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Characterization of bread wheat germplasm for yield traits

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Abstract

High yield in wheat is determined by various yield parameters including number of spikelet per spike, 1000-kernal weight, etc. Thus development of wheat plant containing all the best yield determining parameters in one variety is the ultimate goal of a successful breeding program. This study is aimed at exploring the wheat germplasm to identify wheat genotypes with better yield and yield components contributing towards higher wheat production. A total of 96 diverse wheat genotypes were studied for their morphological parameters which are contributing towards final yield. Genotypes varied in 1000-kernel weight (20.89 to 53 gm), days to flowering (96.33 to 107.50 days) and number of spikelets per spike (7.37 to 23429 spikelets per spike). Analysis of variance exhibited significant differences among mean values of the traits. Least significant difference test showed substantial genetic difference among the 96 genotypes. The present findings suggest that the genotypes investigated have the potential for using in future wheat improvement programs as well as can also be re-sequenced for identifying some novel SNPs which can pave the way for identifying genes and also designing new DNA markers for initiating breeding by design.

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Introduction

Heaxploid wheat, 2n=6x=42,has been grown as staple food crop worldwide. It belongs to the familyPoaceae. The cultivated wheat is a polyploids containing three different genomes in a nucleus. Each set has basic set of chromosome, i.e. 2n=2x-14. Triticumaestivum contains A, B and D sub-genomes, A-genome has been derived from *T. urartu* and B-sub-genome from *Aegilopsspeltoides*, whereas the D-genome has been donated by *Ae. Tauschii* (Ling *et al.,* 2013).

Genetic diversity in wheat is limited as has been indicated in several studies (Mukhtar *et al.,* 2002) that can be used for improving resistance to stresses. For using the diverse genetic resources, it is vital to characterize these resources for planning the future breeding program. In this regard, germplasm has been extensively screened in various agro-climatic zone for studying the response of various traits (Croston and Williams, 1991).

The traits studies were tiller number per plant, 1000-Kernel weight (TKW), time of flowering (ToF), days to heading, spike length (SL), etc. (Zhou *et al.,* 2017).

In several other studies, germplasm has been characterized in several parts of the world, for example, Panjsher valley in Afghanistan (Buerkert *et al.,* 2006), Oman wheat landraces (Al-Maskri *et al.,* 2003) or European winter wheat landraces (Dotlacil *et al.,* 2002). Number of spikes per plant are closely related to tiller number per plant, which determine grain yield in hexaploidwheat (Zanke *et al.,* 2015). Another component that contributes directly and indirectly towards grain weight in wheat is the chlorophyll pigment. More chlorophyll contents will lead to lush green shoots and in turn produce more food, which will ultimately be stored in grain as starch. Wheat varieties with higher grain weight and chlorophyll contents were identified for using in breeding program (Chang *et al.,* 2015).

Grain weight has been found to be an important agronomic character that is usually measured by 1000 kernel weight, and is being utilized for selecting best wheat genotypes (Wu *et al.,* 2018). Relatively big size seed usually has positive impact on on seedling growth and vigour (Botwright *et al.,* 2002). Total grain weight is controlled by alleles mapped on different chromosomes in elite common wheat lines.

Analysis of the grain weight phenotype identified frequently co-segregating alleles with larger grainare genetically and functionally linked in controlling grain weight in cereals (Zhang *et al.,* 2013). Linkage mapping detected a strong association of TKW in a cloned rice orthologue of wheat and its haplotype variants were detected on the basis of gene expression and association analysis (Zhang *et al.,* 2012).Spikelets per spike isanother important yield parameter that can be used as a selection criterion for selecting what lines with high genetic potential (Knezevic*et al.,* 2012). This agronomic character also varies in different genotypes. Number of grains in an ear varies from 70-80 and the number of spikes in bread wheat range from 6-9 in different cultivars (www.researchgate.net).

To achieve the maximum yield potential from crops in any environment, the cultivars should reach maturity within least number of day's duration (Snape *et al.,* 2001). Variation in flowering time is reported to cause yield fluctuations in wheat genotypes (Ginkel *et al.,* 1998). To study variation in morphological yield parameters like TKW, number of spiklets per spike and days to flowering in different genotypes is the ultimate goal of wheat breeding program.

Materials and methods

The present investigation was carried out at the Plant Genomic & Molecular Breeding Lab (PGMB), Agricultural Biotechnology Division (ABD), and National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad Pakistan.

