

**Assessment of high yielding local Pakistani bread wheat genotypes for improved chapati making quality****Jabeen Farheen *, Farzana Nasir Naqvi**Department of Genetics, KU-Circular Road, Faculty of Science, University of Karachi, Main University Road, Karachi-75270, Sindh, Pakistan
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ABSTRACT

The experiment was conducted to classify the maximum glutenin protein possessed Pakistani bread wheat genotype for superlative chapati making quality by ten yield-related parameters. The studied germplasm was acquired from NARC, Pakistan, and planted in randomized-complete-block-design with four replicates at the screen house of the Genetics Department. Data were assessed via Duncan's test, correlation analysis, SDS-PAGE, and cluster analysis. Duncan's test conceded that Pirsabak-85 had the highest plant height, flag leaf area, biomass, grain yield plant⁻¹, harvest index, and protein content. While, the correlation studies showed that plant height, tillers plant⁻¹ ($r = 0.649$), fertile tillers plant⁻¹ ($r = 0.713$), biomass ($r = 0.861$), spike length (LS), thousand-grain weight and harvest index had a positive higher significant association with grain yield plant⁻¹. The SDS-PAGE analysis resolved 30 diverse high and low molecular weight bands, ranging from 200 kDa to 28 kDa glutenin subunits. Among genotypes, Pirsabak-85 showed maximum protein content and 10 Glu-1 scores. The dendrogram analysis revealed that Pirsabak-85 associated with cluster-II, which was a major and most diverged cluster. The Pirsabak-85 can be utilized to enhance bread wheat production and better chapati making quality.

Keywords: Cluster analysis, correlation analysis, genetic variability, SDS-PAGE, *Triticum aestivum*, glutenin protein.

INTRODUCTION: Bread wheat is the highly nutritive and most valuable source that covers 36% food requirement of the earth's people (Faisal *et al.*, 2017). Its seeds contain 20% calories and 20% protein, which made wheat crop as the major cereal in the world (Khan *et al.*, 2017). After the green revolution, wheat grain yield has been enhanced but not as much as the world population, and demand for protein-rich food is increased (Ward *et al.*, 2019). Pakistan is the 7th major wheat-producing country in the world which produces 25.5 million metric tons (FAO, 2019). Its more than a hundred wheat genotypes have great genetic variability, but their chapati making potential is not carefully examined that needs further research which in turn steadily improves the grain quality. Improvement in yield of wheat and its grain quality is most important for every country's economy; but traits are very complicated which are directly and indirectly influenced by the number of genes, environment, and their interactions (Qaseem *et al.*, 2017). Therefore, the development of high yielded bread wheat with good quality is a challenging task (Kaya and Akcura, 2014). The previous literature revealed that the major endosperm gluten protein is closely associated with better dough quality of wheat (Kaya and Akcura, 2014). The gluten protein accumulates and builds a network during the initial stage of growth (Baslar *et al.*, 2012). It consists of glutenin, gliadins, and two prolamines groups. Glutenin composed of 10% high-molecular-weight (HMW) and 40% low-molecular-weight (LMW) glutenin subunits (GSs) that accounts for 40-50% of total bread flour storage protein (Kaya and Akcura, 2014). The HMW-GSs composed of Glutenin-A1, B1, and D1 that are encoded by Glu-A1, Glu-B1 and Glu-D1 loci of chromosomes 1A, 1B and 1D respectively (Payne and Lawrence, 1983; Payne *et al.*, 1984). Similarly, LMW-Glutenin subunits are encoded by Glu-A3, Glu-B3, and Glu-D3 loci (Gupta and Shepherd, 1990).

OBJECTIVES: In short, the dough making quality information through agro-morphological traits and qualitative analysis can play a key role to embellish the effectiveness of crop breeding programs for getting maximum quality production in minimum time and expense. Thus, through yield-related parameters, breeders can enhance acre⁻¹ wheat chapati making quality. In the view of the importance of grain yield and enhance chapati making quality; the present research finding was conducted to evaluate and screen potential high yielded genotypes for improved dough quality in the upcoming bread wheat breeding program under the irrigated condition of Pakistan.

MATERIALS AND METHODS: **Experimental germplasm:** The study material comprised of fourteen local *T. aestivum* genotypes in which Abadgar, Bhattai, Bakhtawar-94, Fareed-06, Khirman, Khyber-87, Kiran-95, Margalla-99 Pirsabak-85, Pirsabak-91, Saher-06, Tatara, TD-I, and TJ-83 were acquired from National Agricultural Research Centre (NARC), Islamabad, Pakistan (table 1).

Experimental design: The study was carried out in the screen house of the Department of Genetics, Faculty of Science, and University of Karachi, Pakistan. The earthen pot contained 9 kg of soil that was prepared by mixing manure and sandy loam in equal quantity. Then earthen pots of 40 x 40 cm size were placed in the well-constructed iron road base screen house where no blockage of air and sunlight (figure 1). The seeds (five seeds pot⁻¹ replication⁻¹) were sown manually in each pot with a space of two inches apart and one inch in depth. Water was supplied every alternative day and Urea provided as Nitrogen source at the stage of tillering. The Malathion spray and weeding was done by hand whenever needed.

