

HIGH MOLECULAR WEIGHT GLUTENIN SUBUNITS (HMW-GS) OF SYNTHETIC HEXAPLOID-DERIVED (SYN-DER) WHEATS IN RELATION TO THEIR QUALITY ATTRIBUTES

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Abstract

Studies for evaluations of replications of ancestral cross among tetraploid wheat and the diploid species *Aegilops tauschii* undertaken for diversified synthetic hexaploid derived (SYN-DER) wheat. *Ae. tauschii* is well versed as resourceful ancestral genetic material that majorly alters at D-genome of hexaploid wheat, thus imparting better bread making performance of wheat. HMW-GS profiles were determined for thirty-five (35) samples. Highest distributed alleles were Ax Null, Bx13+By16 and Dx5+Dy10 at *Glu-A1*, *Glu-B1* and *Glu-D1*, respectively. The k-mean clusters were formed on quality traits basis for 35 genotypes. At *Glu-A1*, *Glu-B1* and *Glu-D1*, each subunit was explored with respect to quality traits that interprets its end-use suitability. Main Quality attributes investigated were comprised of Protein Content (PC), Wet Gluten (WG), Ash, Hardness Score (HS), Gluten Index (GI), Thousand Grain Mass (TGM), Moisture Contents (MC) and Falling Number (FN). At *Glu A1*, genotypes containing Ax1 allele possessed the higher values for FN, PCs, WG and TGM than Null allele containing genotypes. At *Glu D1*, Dx5+Dy10 showed higher values for GI and significantly varied from Dx2+Dy12 at $p<0.05$. The study revelations would be highly useful for breeders and researchers that are targeting the *Ae. tauschii* progenitor crosses for contributing the HMW-GS profiles diversification of bread wheat and also may be helpful for end product manufacturer.

Key words: *Aegilops tauschii*; HMW-GS; Wheat-Quality; Synthetic hexaploid derived (SYN-DER) wheat.

Introduction

Triticum aestivum L. (Wheat) is among the most common global staple food. The demands of the baking industry and diverse consumer preferences have led wheat breeders to prioritize grain quality traits as a key focus in ongoing research. (Kiszonas & Morris, 2017). Several important characteristics of wheat that influences the end use quality and higher variability in these traits is a paramount to produce variety of specific end-use products. Among these end use quality traits, the main emphasis entails on quality and quantity of gluten proteins which are key components of the endosperm. Wheat gluten provides elasticity and extensibility, making it a crucial factor for bread making, baked goods, and extruded products such as pasta and noodles (Shewry, 2019).

Gluten is a composite of monomeric proteins, mainly glutenins and gliadins. Glutenins are categorized into High Molecular Weight Glutenin Subunits (HMW-GS) and Low Molecular Weight Glutenin Subunits (LMW-GS), which play key roles in enhancing dough strength and extensibility, respectively (Lafiandra & Shewry, 2022; Rai & Han, 2023). Moreover, allelic variation in HMW-GS acts as a dependable genetic marker for evaluating genetic diversity in wheat. Hence, analyzing HMW-GS is essential and serves as a crucial factor in wheat breeding to enhance its suitability for end-product manufacturing. It is broadly

recognized that strengthening the ability to capture and utilize genetic diversity remains crucial. The effective use of genetic diversity in traditional breeding programs remains constrained. Therefore, leveraging closely related diploid wheat species through meiotic recombination is crucial, as it can play a significant role in generating genetic diversity (Blary & Jenczewski, 2019). Substantial enhancements in genetic diversity can be achieved by reintroducing wild species into breeding programs (Pour-Aboughadareh *et al.*, 2021). CIMMYT, has widely adopted this approach, capturing new genetic diversity through SYN-DER, which is developed by recreating the ancient cross between tetraploid wheat and the diploid species *Aegilops tauschii*. The D genome from *Aegilops tauschii* confer to better dough and end use specificity in hexaploid wheat species (Delorean *et al.*, 2021). Although the results from the continuous researches on SYN-DERs suggested prominent advantages in respect of resistance and even yield improvement (Kumar *et al.*, 2019) and the effects of the SYN-DERs on the quality have been the point of the concerns (Yang *et al.*, 2009). However, Guzmán *et al.*, (2016) proposed that incorporating SYN-DERs into breeding programs can also contribute to enhancing grain quality. The end-product quality and functionality of wheat largely depend on traits such as kernel texture, protein content, ash content, wet gluten content, gluten strength, hardness score, and α -amylase activity. Zhao *et al.*, (2020)

also emphasized the importance of evaluating recombinant inbred lines (RILs), near-isogenic lines (NILs), and doubled haploid lines (DHs), as they are highly effective in assessing the impact of quality-related subunits.