Plant material consisted of 96 wheat genotypes for dissecting the phenotypic traits. A total number of 185 wheat genotypes were sownat Farms of NIBGE Faisalabad in 2011 by adopting standard agronomic practices.

The plot size was 100 sqft (5 rows 20 ft long). We selected 96 genotypes for morphological studies. Hereafter, all genotypes/accessions/cultivated varieties/obsolete varieties will be denoted by genotypes throughout the document (Table 1).

All the genotypes were sown for three normal wheat growing seasons (2011–2013) to characterize various traits. For this, we selected a uniform piece of landfor managing these trials for avoiding any type of experimental error. All these trials were arranged in a randomized complete block design (RCBD). Each of the experiment was arranged in two replications of 37 incomplete blocks containing five entries (genotypes) in each block.

The randomization was undertaken using alpha program and the plot size for each entry was kept 21m2 (6 rows4ft long with plant to plant distance 7.5 cm). The sowing was largely done in between November 15 to 30 each year by using hand drill. The row to-row distance was kept 30 cm apart. The trial was provided with adequate number of irrigations throughout the season.

All sets of experiments conducted each year were provided with uniform dose of fertilizer, for instance, 50kg ha−1 of NPK at the time of sowing followed by 50kg ha−1 of N application each at booting and grain formation stage. Also, hoeing was done for controlling weed infestations. While chemicals were not sprayed for controlling the aphid. All other agronomic practices were applied evenly on all experiments conducted for three consecutive years (2011–2013).

Measurements of morphological data

Flowering date

At flowering stage, the date wererecorded when 50% of the plants in each entry started anthesis started. The days to flowering was noted for all the studied wheat genotypes.

Number of spikelets per spike

At maturity, number of spikelets (NSS) from each tagged spike (taken the average of five in each entry) were counted. Average number of spikelets were calculated for analysis of means and further data interpretations.

Thousand kernel weight (TKW)

The TKW was measured from 1000 healthy kernels of each genotype. 1000-healthy grains were placed on weighing balance and data for TKW was recorded. Means were computed for further data analysis.

Phenotypic data analysis

Test statistics were used to compute the values for each parameter against averages/means, standard deviation (S.D) for each value and standard error (S.E) of means, Coefficient of variation (C.V) was calculated for each Analysis of Variance (ANOVA) table against each parameter. Critical difference for comparison among means was done to find out the variation and differences in genotypes for each selected trait using Statistix 8.1 software.

Results

Genetic diversity for various traits in wheat genotypes

Days to flowering

This trait was measured from the day anthesis started i.e. when yellowish anthers protruded out of the glume and could be seen easily. Data pertaining to the days-to-flowering was collected from 96 wheat genotypes. Highly significant genetic differences for number of days to flower were observed among all the tested genotypes. Analysis of variance exhibited highly significant differences for means of days to flowering and its interaction over three years. Split plot design was used to analyze the seasonal effect of three years data as per assumptions of the experimental design. Highest number of days to flowering were 107.50 days while the lowest were 96.33 days (Table 1). Results from the ANOVA data was further utilized to test the individual differences among all the genotypes for means of days to flowering through LSD. Dissection of the genetic differences in flowering days revealed 71 groups (A, B, etc.) where non-significant differences in the means were found (Table 2).

Table 1. Genotypic differences among wheat genotypes for days to flowering.

Genotypes	Means	Scoring/Lettering
Syn-7-24	107.50	A
Syn-7-4-1	107.50	A
Syn-7-55	107.00	AB
Syn-3-19	106.67	ABC
Syn-7-75	106.17	BCD
Syn-6-14	106.00	BCDE
Syn-7-31	105.83	CDEF
Syn-7-39	105.67	CDEFG
Syn-6-2-1	105.50	DEFGH
Syn-7-21	105.50	DEFGH
Syn-3-3-2	105.50	DEFGH
KHYBER-79	105.00	EFGHI
Syn-3-9	104.83	FGHI
Syn-3-35	104.67	GHI
Syn-6-73	104.50	HIJ
Syn-3-17	104.33	IJK
Syn-3-3	104.00	IJKL
Syn-7-50-1	103.50	JKLM
Syn-7-5	103.33	KLMN
POTHWAR-93	103.00	LMNO
RHYBER-87	102.67	MNOP
Syn-7-30-2	102.50	MNOP
Syn-7-47	102.50	MNOP
NIBGE 5	102.50	MNOP
Syn-7-65	102.33	NOP
Syn-7-22	102.33	NOP
Syn-3-40	102.33	NOP
NIBGE 4	102.33	NOP
Syn-7-46	102.17	OPQ
NURI-70	102.17	OPQ
Syn-3-32	102.17	OPQ
NR-214	102.00	OPQR
Syn-7-74	101.83	PQRS
NN 1	101.83	PQRS
NN 2	101.83	PQRS
Syn-6-2	101.17	QRST
Syn-7-41	101.17	QRST
SHAHKAR-95	101.17	QRST
MARGALLA-99	101.00	RSTU
FBD-85	101.00	RSTU
CYP-73	101.00	RSTU
Syn-7-32	101.00	RSTU
SINDH-81	100.83	STUV
KOHSAR-95	100.83	STUV
TANDOJAM-83	100.83	STUV
SULTAJ-86	100.67	TUVW
INDUS-79	100.67	TUVW
ROHISTAN-97	100.50	TUVWX
NOWSHERA-96	100.50	TUVWX
NR-228	100.50	TUVWX
MH-97	100.50	TUVWX
C-591	100.50	TUVWX
CH-86	100.50	TUVWX
PUNJAB-85	100.33	TUVWX
IQBAL-2000	100.33	TUVWX
CHENAB-79	100.33	TUVWX
BHWP-79	100.33	TUVWX