All the uniform standard plant protection and agronomic practice were applied for the entire experimental unit during

Code	Names	Pedigree	Origin
A	Abadgar	Yaktana 54 x Norin 10-Bervor x Son64	Sindh
Bhi	Bhittai	VEE/TRAP/1 SOGHOT-90	Sindh
Bak	Bakhtawar-94	KAUZ"S" not known	NWFP
F	Fareed-06	not known	NWFP
Khi	Khairman	SI-91195	Sindh
Khy	Khyber-87	CM43903-H-4Y-1M-1Y-3M-2Y-0B	NWFP
Kir	Kiran-95	WL711 x Crow 'S' (Mexican origin)	Sindh
M	Margalla-99	not known	NWFP
P85	Pirsabak-85	CM33027-F-15M-4Y-4M-2Y-1M	NWFP
P91	Pirsabak-91	FR2208-7F-1F-0F	NWFP
S	Saher-06	Not known	NWFP
Ta	Tatara	Not known	NWFP
TD	TD-1	H68 x MIAS x Norteno	Sindh
TJ	TJ-83	CMS287-J-IY-2M-2Y-3M-0Y	Sindh

Table 1: List of local Pakistani bread wheat genotypes with their pedigree.



Figure 1: View of 14 local Pakistani bread wheat genotypes at the screen house of the Department of Genetics, University of Karachi.

the two years of the study. After physiological maturity, the height of plants (PH) was measured from the base to the tip of the main tiller's spike exclusive of awns via measuring tape. The number of tillers and fertile tillers plant⁻¹ were counted and used the average for statistical analyses. Flag leaf length (FLL) was taken of all the spikes of plant in centimeters (cm) from the beginning of ligula to the edge of the flag leaf tip whereas flag leaf broadness (FLB) was noted at the mid from the widest part of the flag leaf in cm then flag leaf area (FLA) was calculated (Simpson, 1968) i.e.

$$FLA = FLL \times FLB \times 0.75 \quad (1)$$

After calculating all plant's FLA, the average FLA was taken out and used for ANOVA analyses. After harvesting crop, whole plant weight was taken in grams (g) using Kern D-72458 electronic balance, Germany. The spikes of the plant were measured at the base of the last spikelets to the apex of the first

spikelets excluding awns then average mean was worked out for spike length (LS). For grain yield plant⁻¹, total seeds from each plant were weighted on Sartorius GMBH Electronic Balance, Germany separately while, thousand-grain weight (TGW) and harvest index were computed via following formulae;

$$TGW = 10 \text{ seeds weight} \times 100 \quad (2)$$

$$HI = \frac{\text{Total weight of seeds / plant}}{\text{Fresh biomass}} \times 100 \quad (3)$$

Protein Profiling and Interpretation of Bands: The total protein content was quantified (Lowry *et al.*, 1951), and SDS-PAGE for glutenin proteins was assayed on 4% stacking and 10% resolving gels (Farheen and Mansoor, 2020) via Owl P8Ds separation system, Germany. The glutenin subunits pattern of each genotype was identified by using the nomenclature (Payne and Lawrence, 1983). The allelic diversity and *Glu-1* score were calculated as detailed Payne (1987) and Nei (1973).

Statistical design: The pot study was planned in RCBD having four replications. All morphological traits were analyzed through multivariate analysis of variance (MANOVA) where varieties were the dependent variable and morphological parameters were independent variable at 0.01% probability levels and means were compared via Duncan's Multiple Range test (Steel and Torrie, 1986) and Pearson Correlation Coefficient (r). The protein bands data of fourteen local wheat genotypes were triplicated and analyzed through analysis of variance at 0.05% probability level thereat, the protein bands diversity, and their molecular weights were computed on excel-2016 and the cluster analysis was constructed by SPSS v.19.

RESULTS AND DISCUSSION: Plant height:

Agromorphological characterization is the critical step to screening diversity in any genetic material for improving grain yield. Grain yield is the outcome of several growths and physiological process occur during the development of wheat plants (Guendouz *et al.*, 2014). In which, PH is a very critical parameter in terms of lodging resistance. It is evident from table 2 that the plant height has a great coefficient of variability (11.3%), among these Pirsabak-85 (85 cm) and Khyber (82 cm) attained maximum PH while, variety Fareed (65.53 cm), Bakhtawar (68.43 cm) and TD-1 (68.21 cm) attained minimum PH (figure 2).

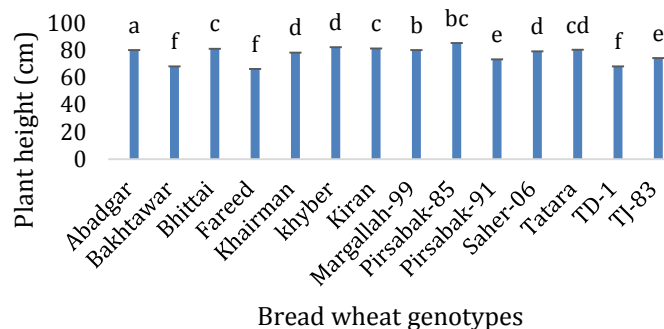


Figure 2: Performance of 14 local Pakistani bread wheat genotypes for plant height under irrigated condition. Duncan's test values are significant at 1% significance level that is indicated in letters ^{a-f} on each standard error bar (n=4).