The main aim of this study was to explore the diversity in SYN-DER wheat accessions concerning HMW glutenin subunit composition and its correlation with grain quality parameters.

Materials and Methods

Sample collection: Thirty-five advanced SYN-DER lines, developed by replicating the ancestral cross between tetraploid wheat and the diploid species *Aegilops tauschii* and sourced from CIMMYT, were obtained from the WWCC (Wheat Wide Crosses and Cytogenetics) Program at the NARC, PARC, Islamabad. All samples were thoroughly mixed using a precision electronic divider and then manually cleaned.

Glutenin extraction and SDS-PAGE: For extraction of glutenin subunits the method reported by Rasheed *et al.*, 2014 was adopted with minor modifications. Piston mortar was used to crush a single grain to powder from each genotype was ground into powder using a piston mortar, with the procedure repeated in triplicate. A microtube containing 10 mg of crushed powder was supplemented with 300 μ L of buffer solution (0.05 M Tris, 0.2% SDS, and 5 M Urea), adjusted to pH 8.0 using HCl, and allowed to sit for 20 minutes. Next 10 μ L of mercaptoethanol was mixed into each microtube and vortexed for uniform mixing. Afterwards the centrifugation at 12,000 rpm for 3 minutes were undertaken and the supernatant was collected for further analysis of HMW-GS using SDS-PAGE (slab-type). Polyacrylamide gel was formed at 7.5% concentration at 10 x 10 x 0.1 cm gel dimensions. A micro-syringe was used to dispense 12 μ L protein extract to every well. Electrophoresis was performed on 200 V till the blue marker line got the bottom of the gel plates. The gels were then detached from the plates. Coomassie Brilliant Blue, 0.25% (w/v), was used to stain the gels in a solution of methanol 50% and acetic acid 10% for approximately half an hour on a slow-speed shaker. De-staining was carried out by methanol (5%) solution in acetic acid (7.5%).

Allelic classification: The compositional profiles at the Glu-A1 and Glu-B1 loci were categorized based on the criteria established by Payne & Lawrence (1983), while the Glu-D1 alleles were recognized conferring to the classification system outlined by William (1993). The allelic classification of each subunit was based on the system described by MacGenes (McIntosh *et al.*, 2014).

Quality characteristics of wheat & wheat flour: Samples free from shriveled & broken kernels were taken and analyzed in triplicate for further quality parameters. TGM (Thousand Grain Mass) was determined according to standard method ISO 520-1977 (E) using the Electronic Seed Counter (Seedburo Model no. 801). The moisture content (MC) of grains was measured using a digital moisture tester (Burrows Model 700), which was calibrated

following the air oven method (AACC 44-15A, 1999). Grains of each sample were milled through Perten Laboratory mill 3100 installed with 0.8mm sieve. Alpha amylase activity was determined as FN in seconds according to AACC method 56-81B (AACC 2000) using Perten 1500 Falling Number. Protein contents (PC) were estimated according to Method ICC No. 105/2 using Auto kjeldhal unit (Buchi 370) and Digestion unit (Buchi K-435) on dry mass basis (dmb). Protein percentage was calculated using Nitrogen (N) factor of 5.7 as $N \times 5.7$. HS was analyzed by using NIR technique (Inframatic 8620A). Gluten contents were determined according to ICC/No. 137/1, 155 & 158 for determination of including WG, dry DG and GI using PertenGlutomatic® 2000 System for Gluten Analysis. Ash contents were determined according to the AACC Method No. 08-01 using Electric muffle furnace-Carbolite (AAF-1100).