LU-26	100.17	TUVWX
FPD-83	100.17	TUVWX
PARWIAZ-94	100.00	UVWXY
DIRK	100.00	UVWXY
GA-2002	100.00	UVWXY
C-518	100.00	UVWXY
MARVI-2000	99.83	VWXYZ
CHENAB-70	99.83	VWXYZ
PIRSABIR-91	99.83	VWXYZ
MARWAT-JOL	99.67	WXYZa
NR-204	99.67	WXYZa
NR-212	99.67	WXYZa
CHENAB-2000	99.67	WXYZa
ARZ	99.67	WXYZa
Syn-6-11	99.67	WXYZa
SONAURA	99.50	XYZab
ROHTAS-90	99.50	XYZab
SANDAL	99.50	XYZab
NR-150	99.50	XYZab
B-SILVER	99.50	XYZab
ANMOL-91	99.50	XYZab
BARANI 70	99.50	XYZab
SALEEM-2000	99.00	YZabc
PUNJAB-81	99.00	YZabc
NR-180	99.00	YZabc
MEHRAN-88	99.00	YZabc
C-271	99.00	YZabc
SARSABZ	98.83	Zabc
SHALIMAR-88	98.67	Abcd
NR-230	98.67	Abcd
KAGHAN-93	98.67	Abcd
C-288	98.67	Abcd
BARANI-83	98.67	Abcd
NR-232	98.50	Bcd
NR-235	98.17	Cd
PAK-81	98.00	Cd
AUQAB-2000	98.00	Cd
Syn-2-6	97.67	D
PARI-73	96.33	E

Number of spikelets per spike

Number of spikeletsper spike was calculated by counting spikeletsof each spike. Significant genotypic differences were observed among all the wheat genotypes grown for this trait. The results from ANOVA showed significant differences in the means for number of spikelets per spike among the studied genotypes (Table 3). Maximum number of spikelets per spike were23.429 while least number of spikelets were counted as 7.374 (Table 4). Results from ANOVA were utilized further for finding the individual differences among the genotypes. LSD test revealed 56 groups (A, B, etc.) where non-significant differences among the means were found in all tested genotypes.

Thousand Kernel Weight

In total, 96 wheat genotypes were studied for various traits including TKW. At maturity, wheat seed were threshed from each of the genotype. In total, 1000 seed were counted and weighed using a balance. Two replications were used to analyze the genetic variation in grain weight. Analysis of variance depicted highly significant differences in grain 1000-weight in all the tested genotypes (Table 5). Analysis of means exhibited a wide range for this trait, i.e. lowest TKW was 20.894 while the maximum TKW was 53.002 gm with an average value 32.108 gm (Table 6). Results from the analysis of variance were further used to investigate the individual differences among the genotypes.

Table 2. Analysis of days to flowering using analysis of variance (ANOVA). The split plot design (Multifactorial) is used for finding the variation.

Source	DF	SS	MS	F	P
V003	1	13.44	13.44		
Genotypes (G)	95	3450.31	36.32	40.33	0.0000
Error (G × R)	95	85.56	0.90		
Year	2	3277.19	1638.60	1829.13	0.0000
Genotype × Year	190	1924.81	10.13	11.31	0.0000
Error G × Y × R	192	172.00	0.90		
Total	575	8923.31			

Grand Mean 101.28.