This difference among genotypes for PH was attributed due to their diverse genetic makeup. Similar findings were noted by the previous researcher (Sultana *et al.*, 2013; Bibi *et al.*, 2017). Khan *et al.* (2013), also observed that tall plants gave more

numbers of wheat kernels and showed high yield. It may be due to the longer period available for the accumulation of photosynthetic assimilates in the grains which enhance the number, size, and weight of seed (Khan *et al.*, 2013). These findings best met with this study results, where Pirsabak-85 plants gave higher GYP values. The data of table 3 revealed that the height of the bread wheat plant was more significant and positively associated with FLA, BIO, LS, GYP, and PC ($r = 0.640$) while significant with TGW (table 3). These findings were agreed with the earlier work of Bibi *et al.* (2017), Qaseem *et al.* (2017), and Ward *et al.* (2019). Bibi *et al.* (2017), suggested that

the PH, TP, and FLA are the yield-related parameters due to their positive association with grain yield plant.

Total tillers plant⁻¹: The tillering ability in the genotypes is the interplay of genes and environment which showed higher genetic variations (Behmanesh *et al.*, 2013; Bibi *et al.*, 2017). Our results indicated a higher variability of about 41% among studied Pakistani genotypes (table 2). The DMR test of studied genotypes revealed that TD-1 (4.25), Pirsabak-85 (4.20), and Khyber (4.14) produced maximum total tillers plant⁻¹, where genotype Fareed (2.44) gave a few numbers of tillers plant⁻¹ (figure 3).

SV	Df	PH	TP	FTP	FLA	BIO	LS	TGW	HI	GYP	PC
Geno	13	2.9**	0.8**	0.6**	2.1**	0.8**	6.2**	2.0**	2.6**	1.2**	5.0**
CV%		11.3	40.8	24.1	2.5	39.0	10.2	11.8	29.7	12.0	1.3

Table 2: Mean squares and their significance of ten agro-morphological traits of 14 local Pakistani bread wheat genotypes

*= P<0.05, **=P<0.01

SV = Sources of variations, Geno = Genotypes, CV = Coefficient of variability, Df = Degree of freedom, PH = Plant height, TP = Tillers plant⁻¹, FTP = Fertile tillers plant⁻¹, FLA = Flag leaf area, BIO = Biomass, LS = Length of Spike, TGW = Thousand-grains weight, HI = Harvest index, GYP = Grain yield plant⁻¹, PC = Protein content.

	PH	TP	FTP	FLA	BIO	LS	TGW	HI	GYP	PC
PH	1	0.112	0.121	0.318**	0.299**	0.464**	0.235*	-0.153	0.246**	0.640**
TP		1	0.860**	-0.078	0.754**	0.168	0.219*	-0.118	0.649**	0.171
FTP			1	-0.094	0.807**	0.135	0.169	-0.099	0.713**	0.138
FLA				1	0.139	0.477**	-0.076	-0.042	0.153	0.294**
BIO					1	0.311**	0.156	-0.177	0.861**	0.307**
LS						1	-0.082	-0.101	0.252**	0.554**
TGW							1	0.065	0.226*	0.900**
HI								1	0.268**	0.340**
GYP									1	0.532**
PC										1

Table 3: Correlation of yield-related traits of 14 local Pakistani bread wheat genotypes for improved chapati quality.

*= P<0.05, **=P<0.01

PH = Plant height, TP = Tillers plant⁻¹, FTP = Fertile tillers plant⁻¹, FLA = Flag leaf area, BIO = Biomass, LS = Length of Spike, TGW = Thousand-grain weight, HI = Harvest index, GYP = Grain yield plant⁻¹, PC = Protein content.

In the case of coefficient of correlation, FTP ($r = 0.860$), BIO ($r = 0.754$) and GYP ($r = 0.649$) were highly significant while, TGW was positive and significantly associated with TP (table 3). The more fertile tillers in the plant increase the overall biomass of the plant. While, the non-significant relationship with FLA, LS, and HI maybe the presence of infertile spikes. These findings are supported by the outcomes of Qaseem *et al.* (2017). Behmanesh *et al.* (2013), also showed a high correlation for BIO, and GYP with TP that was similar to the current experiment. The greater number of tillers in the genotype enhances the chances of the presence of distal florets that elevate the number of grains which untimely increase crop yield (Slafer *et al.*, 1996).

significant at 1% significance level that is indicated in letters a-f on each standard error bar (n=4).

Fertile tillers plant⁻¹: Data analysis exhibited that there were higher significant differences among 14 Pakistani genotypes in producing fertile tillers plant⁻¹ with 24% of the coefficient of variability (CV) (table 2). The highest number of fertile tillers plant⁻¹ was produced by TD-1 (4 FTP), Khyber, and Pirsabak-85 (3.4 FTP), whereas Fareed (2.11 FTP) had lesser FTP (figure 3). The number of fertile tillers plant⁻¹ exhibited positive and significant relationship with BIO ($r = 0.807$) and GYP ($r = 0.713$) at 1% probability level whereas, rest of the studied traits were non-significant with fertile tillers plant⁻¹ (table 3). These experimental results were according to the Behmanesh *et al.* (2013) who worked on 13 advanced endemic durum genotypes. Also Sultana *et al.* (2013), observed the higher significant positive correlation between FTP and GYP. Thus, fertile tillers are the important constituent of the crop grain yield. It is directly linked with biomass and grains plant⁻¹. Therefore, greater numbers of FTP will ensure a higher yield of the wheat crop. Whereas at drought and salinity the growth of tillers is greatly affected due to the lesser moisture accessibility (Sultana *et al.*, 2013; Khan *et al.*, 2017). Hence, selection depends on higher FTP exhibiting genotypes will help to improve grain yield in *T. aestivum*.