Statistical analysis: All analysis was carried out in triplicate. SPSS software (SPSS version 21, USA) was used for analysis of Duncan's multiple range test (DMRT) Table 1. Genetic diversity at each Locus was measured in terms of Nei's Index (Nei 1973). *Nei's Index* measured by employing the formula: $H = 1 - \sum P_i^2$, where H and P_i denoted the genetic variation index and the frequency of the number of alleles at the locus, respectively. Allelic frequencies were determined by summing the occurrences of each allele across all individual accessions and then dividing by the total number of accessions, irrespective of their genetic makeup (see Table 2). For data analysis, K-Means Cluster, Correlogram-Scatter matrix, and Whisker box plots were generated using R (Version 4.1.3) with the factoextra, performance analytics, corrplot, and ggplot2 packages. A K-Means cluster diagram was specifically created to illustrate three distinct clusters based on various quality traits. i.e. PC, Ash, TGM, FN, WG, GI, MC of 35 SYN-DER accessions denoted as DH-1 to DH-35 was plotted Fig. 1. At each subunit the averages of each quality trait were analyzed to plot whisker box plots diagram employing one-way analysis of variance; Tukey's test Fig. 2. Data is given in supplementary materials. Correlation-Scatterplot Matrix Fig. 3 was generated among quality traits employing Pearson's correlation.

Results and Discussion

Quality traits of SYN-DER wheat: The quality parameters of 35 SYN-DER genotypes are presented in Table 1 and grouped in three clusters. On the basis of quality traits 35 genotypes were found to cluster in three distinct clusters Fig.1. Groups. Cluster 2 containing genotypes were found to have the highest mean values for PC, TGM, HS and WG, while lowest values for GI, MC, Ash and FN among other two Clusters. FN of these genotypes had an average of 417sec. Most of them reflected sound grains with very low or no α -amylase activity as around 85% samples possessed FN >300sec. PC was found to range from 11.5 to 16.03% except one genotype with 9.70% PC. The broad sense heritability was recorded 0.71 which indicated less role of environment in transfer of this trait. Storage proteins largely narrate the

dough properties and ultimately the end product suitability (Sharma *et al.*, 2020). PC of wheat is regulated by genetic makeup, growing practices and environmental conditions (Siddiqi *et al.*, 2021). PC of Pakistani wheat varieties ranged from 8-15% (Khurshid *et al.*, 2022) while Shewry (2009) narrated protein range from 7-22% in different wheat grains and flour. The inverse relationship between grain yield and protein content poses a significant challenge in breeding programs. Nevertheless, identifying lines that exhibit both high yield and elevated protein levels is crucial. Advanced lines demonstrating promising grain yield performance can serve as valuable sources for

screening higher protein content. The average WG was 26.65% with two extreme values of WG contents significantly ($p<0.05$) varied at 37.7% and 17.05%. Wheat wet gluten significantly influences dough properties, including rheology, gas retention, controlled expansion, structural enhancement, and water absorption and retention (Marchetti, 2012). Gluten strength in terms of Gluten Index showed wider range i.e., 11 to 96 GI were obtained. GI is very important trait that significantly showed its impact on end use suitability. It imparts significant positive effect on bread making while vice versa for chapatti making (Yousaf *et al.*, 2020).

Table 1. Quality characteristics of SYN-DER wheat genotypes.

