S/m²					-	0.16	0.34	0.71**	0.03
Sp/S						-	-0.37	0.17	0.01
TGW							-	0.27	0.14
GY								-	0.65**

,= Significant at 5% and 1% probability level, respectively. Whereas DH: days to heading; DM: days to maturity; FLA: flag leaf area; PH: plant height; T/m²: tillers/m²; S/m²: spikes/m²; Sp/S: spikelets/spike; TGW: 1000-grains weight; GY: grain yield.*

estimating genotype stability introduced by Pinthus (1973). A higher R^2 value indicates favourable responses of genotypes to the environmental variation and hence >70% of R^2 value for a plant trait is valid, and vice versa (Mekbib, 2003; Parviz *et al*., 2015). Mean scores showed that spring wheat genotype *Janbaz* had maximum spikelets per spike (22.33) as well as grain yield of 3141.2 kg ha^{-1}. Whereas, durum genotypes *DWE8* and *DWE7* showed maximum count for spikelets per spike and grain yield, respectively (Table 4). These results confirm the trends found in studies by Singh and Upadhyay (2013). Ramzan *et al*. (1994) have also suggested grain yield as one of the selection criteria for wheat crop. Importantly, grain yield is always a top priority for a plant breeder during population improvement programs. High values for both the traits are appreciated by plant breeders to achieve high yield targets.

1000-grain weight (TGW) and harvest index (HI)

Thousand grain weight and harvest index are two of the highly important yield traits in wheat. TGW is not only influenced by genetic factors but it is highly dependent on environmental factors as well (Hadjichristodoulou, 1990). Larger values for both the traits are expected for wheat genetic improvement programs. ANOVA showed highly significant differences (P= 0.01) among the genotypes of wheat for TGW as well as for harvest index (Table 3). Similar trend has been observed for durum genotypes for both the traits. On contrary, no such genetic differences have been observed for spring wheat genotypes. A single degree of freedom contrast among the two wheat genotypes *i.e.* durum and spring wheat, revealed visibly significant differences for both the traits at 5% probability (Table 3). The coefficient of variation (CV%) and coefficient of determination (R^2) for TGW was 14.86% and 0.58 while for harvest index was 18.92% and 0.77, respectively (Table 3). Mean values indicated that *Janbaz* had maximum TGW of 47.25gm in contrast to durum genotype *DWE8* (28.06 gm). On the other hand, *Shahkar* and *DWE 7* had the maximum harvest index (0.64 %) (Table 4). The findings similar to the current study were reported by Majumder *et al*. (2008). Grain weight in cereal crops compensates for earlier stresses in circumstances where favourable environment predominates during the grain filling period (Evans and Wardlaw, 1976). Therefore, apart from contributing to final yield, thousand grain weight has a role in resilience of cereal crops.

Correlation analysis

Coefficient of correlation refers to the measure of degree of association between two variables. The importance of correlation studies is imperative in plant breeding and ease the plant breeders' job by enabling indirect selections. It also gives an understanding of how the improvement of one trait can bring simultaneous improvement in the other trait as well (Adhikari *et al*., 2018). Pearson's product-moment correlation coefficient (r) values are presented in Table 3. The plant height depicted a visibly positive correlation with spikes m^{-2} (r = 0.63, P= 0.01) and grain yield (r=0.54, P= 0.05). Khan *et al*. (2015) also reported similar findings in terms of plant height and grain yield. Wherever, tillers m^{-2} had significantly positive relationship with spikes m^{-2} (r = 0.75, P= 0.01) and grain yield (r = 0.67, P= 0.01). The mentioned findings are also confirmed by the results of Khan *et al*. (2015). If the number of productive spikes is more, the yield per plant will increase eventually. Spikes m^{-2} were positively associated with grain yield (r = 0.71, P= 0.01). Whereas, grain yield has shown a strongly positive association with harvest index (r= 0.65, P= 0.01). Similarly, positive correlation between the parameters like spikes m^{-2} and grain yield of wheat can also be seen in the findings of Degewione *et al*. (2013), Khan (2013). A non-significant positive association was observed between 1000-grain weight and grain yield (r= 0.27). Similar non-significant correlation between these two traits has been previously reported by Fatih (1986), Yousaf *et al*. (2008), Iftikhar *et al*. (2012) and Puri *et al*. (1982) indicating the presence of positive relation among grain yield and 1000-grain weight.

Conclusions and Recommendations

The results of our experiment have shown a broad range of variability in tested germplasm for all the studied traits, which in turn gives an opportunity to select and/or recommend for better performing genotypes. In the present study, strong positive correlations have been observed among grain yield, spikes m^{-2} and tillers m^{-2}. Harvest index also exhibited a strong positive correlation with grain yield, demonstrating that selecting one will improve the mean for other in a breeding population. Overall, spring wheat genotypes *Janbaz*, *Barsat* and *Shahkar* were best performers in terms of 1000-grain weight, grain yield as well as for harvest index. On the other hand, durum genotypes *DWE3* and *DWE7* outperformed the other genotypes on the basis of 1000-grain weight, grain yield and harvest index. This study concludes that plant traits such as spikes m^{-2} and tillers m^{-2} can be considered as a suitable selection criterion for the development of high yielding spring and durum wheat varieties. It is also recommended that further investigation on multi-location performance, heritability and genetic advance of the identified genotypes could help plant breeders to make efficient breeding decisions that can lead to the development of high-yielding wheat cultivars in the KPK Province of Pakistan.

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Novelty Statement

Very few diversity analyses of *Triticum durum* L. genotypes has been done previously. The increased demand of durum wheat flour for pasta and bakery products is presumably a motivation towards future breeding of tetraploid wheat. Inclusion of advanced lines of durum wheat in diversity panel will provide the basis for novel durum wheat breeding programs in the area.

Author's Contribution

All authors contributed significantly in manuscript preparation and are in complete agreement with the contents of this manuscript. Attiq ur Rehman originally planned and conducted the experiment also drafted the manuscript. Iftikhar Hussain Khalil acquired plant germplasm, supervised the whole study and critically revised the manuscript. Ihtisham Ali assisted in data collection, data handling and data analysis.

Conflict of interest

The authors declared no conflict of interest.

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