Flag leaf area: FLA is one of the major physiological

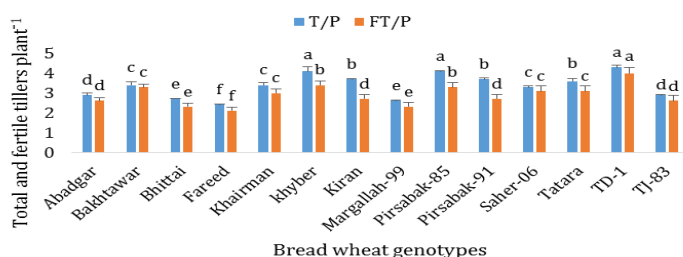


Figure 3: Performance of 14 local Pakistani bread wheat genotypes for total tiller plant⁻¹ (T/P) and fertile tillers plant⁻¹ (FT/P) under irrigated condition. Duncan's test values are

parameters in the selection of high yielded wheat varieties (Sultana *et al.*, 2013). The greater FLA provides the maximum area for photosynthesis within the leaf that afterward translocated in seeds and stored, as a result, the weight of grains will increase. Figure 4 indicated that the maximum leaf area possessed by genotypes Pirsabak-85 and Bhattai that were significantly higher among all genotypes i.e. 43.67 cm², and 42.48 cm², respectively (figure 4). The minimum flag leaf area was recorded from Fareed (21.13 cm²). Qaseem *et al.* (2017) found similar results for Pakistani bread wheat genotypes. Table 3 revealed that FLA had a higher significant positive correlation with LS, and PC. FLA was found to have a non-significant correlation with the rest of the parameters. These findings are concurrent with the observation of Qaseem *et al.* (2017).

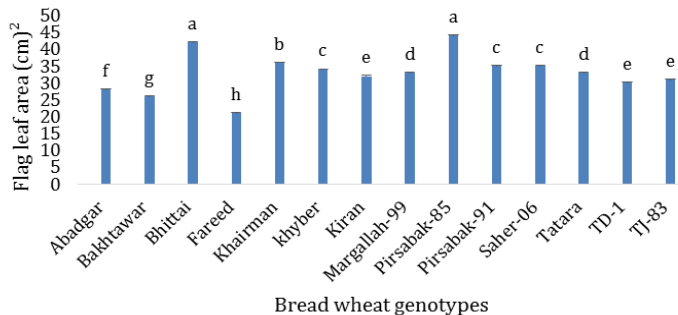


Figure 4: Performance of 14 local Pakistani bread wheat genotypes for flag leaf area under irrigated condition. Duncan's test values are significant at 1% significance level that is indicated in letters a-h on each standard error bar (n=4).

Biomass: Pakistani genotypes were highly diverse for biomass with a 39% coefficient of variability (table 2) that was well supported by Behmanesh *et al.* (2013) and Bibi *et al.* (2017). The maximum biomass was observed from Pirsabak-85 (15.00 g), Bakhtawar (14.86 g), and TD-1 (14.63 g). Where the lowest biomass was noted from Fareed (5.89 g) (figure 5).

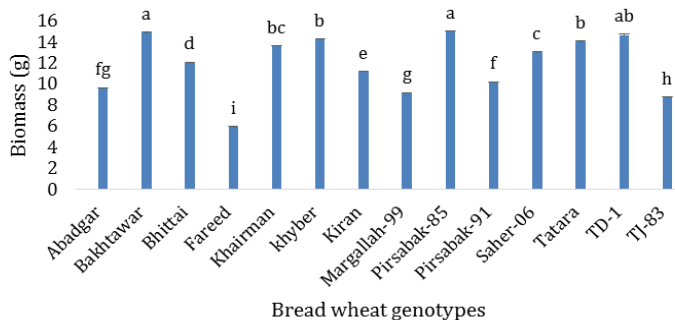


Figure 5: Performance of 14 local Pakistani bread wheat genotypes for biomass under irrigated condition. Duncan's test values are significant at 1% significance level that is indicated in letters a-i on each standard error bar (n=4).

Likewise, it was noted in the earlier report that TD-1 exhibited better fresh and dry biomass (Faisal *et al.*, 2017). The correlation results obtained from harvested plants displayed a positive and significant correlation with LS, GYP (r = 0.861), and PC at 1% significant level. However, TGW and HI had a non-significant correlation with biomass (table 3). Similarly, Behmanesh *et al.* (2013) found that the BIO was highly significantly correlated with GYP and HI that was partial like our study. Also, other previous investigators testified the

current study results (Qaseem *et al.*, 2017). It was suggested earlier that the biomass of the plant is also a secondary measure of photosynthetic assimilates that would ultimately contribute to the grain yield of the crop (Ali *et al.*, 2013).

Length of spike: Mean comparison of genotypes revealed that Bhattai and Pirsabak-85 had the highest mean value of 13.36 cm and 12.95 cm, respectively. However, the least spike length was observed in Saher-06 (9.34 cm), Fareed (9.64 cm), and Pirsabak-91 (9.79 cm) (figure 6). Correlation analysis indicated that the length of the spike was a positive highly significant relationship with GYP and PC (table 3). Similar results of a significant correlation of GYP with LS were observed by Sultana *et al.* (2013) and Qaseem *et al.* (2017). Thus, the highly significant association of LS with GYP might be due to the presence of great variability in the size and weight of genotypes grains.