Clusters ¹	Genotypes	FN (sec)	PC (%)	HS	MC (%)	TGM (gm)	Ash (%)	WG (%)	GI
Cluster 1	DH-6	387.00	12.23*	34.00*	9.57	32.00*	2.00*	28.10	36.00
	DH-10	296.00	11.67*	48.67*	9.78	22.90*	1.90	30.19	37.55
	DH-13	788.00*	12.70	47.33*	9.40*	33.40	1.65	28.03	40.50
	DH-14	375.00	12.37*	42.33*	9.83	27.20*	1.78	26.89	37.00
	DH-15	368.50	12.00*	50.33*	10.73	40.80	1.88	25.49	37.00
	DH-16	465.00	13.27*	47.33*	10.96	37.80	1.59	25.38	92.50*
	DH-18	526.50	12.67	30.00*	10.53	31.60*	1.60*	31.85	62.00
	DH-19	477.00	12.50*	37.33*	10.80	32.20	1.81	28.76	37.35
	DH-20	531.00	12.07*	34.33*	10.62	32.60	1.88	28.97	33.2*
	DH-22	442.50	12.03*	35.33*	10.79	32.80	1.70	22.83	32.00*
	DH-23	636.50*	13.33*	48.67*	11.20*	26.40*	1.82	25.68	47.00
	DH-26	262.00*	14.17*	51.33*	10.62	27.80*	1.90	26.72	66.00
	DH-29	365.00	12.57*	36.00*	10.62	42.80*	1.89	26.93	71.50
	DH-30	299.50	10.80*	48.33*	10.92	38.20	1.79	18.94	93.00
Cluster 2	DH-1	427.00	14.67*	46.67	9.50	29.80*	1.78	33.62	49.80
	DH-2	316.50	13.53*	47.00	9.37*	39.80	1.63	26.74	76.10
	DH-3	490.00	12.77	39.00*	9.53	37.60	1.55	29.36	48.50
	DH-4	266.67*	12.80	49.00*	9.42*	43.00*	1.72	26.10	61.00
	DH-5	393.00	14.73*	45.33	9.41*	38.20*	1.83	25.60	55.00
	DH-7	467.50	13.40*	41.33*	9.57	36.80	1.74	34.26	64.92
	DH-8	528.00	15.40*	51.67*	9.65	35.40*	1.78	27.70	44.50*
	DH-9	339.00	12.47*	51.00*	9.22*	42.20*	1.69	27.05	10.89*
	DH-11	343.67	12.00*	42.33*	9.57	44.20	1.70	28.20	13.96*
	DH-12	389.50	16.03*	48.00*	9.70	38.60	1.72	37.75*	46.50
	DH-24	388.50	14.00*	52.67*	10.62	48.00*	1.59	32.67	46.50
	DH-25	241.00*	12.87	53.67*	10.63	40.00	1.70	27.08	29.50*
	DH-35	345.00	14.47*	48.33*	10.12	34.80	1.66	30.57	90.50
Cluster 3	DH-17	456.50	12.60	37.00*	10.82	32.40	1.77	17.05*	91.00*
	DH-21	537.50	12.43*	47.33*	11.33*	28.80*	1.71	24.06	80.50
	DH-27	478.50	11.70*	46.33	10.74	33.80	1.75	28.45	83.00*
	DH-28	495.00	11.17*	47.33*	10.47	38.00	1.70	19.61	96.00*
	DH-31	283.00	11.67*	47.00	10.63	49.00*	1.94	20.38	70.50
	DH-32	328.00	12.77	47.00	10.70	34.80	1.70	22.24	90.00*
	DH-33	464.50	11.93*	50.67*	10.39	40.00	1.61	22.09	83.00
	DH-34	396.00	9.70*	48.33*	11.03	48.40*	1.52*	19.27	52.00
	DH-35	345.00	14.47*	48.33*	10.12	34.80	1.66	30.57	90.50
	Mean	416.97	12.78	45.10	10.25	36.07	1.75	26.65	56.99
	St. Dev.	113.09	1.28	6.09	0.64	6.45	0.11	4.77	24.29
	Heritability	0.53	0.71	0.62	0.85	0.92	0.65	0.77	0.63

*Significantly different from mean at $p<0.05$. 1 corresponds to clusters in Fig. 1. FN, PC, HS, MC, TGM, WG, GI denotes Falling Number, Protein content, Hardness Score, Moisture content & Thousand Grain Mass

Table 2. Allelic frequencies of HMW-GS at Glu-1 loci in 35 accessions of the SYN-DER genotypes.

Locus	Allele	Subunit	Number of accessions	Frequency (%)	H (Nei's Index)
<i>Glu-A1</i>	A	1	11	31.4	0.5
	B	2*	2	5.71	
	C	Null	22	62.86	
<i>Glu-B1</i>	B	7 + 8	2	5.71	0.78
	C	7 + 9	1	2.86	
	F	13 + 16	12	34.28	
	I	17 + 18	10	28.6	
	D	6 + 8	3	8.57	
	G	20	1	2.86	
	H	14 + 15	2	5.71	
	A	7	3	8.57	
<i>Glu-D1</i>	Ah	1.5 + 10	1	2.86	0.82
	N	2.1 + 10	3	8.57	
	A	2 + 12	5	14.28	
	D	5 + 10	12	34.28	
	H	5 + 12	2	5.71	
	Ar	2 + 10.1	1	2.86	
	Bk	5 + 10.1	1	2.86	
	Bl	5 + 12.2	1	2.86	
	X	2 + 10.2	5	17.14	
	-	5 + 10.2	3	8.57	
	-	2.1 + 10.2	1	2.86	