Coefficient of variation (G × R) 0.94.

Coefficient of variation (G × Y × Y) 0.93.

Table 3. Analysis of no. of spikelets per spike using analysis of variance (ANOVA). The split plot design (Multifactorial) is used for finding the variation.

Source	DF	SS	MS	F	P
V003	1	13.44	13.44		
Genotypes (G)	95	3450.31	36.32	40.33	0.0000
Error (G × R)	95	85.56	0.90		
Year	2	3277.19	1638.60	1829.13	0.0000
Genotype × Year	190	1924.81	10.13	11.31	0.0000
Error G × Y × R	192	172.00	0.90		
Total	575	8923.31			

Grand Mean 101.28.

Coefficient of variation (G × R) 0.94.

Coefficient of variation (G × Y × Y) 0.93.

Analysis of 1000-kernal weight using least significant differences (LSD) test showed substantial genetic differences among all the tested genotypes.

Discussion

Number of spikelets on each spike

Generally, the main goal of all plant breeding program revolves around selecting the productive plants from genetic material displaying high genotypic stability for trait of interest in multiple environments (Litrico and Violle, 2015). Number of spikelets on each spike displayed significant variations among all the wheat genotypes investigated in the present study. Significant genetic differences were observed among the measns of number of spikelets per spike which ranged from 7.374 to 23.429. Mean number of spikelets per spike is associated with the tiller number per plant and therefore contribute in yield per plant too.

On the other hand, plant with fewer tillers and spikelets per spike showed greater seed weight owing to their better food assimilation and grain filling. Considerable genetic diversity has been demonstrated for multiple traits in the selected wheat genotypes. Number of traits including PH, number of tillers per plant, TKW, spikelets per spike, days to maturity and days to flowering showed substantial diversity among the wheat genotypes (Murad *et al.,* 2019). Substantially higher genomic prediction accuracy than QTL-based prediction has been reported in smaller populations too (Norman *et al.,* 2017). Recent

studies utilizing the genetic and phenotypic analysis of wheat spike and kernel traits exhibited geographic patterns as well as long-term trends that arise from breeding progress, especially in context to the fertility of spikelet, for instance number of kernel/spikelet, one of the components of determining final grain yield. Furthermore, the quantitative inheritance of traits contributes additionally by small-effect QTL which is substantiated by the genomic prediction (Wurschum *et al.,* 2018).

Table 3. Analysis of no. of spikelets per spike using analysis of variance (ANOVA). The split plot design (Multifactorial) is used for finding the variation.

Source	DF	SS	MS	F	P
V003	1	13.44	13.44		
Genotypes (G)	95	3450.31	36.32	40.33	0.0000
Error (G × R)	95	85.56	0.90		
Year	2	3277.19	1638.60	1829.13	0.0000
Genotype × Year	190	1924.81	10.13	11.31	0.0000
Error G × Y × R	192	172.00	0.90		
Total	575	8923.31			

Grand Mean 101.28.
Coefficient of variation (G × R) 0.94.
Coefficient of variation (G × Y × Y) 0.93.

Table 5. Analysis of TKW using analysis of variance (ANOVA). The split plot design (Multifactorial) is used for finding the variation.

Source	DF	SS	MS	F	P
V003	1	49.6	49.565		
Genotypes (G)	95	25174.4	264.994	2557.60	0.0000
Error (G × R)	95	9.8	0.104		
Year	2	151.4	75.720	34.70	0.0000
Genotype × Year	190	975.0	5.132	2.35	0.0000
Error G × Y × R	192	418.9	5.132		
Total	575	26779.2			

Grand Mean 36.431.
Coefficient of variation (G × R) 0.88.
Coefficient of variation (G × Y × Y) 4.05.

Thousand kernel weight

Grain weight showed variation among selected wheat genotypes which ranged from 20.894 gm to 53.002 gm per 1000-Kernel.

The differences in grain weight can be directly linked to grain filling and the expansion of cells by uptake of water (Cui *et al.,* 2011). The genotypes having better root growth, root biomass and water uptake are better in grain filling and gaining grain weight. Different parameters like carpel weight at anthesis, individual floret anthesis date and duration of grain filling have been studied to elucidate their impact on TKW (Baillot *et al.,* 2018). Li *et al.,* (2016) have studied that the different positions of spikelets and grain in ear aeffect the grain weight and number of grains.

Their results showed that the position of spikelets and grain effect the grain weight varied with the number of grains in spikelets.

Table 6. Wheat genotypes tested for genetic diversity in grain weight of all the tested wheat genotypes.