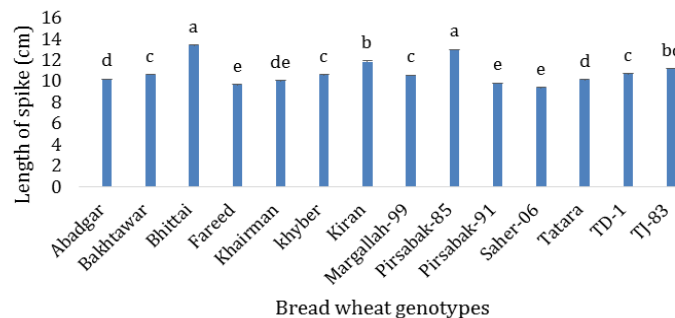


Figure 6: Performance of 14 local Pakistani bread wheat genotypes for length of spike under irrigated condition. Duncan's test values are significant at 1% significance level that is indicated in letters a-e on each standard error bar (n=4).

Thousand-grain weight: Comparisons among genotypes revealed that maximum TGW gave by variety Khyber (107.88 g), Pirsabak-85 (100.44 g), and Abadgar (98.00 g). These three varieties differed significantly from the other eleven genotypes. Furthermore, the lowest grain weights were recorded from Pirsabak-91 (74.36 g), Bakhtawar (75.32 g), and TD-1 (76.05 g) (figure 7).

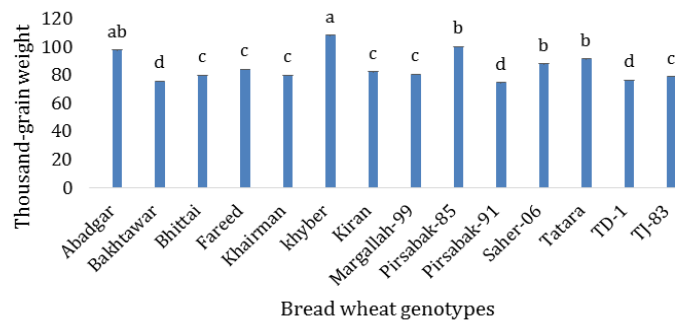


Figure 7: Performance of 14 local Pakistani bread wheat genotypes for thousand-grain weight under irrigated condition. Duncan's test values are significant at 1% significance level that is indicated in letters a-d on each standard error bar (n=4).

Our finding was similar to the report of Qaseem *et al.* (2017), who also conceded the better performance of the genotype for TGW. The decrease in grain weight of the genotypes may be due to the lesser accumulation of photosynthetic assimilates in the seeds (Slafer *et al.*, 1996). In the case of the coefficient of correlation, TGW had a significant association with GYP and PC (r = 0.900) while, non-significant relation with HI (table 3).

Qaseem *et al.* (2017), found a highly significant positive association of TGW with GYP. In contrast, Guendouz *et al.* (2014) observed a negative correlation between GYP and TGW. Also, a non-significant correlation between TGW and HI was observed by Qaseem *et al.* (2017). Although, grain weight is well known as a key component for grain yield (Guendouz *et al.*, 2014).

Harvest index: The HI is the pivotal indicator for the translocation of photosynthetic matters to the wheat kernels (Guendouz *et al.*, 2014; Ali *et al.*, 2013). The study showed the higher genetic variability (29.7%) among Pakistani genotypes for HI (table 2), in which Pirsabak-85, Khyber, and Tatara had the highest harvest index with the mean value of 52.19%, 43.42%, and 43.32%, respectively. Also, genotype Pirsabak-91 (27.07%) had the lowest harvest index (figure 8).

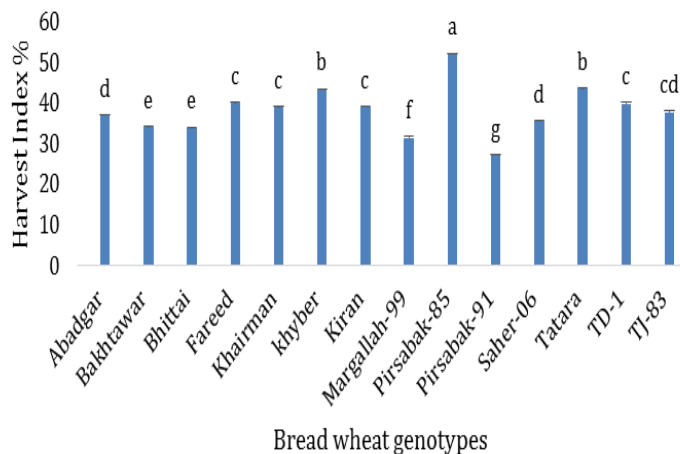


Figure 8: Performance of 14 local Pakistani bread wheat genotypes for harvest index under irrigated condition. Duncan's test values are significant at 1% significance level that is indicated in letters ^{a-g} on each standard error bar (n=4).

HI is positively significantly correlated with GYP and PC. These results support the outcomes of Guendouz *et al.* (2014), who observed a positive correlation between GYP and HI at a 1% level of significance. While, Qaseem *et al.* (2017), found a non-significant positive correlation of HI with GYP. According to the current study, TWG and HI enhanced as GYP increase. It has been concluded that the TGW and HI have a direct relation with grain yield in *T. aestivum*. These results are concurrent with the observation of Behmanesh *et al.* (2013) and Bibi *et al.* (2017), they also observed higher genetic variation among wheat varieties. Therefore, such type of variability plays a key role in the breeding program to fulfil anticipated aims of breeders like breeding for the eradication of disease, pest, improve chapati making grain quality and yield (Qaseem *et al.*, 2017).