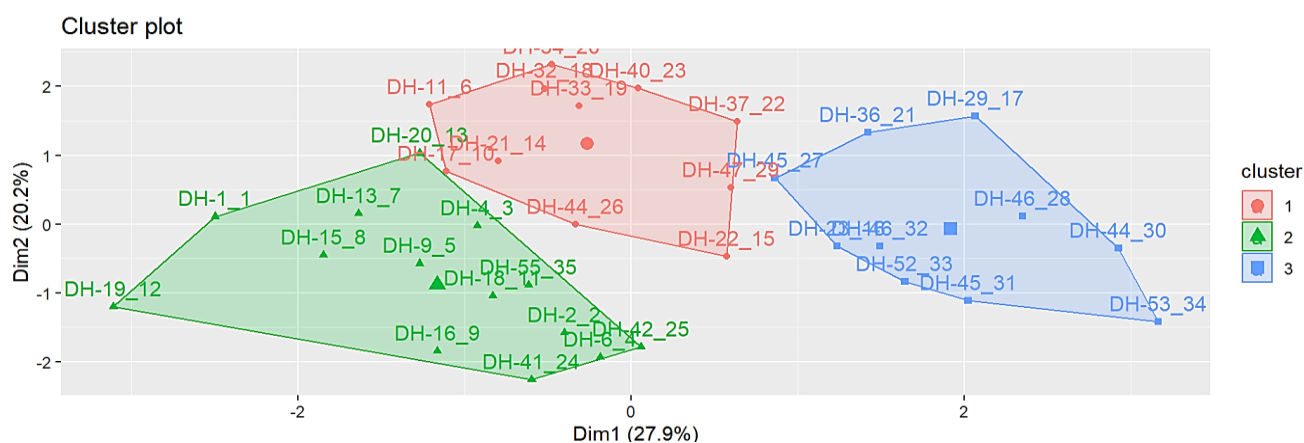


Fig. 1. K-Means cluster Diagram showing clusters at k=3 on basis of quality traits i.e. PC (Protein Content), Ash contents, TGM (Thousand Grain Mass), FN (Falling Number), WG (Wet Gluten content), GI (Gluten Index), MC (Moisture content) of 35 SYN-DER wheat accessions denoted as DH-1 to DH-35.

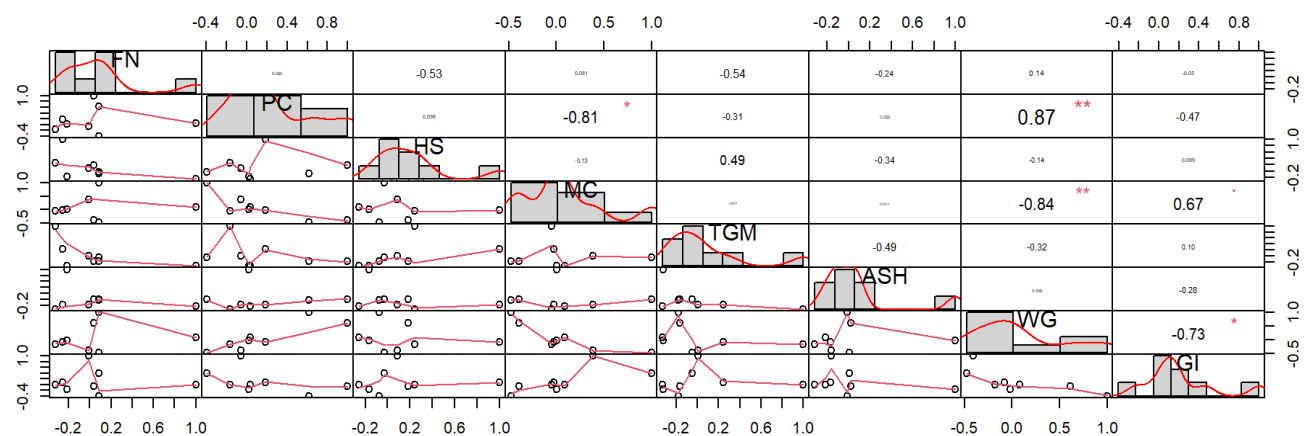


Fig. 3. Correlation-Scatterplot Matrix for eight quality traits i.e. FN (Falling Number), PC (Protein Content), HS (Hardness Score), MC (Moisture Content), TGM (Thousand Grain Mass), WG (Wet Gluten Content), GI (Gluten Index). Matrix is elaborated with histograms on the diagonal, bivariate scatter plots on bottom of the diagonal & value of the correlation plus the significance level as stars on the top of the diagonal. Each significance level is associated to a symbol: p-values (0, 0.001, 0.01, 0.05, 0.1, 1) <=> symbols (***, **, *, ., .)

