Assessment of high yielding local Pakistani bread wheat genotypes for improved chapati making quality

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Authors\' Contribution
Farheen, J. developed the concepts of the study, designed, conducted and statistically analysed the experiment and as well as drafted the research findings;
F. N. Naqvi supervised the study and critically reviewed the research findings.

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ABSTRACT

The experiment was conducted to classify the maximum glutenin protein possessed Pakistani bread wheat genotype for superrelative 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 prolamine 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-Glutten 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, Bhittai, Bakhtawar-94, Fareed-06, Khirman, Khyber-87, Kiran-95, Margalla-99 Pirsabak-85, Pirsabak-91, Saher-06, Tatta, TD-1, 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
Table 1: List of local Pakistani bread wheat genotypes with their pedigree.

| 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  | Kirman     | SI-91195                      | Sindh  |
| Khy  | Khyber-87  | CM43903-H-4Y-1M-1Y-3M-2Y-0B   | NWFP   |
| Kir  | Kiran-95   | WL711 x Crow 'S' (Mexican origin) | NWF    |
| M    | Margalla-99| not known                     | NWFP   |
| P85  | Pirsaibak-85| CM33027-F-15M-4Y-4M-2Y-1M    | NWFP   |
| P91  | Pirsaibak-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-1Y-2M-2Y-3M-0Y       | Sindh  |

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

Table 2:

| 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   |
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| TJ   | TJ-83      | CMS287-J-1Y-2M-2Y-3M-0Y       | Sindh  |

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 # on each standard error bar (n=4).

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 Pirsaibak-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).

 Equation (3)

\[ HI = \frac{\text{Total weight of seeds} \times \text{plant}}{\text{Fresh biomass}} \times 100 \]

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 2D gels 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 conducted by SPSS v.19.
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 Pirsaab-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), Pirsaab-85 (4.20), and Khyber (4.14) produced maximum total tillers plant1, where genotype Fareed (2.44) gave a few numbers of tillers plant1 (Figure 3).

**Fertile tillers plant**1: Data analysis exhibited that there were higher significant differences among 14 Pakistani genotypes in producing fertile tillers plant1 with 24% of the coefficient of variability (CV) (Table 2). The highest number of fertile tillers plant1 was produced by TD-1 (4 FTP), Khyber, and Pirsaab-85 (3.4 FTP), whereas Fareed (2.11 FTP) had lesser FTP (Figure 3). The number of fertile tillers plant1 exhibited positive and significant relationship with BIO (r = 0.807) and GYP (r = 0.649) was 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 (Slafet et al., 1996).

**Flag leaf area:** FLA is one of the major physiological

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

| 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 |
| BIO | 1    | 0.139| 0.477***| -0.076| -0.042| 0.153| 0.294**|
| LS  | 1    | -0.082| 0.156| 0.311**| 0.156| -0.177| 0.861**| 0.307** |
| TGW | 1    | 0.065| 0.226*| 0.065| 0.226*| 0.900**|
| HI  | 1    | 0.268**| 0.340**|
| GYP | 1    | 0.532**|
| PC  | 1    | 1    |

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**Figure 3:** Performance of 14 local Pakistani bread wheat genotypes for total tiller plant1 (TP) and fertile tillers plant1 (FTP) under irrigated condition. Duncan’s test values are significant at 1% significance level that is indicated in letters ± on each standard error bar (n=4).

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

| 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 |

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**Table 3:** Sources of variations, Geno = Genotypes, CV = Coefficient of variability, Df = Degree of freedom, PH = Plant height, TP = Tillers plant1, FTP = Fertile tillers plant1, FLA = Flag leaf area, BIO = Biomass, LS = Length of Spike, TGW = Thousand-grains weight, HI = Harvest index, GYP = Grain yield plant1, PC = Protein content.
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 Bhittai 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–h 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 Bhittai 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–h 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 Tatarahad 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 *s 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, TGW 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 fulfill 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 *s 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 µg 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 *s on each standard error bar (n=4). These outcomes were testified by an earlier report of Azeeem 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

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**Volume Number 5 || Issue Number 3 || Year 2020 || Page Number 5**
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 Pirsa-bak-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 Pirsa-bak-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; Mihalik 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 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | null |
| 2   | 0.12 | 138 | 0 | 1 | 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 |
| 7   | 0.19 | 110 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 7 |
| 8   | 0.21 | 104 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 9 |
| 9   | 0.22 | 99  | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 10 |
| 10  | 0.24 | 94  | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 12 |
| 11  | 0.26 | 87  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 13 |
| 12  | 0.28 | 81  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 16 |
| 13  | 0.29 | 76  | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 17 |
| 14  | 0.31 | 71  | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 18 |
| 15  | 0.33 | 66  | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 19 |
| 16  | 0.34 | 61  | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 |
| 17  | 0.36 | 57  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| 18  | 0.40 | 50  | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| 19  | 0.41 | 47  | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 |
| 20  | 0.43 | 45  | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| 21  | 0.47 | 43  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| 22  | 0.48 | 41  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23  | 0.50 | 40  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 24  | 0.52 | 39  | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 |
| 25  | 0.55 | 37  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| 26  | 0.59 | 34  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 |
| 27  | 0.60 | 32  | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 28  | 0.62 | 31  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| 29  | 0.64 | 30  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 30  | 0.66 | 28  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| 31  | 0.67 | 27  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 32  | 0.69 | 26  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 33  | 0.71 | 25  | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| 34  | 0.72 | 24  | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| 35  | 0.76 | 22  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 36  | 0.78 | 21  | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 37  | 0.79 | 20  | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 38  | 0.81 | 19  | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 39  | 0.83 | 18  | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| 40  | 0.84 | 17  | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 41  | 0.86 | 16  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 42  | 0.90 | 15  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 43  | 0.91 | 14  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 44  | 0.93 | 13  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 45  | 0.95 | 13  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 46  | 0.97 | 12  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 47  | 0.98 | 12  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**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).

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

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

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.

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 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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