Grain yield plant⁻¹: DMRT results indicated that the genotypes Pirsabak-85 (6.5 g grains yield plant⁻¹), and Khyber (6.0 g grains yield plant⁻¹) performed better for maximum grain yield plant⁻¹. In contrast, Pirsabak-91 (2.20 g grains yield plant⁻¹) possessed the lowest grain yield plant⁻¹ (figure 9). Moreover, grain yield plant⁻¹ had a positive and highly significant correlation with PC (table 3). In contrast, Kaya and Akcura (2014) observed a negative relationship between GYP and PC. While, it had been reported that higher grain yield in the *T. aestivum* and *T. durum* is directly related to the number of grains in the plant (Calderini *et al.*, 1999). Correspondingly, Guendouz *et*

al. (2014) noted that the number of grains spike⁻¹ of any plant has the prime importance with kernels' weight and its yield.

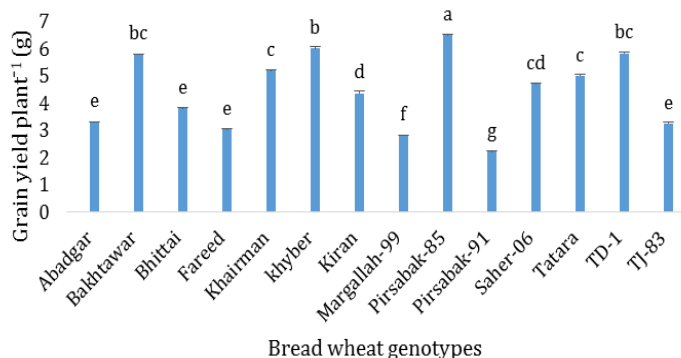


Figure 9: Performance of 14 local Pakistani bread wheat genotypes for grain yield plant⁻¹ under irrigated condition. Duncan's test values are significant at 1% significance level that is indicated in letters ^{a-g} on each standard error bar (n=4).

Protein profiling: Bread wheat gluten characteristics and total protein amount have a decisive role in bread, chapatis, and pasta-making (Begum *et al.*, 2019). To produce high-quality bread, the presence of genetic diversity is the key to increase the end-use quality of wheat. Current protein content analysis revealed that genotype Pirsabak-85 had maximum total soluble seed protein content (1351 $\mu\text{g mg}^{-1}$ fresh weight protein) whereas, minimum protein content was found in Fareed, Khyber, and Pirsabak-91 (figure 10).

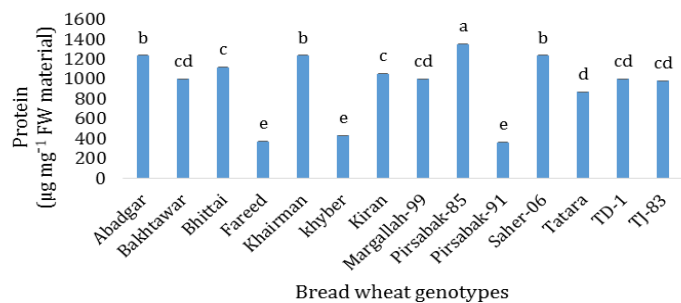


Figure 10: Performance of 14 local Pakistani bread wheat genotypes for protein content under irrigated condition. Duncan's test values are significant at 1% significance level that is indicated in letters ^{a-e} on each standard error bar (n=4).

These outcomes were testified by an earlier report of Azeem *et al.* (2019). Thus, it is concluded that the protein content in the wheat kernel was the vital parameter to improve the rheological properties of chapati dough, which was directly associated with its dough volume (Laidig *et al.*, 2017).

To understand the genetic association between bread wheat cultivars, cluster analysis was performed. Where dendrogram grouped the 14 genotypes into two major clusters according to the percent homology (figure 11). Cluster-I was smaller, composed of five genotypes that further divided into I-A, I-B, and I-C. Sub-cluster I-A further divided into two classes that were class-1, and 2. The class-1 has Pirsabak-91 and TD-1 that was 96% similar where class-2 has TJ-83 that was 84% similar to class-1. The sub-cluster I-B and I-C contained only one genotype that was Khyber-87 and Kiran-95, respectively showed 68% similarity with I-A sub-cluster. The major cluster-II comprised of 9 genotypes and sub-divided into II-A, II-B, and II-C sub-clusters. Abadgar and Bakhtawar-94 were in the II-A

cluster that showed a 44% similarity. While II-B further divided into two classes. The class-1 has Fareed-06 and Khirman and class-2 has Saher-06, and Tatara. Where class-1 genotypes were 92% and class-2 genotypes were 84% similar. Furthermore, class-1 was 60% similar with class 2. Similarly, sub-cluster II-C further sectioned into two classes where class-1 possessed Margalla-99 and Pirsabak-85 where other classes had Bhittai only. However, within class-1 cultivars showed 84% similarity and class-2 had 44% homology with class-1 (figure 11). The

NWFP cultivars such as Pirsabak-85 and Sindh cultivars as Bhittai was not separated maybe due to the facts that they belong to the same species and *de novo* genetic variability in cultivars of bread wheat happened subsequently due to the hybridisation of AABB genome from *Triticum turgidum* and DD genome from *Aegilops tauschii* (Liu *et al.*, 2009; Mihálik *et al.*, 2012). In this study, the HMW-GS alleles, compositions, and frequencies of all the genotypes revealed through SDS-PAGE that was based on the mobility of HMW-GS bands (table 4).