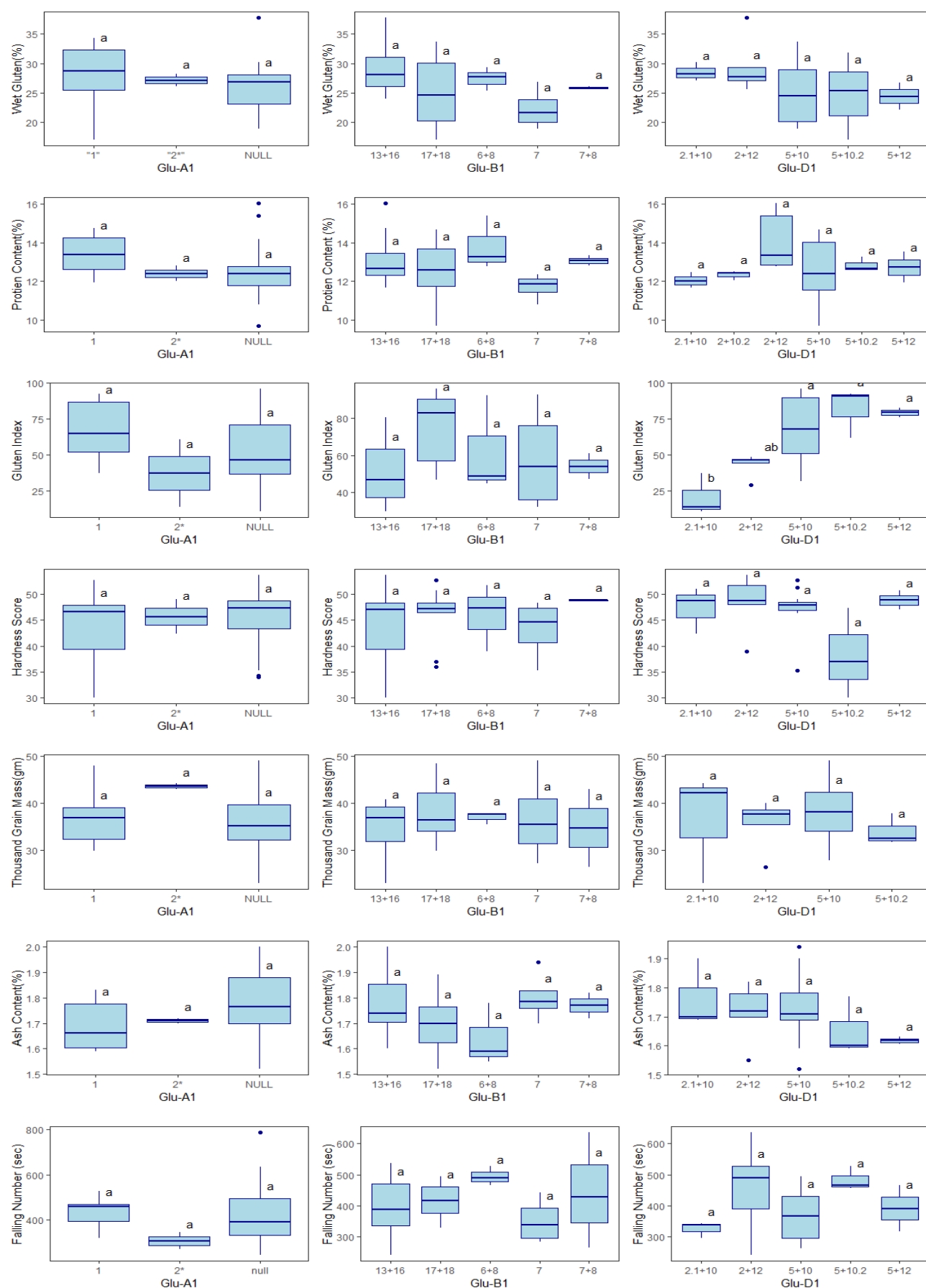


Fig. 2. Box and Whisker plot representing the interquartile range and whiskers the minimum and maximum for each subunit at three Loci i.e. GluA1, GluB1 & Glu D1 for their average means of PC (Protein Contents), Thousand Grain Mass (TGM), Ash, WG (Wet Gluten), GI (Gluten Index), MC (Moisture Content), & FN (Falling Number). Means of groups with different small letters are significantly different (one-way analysis of variance; Tukey's test; $p < 0.05$).