Genotypes	Means	Scoring/Lettering
Syn-7-74	53.002	A
Syn-3-19	51.452	B
Syn-7-41	50.592	C
Syn-3-3	48.935	D
Syn-7-30-2	47.965	E
Syn-7-55	47.618	EF
Syn-6-2-1	47.468	FG
Syn-7-32	47.202	GH
Syn-7-4-1	47.138	GH
Syn-7-24	47.072	H
Syn-6-14	46.698	I
NN 2	45.333	J
NIBGE 5	45.333	J
Syn-7-47	45.295	J
NIBGE 4	45.167	J
Syn-7-65	44.622	K
Syn-7-50-1	44.382	K
Syn-2-6	43.742	L
Syn-3-17	43.128	M
Syn-7-31	42.545	N
Syn-6-2	42.378	N
NN 1	42.317	N
PARWIAZ-94	41.789	O
Syn-7-46	40.708	P
Syn-6-73	40.608	P
Syn-3-3-2	39.942	Q
AUQAB-2000	39.382	R
ROHISTAN-97	38.957	S
Syn-7-21	38.718	ST
BARANI 70	38.715	ST
NR-230	38.466	T
NR-228	37.891	U
NR-235	37.887	U
PAK-81	37.518	V
NR-232	37.157	VW
ANMOL-91	37.034	WX
Syn-3-40	37.028	WX
SHALIMAR-88	36.850	WXY
C-271	36.850	WXY
TANDOJAM-83	36.790	WXY
PIRSABIR-91	36.677	XYZ
LU-26	36.639	YZ

POTHWAR-93	36.577	YZa
CHENAB-70	36.570	YZa
Syn-3-9	36.335	Zab
SANDAL	36.243	ab
C-288	36.038	b
NR-204	35.375	c
NOWSHERA-96	34.929	d
SALEEM-2000	34.897	d
MARGALLA-99	34.816	de
Syn-7-5	34.725	de
PUNJAB-81	34.522	e
ARZ	33.999	f
NR-180	33.960	fg
MARWAT-JOL	33.922	fg
KHYBER-79	33.884	fg
PUNJAB-85	33.737	fg
FPD-83	33.617	gh
BHWP-79	33.299	hi
MARVI-2000	33.152	ij
ROHTAS-90	33.123	ij
PARI-73	32.884	j
NR-214	32.414	k
CYP-73	32.374	kl
NR-150	32.248	klm
B-SILVER	32.052	klmn
BARANI-83	32.027	lmn
CHENAB-79	31.926	mno
SULTAJ-86	31.810	nop
MEHRAN-88	31.804	nop
C-518	31.702	nopq
SARSABZ	31.597	opqr
MH-97	31.587	opqr
KOHSAR-95	31.482	pqrs
Syn-7-75	31.368	qrst
Syn-3-35	31.277	rstu
RHYBER-87	31.258	rstu
Syn-7-22	31.132	stuv
Syn-6-11	31.022	tuv
SONAURA	30.927	uvw
Syn-7-39	30.795	vwx
Syn-3-32	30.595	wxy
IQBAL-2000	30.519	xy
SHAHKAR-95	30.377	y
CHENAB-2000	29.891	z

INDUS-79	28.343	A
GA-2002	27.882	B
KAGHAN-93	27.768	B
C-591	27.643	B
CH-86	27.036	C
NR-212	26.866	C
NURI-70	25.913	D
SINDH-81	25.870	D
FBD-85	25.316	E
DIRK	20.894	F

Days to flowering

Genetic diversity was also observed in days to flowering in investigated wheat genotypes, ranging from as early as 96 days to 107 days as mean days to flower. Significant differences were observed (alpha = 5 % and standard error for comparison 0.5479) at critical T value of 1.985 (critical value for comparison =1.0877) in tested wheat genotypes. Genes are known in wheat that are responsible for variation in flowering time. Mutations in *Ppd-1* genes loci is reported to cause early flowering and the alleles of three *Ppd-1* genes have been identified and reported to cause different levels of photoperiod insensitivity (Mohler *et al.,* 2004; Beales *et al.,* 2007; Wilhelm *et al.,* 2009). A number of QTLs studies in wheat have been carried out for days to heading (Hanocq *et al.,* 2004; Bogard *et al.,* 2011; Carter *et al.,* 2011). Days to heading also varies in different environments. It is reported that days to flowering varied from 5 to 9 days for a wheat population grown in 10 different environments (Bogard *et al.,* 2011).

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