No.	RF	MW	A	Bhi	Bak	F	Khi	Khy	Kir	M	P85	P91	S	Ta	TD	TJ	HMW
1	0.10	147	1	0	1	1	1	0	0	1	0	0	1	1	0	0	null
2	0.12	138	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1
3	0.14	130	0	0	0	0	0	1	1	0	0	1	0	0	1	1	2*
4	0.16	120	0	0	0	0	0	1	1	0	0	1	0	0	1	1	2
5	0.17	115	1	1	1	1	1	0	0	1	1	0	1	1	0	0	5
6	0.19	110	0	1	1	1	0	0	0	0	0	0	1	1	0	0	7
7	0.21	104	0	1	0	0	0	0	0	0	0	0	1	0	0	0	9
8	0.22	99	1	1	1	1	1	0	0	1	1	0	1	1	0	0	10
9	0.24	94	0	0	0	0	0	1	1	0	0	1	0	0	1	1	12
10	0.26	87	0	0	0	0	0	0	0	1	1	0	0	0	0	0	13
11	0.28	81	0	0	0	0	0	0	0	1	1	0	0	0	0	0	16
12	0.29	76	1	0	0	0	1	1	1	0	0	1	0	0	1	1	17
13	0.31	71	1	0	0	0	1	1	1	0	0	1	0	0	1	1	18
14	0.33	66	0	0	1	0	1	0	0	1	0	0	0	1	0	0	
15	0.34	61	1	1	1	1	1	1	1	0	1	1	0	0	1	0	
16	0.36	57	0	0	0	0	0	0	0	0	0	1	0	0	0	1	
17	0.40	50	1	0	0	0	1	0	0	0	0	0	1	1	0	0	
18	0.41	47	0	1	0	1	0	0	0	1	1	1	0	0	0	1	
19	0.43	45	1	0	1	0	0	1	0	0	0	0	0	1	1	0	
20	0.45	44	0	0	0	0	0	0	1	1	1	0	0	0	0	0	
21	0.47	43	0	0	0	0	0	0	0	0	0	0	0	1	0	0	
22	0.48	41	0	0	1	0	0	0	0	0	0	0	0	0	0	1	
23	0.50	40	0	1	0	0	1	0	0	0	0	1	0	0	0	0	
24	0.52	39	0	0	0	0	0	1	0	1	1	0	1	1	0	0	
25	0.55	37	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
26	0.59	34	0	0	0	1	1	1	0	0	0	1	1	0	1	1	
27	0.60	32	1	1	0	0	0	0	0	0	0	0	0	0	0	0	
28	0.62	31	1	0	1	0	0	0	1	0	0	0	0	1	0	0	
29	0.64	30	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
30	0.66	28	0	0	0	1	1	0	0	0	0	1	0	1	1	0	
31	0.67	27	0	1	0	0	0	0	0	0	0	0	0	0	0	0	
32	0.69	26	1	0	1	0	0	0	0	0	0	0	0	0	0	0	
33	0.71	25	0	0	0	0	0	1	0	0	1	0	1	0	0	0	
34	0.72	24	0	1	0	1	1	0	1	1	0	1	0	0	1	1	
35	0.76	22	0	0	1	0	0	0	1	0	1	0	0	1	0	0	
36	0.78	21	1	0	0	0	0	1	0	0	0	0	0	0	0	0	
37	0.79	20	0	0	0	1	1	0	0	0	0	1	1	0	1	1	
38	0.81	19	1	0	0	0	0	0	0	0	1	0	0	0	0	0	
39	0.83	18	0	1	1	0	0	0	0	1	0	0	0	0	0	1	
40	0.84	17	0	0	0	0	0	1	0	0	0	0	1	1	0	0	
41	0.86	16	0	0	0	0	0	0	0	0	1	0	0	0	0	0	
42	0.90	15	1	0	0	0	0	0	1	0	0	0	0	0	0	0	
43	0.91	14	0	0	0	0	0	1	0	0	0	0	0	0	1	1	
44	0.93	13	0	1	0	0	1	0	0	1	1	1	1	1	0	0	
45	0.95	13	0	0	1	0	0	0	0	0	0	0	0	0	0	1	
46	0.97	12	0	1	0	0	1	0	0	0	0	1	0	0	0	0	
47	0.98	12	0	0	1	0	0	0	0	0	0	0	0	0	1	0	
Total bands			15	14	14	10	15	13	11	12	14	15	12	14	13	15	
<i>Glu-1</i> score			8	9	6	6	8	8	8	8	10	8	7	6	8	8	

Table 4: High and low molecular weight glutenin subunits of 14 local Pakistani bread wheat genotypes.

The HMW glutenin band 1 having the slowest electrophoretic mobility and the rest have been numbered successively with the increasing order of mobility. The total of 9 alleles and 7 different compositions were identified at the *Glu-1* loci in which null, 1, and 2* at *Glu-A1*, 7, 7+9, 13+16, and 17+18 at *Glu-B1* and 2+12 and 5+10 found at *Glu-D1* locus (table 5). The most frequent allele at the *Glu-A1* locus on the chromosome 1A and *Glu-B1* locus on the chromosome 1B was null (allele c) having 147 kDa MW and 17+18 (allele i) having 76+71 kDa MW respectively, that was present in the 50% of the studied cultivars (table 3).