Grain texture is the most important trait which determines hardness or softness of wheat. Hardness score ranged from 30-54 with an average of 45. Grain hardness is a primary factor in wheat classification and significantly influences the quality of end products (Hourston *et al.*, 2017; Abid *et al.*, 2024). Grain hardness significantly affects the rheological properties of dough. A key physical distinction between the endosperm of hard and soft wheat is the degree of adhesion between starch granules and the surrounding protein matrix (Hourston *et al.*, 2017; Wang & Jeronimidis, 2008). These wheat genotypes are classified within the medium-soft to medium-hard range. Acar, Sanal & Köksel, 2019 & Iqbal *et al.*, 2022 reported significant impact of kernel size on the hardness and ultimately the yield of the grains. MC ranged from 9.22-11.33% with an average of 10.25%. Moisture content (MC) in wheat grains is significantly affected by various factors, including processing methods, grinding techniques, and climatic conditions during harvest. Variations in these factors can lead to differences in MC, which in turn influence the quality and storage stability of the grains. TGM was found to range from 22.90 to 48.40 g with an average of 36.07 g. This characteristic depends on grain size and density, which ultimately affect yield. Wheat kernels are categorized by weight as follows: very small (15–25 g), small (26–35 g), medium (36–45 g), large (46–55 g), and very large (over 55 g) According to this scale most of the genotypes are in the category of medium grains. (Zanetti *et al.*, 2001) reported TGM in the range 42.4-48.7 g of 128 wheat varieties while thousand grain mass found in the range 31.43-37.28 g in Pakistani wheat varieties (Anjum *et al.*, 2002). Ash content of these genotypes ranged from 1.52% (DH-34) to 2.0% (DH-6) with an average of 1.75%. Ash content significantly affects flour extraction rates, as higher ash levels indicate increased inclusion of bran and germ during milling. This elevated mineral content can influence the color and quality of both the flour and the final baked products. High ash content in wheat can negatively impact flour yield during the milling process as the presence of minerals and other non-flour components can reduce the amount of flour that can be extracted from the grain. Higher ash contents also reveal lesser flour yield (Iqbal, Pasha, Abrar, Masih, & Hanif, 2015).

Correlation among the quality traits as depicted in Fig.3 were determined between different traits. WG was found to be correlated to PC positively and MC negatively, both at significant levels of $p < 0.01$. PC was found also revealed to significantly negatively related to MC at $p < 0.05$. GI was negatively related to WG at $p < 0.05$. Generally, MC and PC correlation may vary depending on several factors, including the wheat variety, growing conditions, and post-harvest handling etc.

At *Glu-1* Loci, HMW-GS composition and association with quality characteristics in SYN-DER wheat: HMW-GS profiles were evaluated at three loci i.e., *Glu-A1*, *Glu-B1* and *Glu-D1*, separately for each quality trait to reveal impacts on each subunit Fig.2. HMW-GS decodes its distinct impact on processing and ultimately the end use quality (Rai & Han, 2023). Each locus is separately discussed below.

Genotypes were segregated on the basis of their *Glu-A1* composition and results for quality traits are summarized in Fig. 1. At *Glu-A1*, 0.5 H (Nie's Index) was calculated (Table 2). Frequency of AxNull and Ax1 subunits were 62.9 and 31.4 %, respectively. Only 6% of accessions were found to carry Ax2* subunit. In current study, Ax1 and AxNull possessing alleles impact on wheat quality trait i.e. FN, PC, WG, GI, TGM were found superior in former group except HS that was slightly higher for latter one. These results were in agreement to Vancini *et al.*, (2019), that allele Ax1 were superior to those with the Ax2* and AxNull alleles in terms of sedimentation volume, gluten strength, dough tenacity, water absorption, farinograph stability and grain hardness as well. Oury *et al.*, (2010) also reported better bread wheat characteristics of Ax1 and Ax2* possessing alleles than AxNull alleles. No significant variation was evident among subunits for all traits. While for PC Ax1 and Ax2* alleles found to show significant variations at $p < 0.05$ (data not shown). Gluten strength in terms of GI showed lower ranges for Null alleles possessing genotypes than 1 allele that is agrees with (Rai & Han, 2023) which ranked Ax1 > Ax2* > AxNull for dough strength. Since the frequency of AxNull and Ax1 subunits were 62.9 and 31.4 %, respectively. Most of these genotypes may rank to be suitable for noodles while Ax1 allele possessing may be suitable for bread making (Rasheed *et al.*, 2014).

At *Glu-B1* Nie's index was calculated as 0.78 (Table 2). Major allelic composition at *Glu-B1* was found to be comprise of Bx13+By16, Bx17+By18, Bx6+By8, Bx7, Bx14+By15, Bx7+By8, Bx7+By9 and Bx20 at frequency of 34.3, 28.6, 8.6, 8.6, 5.7, 5.7, 2.9 and 2.9 percent, respectively. Mean FN, HS, TGM & GI were exhibited higher ranges for Bx17+By18 containing alleles in comparison to Bx13+By16 alleles. While WG & PC showed higher contents in Bx13+By16 containing alleles. These findings align with Li *et al.*, (2010), who identified the suitability of Bx13+By16 for noodles, steamed bread, and instant noodles, products known to require elevated protein and gluten levels (Rai & Han, 2023; Xiong *et al.*, 2021). Furthermore, the higher levels of FN, PC, HS, TGM, and WG observed in Bx14+By15 containing alleles indicate its suitability for bread making, a conclusion consistent with research by Vancini *et al.*, (2019) and Zhao *et al.*, (2020). While, FN, PC & WG lowest values were shown in 'Bx7' possessing alleles and in line with the findings of (Branlard *et al.*, 2004; Chuanxi, Feng & Yunzhi, 1995) that reported lowest values for Zeleny sedimentation value (Zel) and bread volume in Bx7 containing alleles at *Glu-B1*.

The unique pool of alleles at *Glu-D1* are contributed through *Ae. tauschii* that plays an important role in governing the improvements in wheat breeding programs, employing the impacts on end use quality (Delorean *et al.*, 2021; Kumar *et al.*, 2019). *Glu-D1* revealed highest impacts on processing quality of wheat in comparison to *Glu-A1* and *Glu-B1* (Yousaf *et al.*, 2022). Highest frequency of 34.3% was possessed by the genotypes containing Dx5+Dy10 sub units at *Glu-D1*. Subunits Dx2+Dy12, Dx2+Dy10.2, Dx2.1+Dy10, Dx5+Dy10.2, composed 14.3, 17.14, 8.6, 8.6% of the genotypes at *Glu-D1*. Nie's Index at *Glu-D1* was calculated as 0.82. Results

revealed the higher mean values for FN, HS, PC & WG for Dx2+Dy12 containing alleles, as previously also reported for WG and FN by (Vancini *et al.*, 2019). While GI and TGM were higher in Dx5+Dy10 possessing alleles. Significant variation at $p < 0.05$ was found at Dx2+Dy12 for GI Fig.2. Significant variations at $p < 0.005$ were found among Dx5+Dy10 and Dx2.1+Dy10 and Dx5+Dy10.2 and Dx2.1+Dy10 subunits while variation for Dx5+Dy12 and Dx2.1+Dy10 were found at $p < 0.05$ (data not shown). Higher GI, TGM & HS were found in Dx5+Dy12 containing alleles than Dx5+Dy10 as also evident by (Pena *et al.*, 1995) reported that found better contribution for dough strength and bread making by 5+12.

The data generated from this study provides novel insights and highlights the necessity for further investigation into the combined effects of various alleles across different recombinant inbred lines (RILs), near-isogenic lines (NILs), and doubled haploids (DHs). A comprehensive analysis of promising lines exhibiting elevated protein content (PC) and increased test grain mass (TGM) is crucial for effectively utilizing these accessions in recombination breeding programs. Such an analysis will facilitate the identification of desirable traits and the development of improved wheat varieties.

Conclusion

The evaluated accessions of wheat (*Triticum aestivum*) that entails its specialty of advancement by ancestral *Ae. tauschii* presented genetic diversity at *Glu-1*. Diversified composition of alleles at *Glu-D1* was shown that may highly relate to the D-subgenome contribution from *Ae. tauschii* species. Impacts of HMW-GS at three Loci i.e. *Glu-A1*, *Glu-B1* and *Glu-D1* on quality traits were evident. Gluten strength in terms of gluten index that highly relates to breadmaking characteristics were found highest in Dx5+Dy10 following Dx5+Dy10.2, Ax1 and Bx17+By18 possessing accessions at *Glu-A1*, *Glu-B1* and *Glu-D1*, respectively. Correlation among quality traits showed TGM and FN, WG and GI, WG and MC, GI and MC were significantly correlated at $p < 0.05$. Findings are beneficial for the breeders w.r.t incorporating the quality traits for end use suitability and also helpful for early generation selection of quality traits in wheat.

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