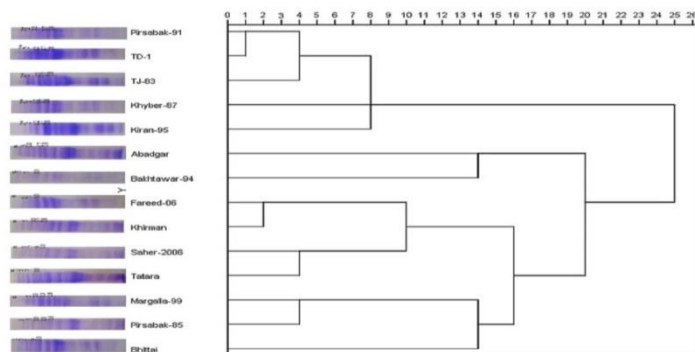


Figure 11: Phylogenetic relationships among 14 local bread wheat genotypes for total soluble seed protein.

At *Glu-D1* locus on chromosome 1D, 5+10 (allele d) having 115+99 kDa MW were the highly occurred subunit with the frequency of 64% (table 4 and 5). Current study results are similar with the findings of Mihalik *et al.* (2012) and, Kaya and Akcura (2014) who reported that at locus *Glu-B1* subunits 17+18 (allele i) and *Glu-D1* subunits 5+10 (allele d) are

Locus	Allele	Subunit composition	Frequencies	%	Genotypes
<i>Glu-A1</i>	a	1	2	14	Bhattai, Pirsabak-85
	b	2*	5	36	Khyber, Kiran, Pirsabak-91, TD-1, TJ-83
	c	null	7	50	Abadgar, Bakhtawar-94, Fareed-06, Khirman, Margalla-99, Saher-06, Tatara
<i>Glu-B1</i>	a	7	3	22	Bakhtawar-94, Fareed-06, Tatara
	c	7+9	2	14	Bhattai, Saher-06
	f	13+16	2	14	Margalla-99, Pirsabak-85
	i	17+18	7	50	Abadgar, Khirman, Khyber-87, Kiran, Pirsabak-91, TD-1, TJ-83
<i>Glu-D1</i>	a	2+12	5	36	Khyber-87, Kiran, Pirsabak-91, TD-1, TJ-83
	d	5+10	9	64	Abadgar, Bhattai, Bakhtawar-94, Fareed-06, Khirman, Margalla-99, Pirsabak-85, Saher-06, Tatara

Table 5: Alleles, subunit composition and their frequencies of high molecular weight glutenin subunits of 14 local Pakistani bread wheat genotypes.

CONCLUSION: The great extent of genetic variability existed in the local studied genotypes. The information of kernel yield along with plant height, tillers plant⁻¹, fertile tillers plant⁻¹, biomass, spike length, grains plant⁻¹, thousand-grain weight, and harvest index will assist in breeding and selection of genotypes for improving chapati making quality. Among genotypes, Pirsabak-85, Khyber, and TD-1 performed optimum for grain yield and other quality-related parameters. Also, Pirsabak-85 found to be quite promising for better biscuits, bread, pasta, and chapatis making as judged by their HMW-GS and LMW-GS SDS-PAGE bands and their quality scores. These cultivars will be used as parents for high quality in the future breeding program. Further investigations are needed to drive sounder conclusion for the development of high yielding and best quality varieties, to bring the massive change

associated with the good bread-making quality, while the presence of the “a” at *Glu-A1*, “a” and “c” at *Glu-B1*, and “a” at *Glu-D1* predicted as poor quality for bread products. This indicated that the wheat breeders of Pakistan can develop cultivar with superior quality by replacing the “null” with 2* allele, which imparts the better quality of wheat flour (Sajjad *et al.*, 2012).

Similarly, the LMW glutenin subunits are encoded by *Glu-A3*, *B3*, and *D3* which plays a pivotal role in the dough extensibility and strength of bread wheat (Kaya and Akcura, 2014; Dhaka and Khatkar, 2015). Nadeem *et al.* (2016), suggested that the molecular weight of polypeptides below 70 kDa and above 27 kDa is low molecular weight glutenin subunits. In contrast, protein bands present above 70 kDa are high molecular weight glutenin. Moreover, the highest number of polypeptides was reported in the LMW-GS region of subunits. The wide variations of HMW-GS and LMW-GS bands were also found in studied bread wheat cultivars.

The rare glutenin subunit compositions of null, 13+16, 5+10; null, 7+9, 5+10; 1, 7+9, 5+10, and 1, 13+16, 5+10 were detected only in Margalla-99, Saher-06, Bhattai and Pirsabak-85, respectively (table 5). In general, most of the cultivars express five HMW-GSs but some cultivars possessed four HMW-GSs. These results are consistent with the research findings of Kaya and Akcura (2014) Judging by HMW-GS polypeptide molecular weight and compositions, only Pirsabak-85 and Bhattai had better quality due to the presence of 1, 13+16, 5+10 and 1, 7+9, 5+10 subunits, respectively. These findings are well supported by Payne (1987) and, Kaya and Akcura (2014).

in wheat cultivation. Such types of breeding programs will lead to an increase in the economic state of Pakistan and as well as the world.

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