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Predictive Genetic Profiling of High Molecular Weight Glutenin Subunits in Indian Wheat Varieties for Bread-Making Quality

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ABSTRACT

This study aimed at providing a predictive assessment of bread-making quality of 25 Indian bread wheat genotypes and the German variety Bussard based on the characterization of their established high-molecular-weight (HMW) glutenin subunits quality scores using SDS-PAGE, without direct rheological or baking validation. HMW glutenin subunits at the Glu-A1, Glu-B1, and Glu-D1 loci were identified and classified, and Glu-1 quality scores (1-10 scale) were calculated based on SDS-sedimentation associations. High-molecular-weight glutenin profiles were used to compute similarity indices and construct a dendrogram using Genstat. The genotypes Bussard, C-591, C-306, and WH-533 exhibited the highest Glu-1 quality scores, associated with favourable alleles such as Glu-D1 (5+10) and Glu-A1 (1 or 2*) in combination with Glu-B1 (7+8 or 7+9), conferring association with superior dough strength and bread-making quality. Genetic similarity coefficients among the 26 genotypes ranged from 0.53 to 1.00, and the dendrogram separated them into two major clusters, each with two sub-clusters; C-591 and C-306 grouped in SG2b, whereas WH-533 and Bussard clustered in SG1a. The genetic similarity in glutenin composition, based on the clustering, indicates the quality potential of bread wheat cultivars without measuring the functional performance.

Keywords: Glutenin subunits, Glu-1 quality score, bread-making quality, wheat genotypes, SDS-PAGE, genetic similarity

Introduction

One of the important breeding goals in bread wheat is its end-use quality as it determines the quality of products. The end use quality in wheat is determined by gluten as it confers specific viscoelastic characteristics to wheat dough (Islam et al., 2019). Glutenins in general and high molecular weight glutenin subunits (HMW-GS) are considered important to determine processing quality in wheat (Sharma et al., 2020). High-molecular-weight (HMW) subunits of glutenin are encoded by genes at major three homologous loci viz. Glu-A1, Glu-B1, and Glu-D1 positioned on the stretched arms of chromosomes 1A, 1B and 1D, respectively (Nimbal et al., 2017). Since, the glutenins are major polymeric component of gluten, therefore, the differences in glutenins determine physicochemical (elasticity) and rheological (extensibility) properties of the dough (Abedi and Pourmohammadi, 2021). Two major seed storage protein groups namely Glutenins and gliadins are present in *Triticum aestivum* L. (hexaploid wheat) whose biochemistry as well as the genetics have been broadly studied, revealing both as major determinants of bread-making quality (BMQ) (Li et al., 2021;

Shewry, 2023). Well-characterized inheritance and high polymorphism have made them invaluable for wheat breeding and genetic research.

Quality scores assigned to individual or paired HMW glutenin subunits enable evaluation of bread-making quality (BMQ) potential based on Glu-1 patterns (Nimbal et al., 2017). Studies in European and Indian wheat varieties indicate that HMW glutenin subunit composition accounts for 33–50% of BMQ variation (Wang et al., 2022), with Glu-1 scores positively correlating with bread-making and negatively with biscuit-making qualities. Thus, Glu-1 scores serve as a valuable selection criterion in wheat breeding as a predictive and prescreening mechanism (Jain et al., 2002), while end-use quality (e.g., chapati, bread) classification relies on direct rheological and baking tests such as dough strength, SDS-sedimentation values, solvent retention capacity and gluten index (Coventry et al., 2011). The present study utilizes the glutenin subunit quality scoring in 26 wheat genotypes and their clustering via genetic similarity matrices, to evaluate the genetic diversity and their quality potential, which can be used as a predictive and prescreening tool in wheat improvement programs by the breeders. The selected wheat genotypes in present study were chosen as they thrive well in different agronomic management like Bussard in intensive input conditions, C-591 and C-306 in medium input conditions and WH-533 in water deficit conditions. These genotypes may be involved in recombination breeding to develop high yielding high quality wheat cultivars, based on their association with superior bread-making quality interpreted through Glu-1 quality score.

Materials and Methods

For conducting the present study on profiling of glutenin subunits using SDS-PAGE to predict bread-making quality potential based on established Glu-1 scoring systems, seed samples of twenty-six wheat varieties were used in this study (table 1). These varieties were Bussard (German Wheat variety) and 25 Indian hexaploid wheat genotypes namely C-306, C-591, CS, HD 2009, HD 2204, HD 2285, HIG 17, HUW 134, K 68, KS, Norin 10, Raj 3077, Sonalika, UP 262, UP 368, WH 147, WH 147M, WH 157, WH 283, WH 291, WH 331, WH 416, WH 533, WH 542, and WH 553. SDS-PAGE analysis for gluten proteins of these samples was conducted by extracting wheat flour (30mg) in 400 µL of buffer (1M Tris-HCl, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol and 1% bromophenol blue), heating the extract at 80°C for 30 min, cooling and mixing it with 30 µL tracking dye and then centrifuging it at 10000 rpm at 10 mA for 15 minutes to obtain the supernatant. The electrophoresis was carried out by loading 35 µL of the obtained sample on a 12% running gel and 5% stacking gel (1.0 mm thick). The gel was run at 10-20 mA for the dye to reach the gel's bottom (Nimbal et al., 2010). Following electrophoresis, gel was separated and stained with Coomassie Brilliant Blue R-250 and destained for identification of the HMW glutenin subunits. The methods of Payne and Lawrence (1983) were used for identification and nomenclature of the high molecular weight glutenin subunits at the Glu-A1, Glu-B1 and Glu-D1 loci and for classification of each subunit or subunit pair according to their standard allele designations obtained on banding patterns in SDS-PAGE. Samples of these varieties were scored for High and Low molecular weight profiles of glutenin subunit patterns following (Rogers et al., 1989), and Glu-1 quality scores were calculated according to Payne et al. (1987) and Wang et al. (2022) by assigning a numerical quality value to each observed HMW glutenin subunit allele at Glu-A1, Glu-B1 and Glu-D1 based on their SDS-sedimentation associations and then adding the three locus specific values to obtain a single Glu-1 score for each variety. In the applied scoring system, the quality score values range from 1 to 10, where a score of 10 denotes the highest gluten and better break making quality, and 1 denotes the lowest quality associated with weak dough and lower bread-making quality. Similarity index analysis was performed on the Glu-1 score–based HMW-GS compositions and dendrogram were prepared using Genstat computer programme. The dendrogram was inferred as a representation of genetic similarity of glutenin composition and not the indicator of functional quality performance. Moreover, the low-molecular weight (LMW) glutenin bands were recorded for comprehensiveness of the protein profiles and were not used in quality scoring or clustering as it is exclusively based on HMW-GS composition due to their established role in Glu-1 quality assessment.

Results and Discussion

The documentation of bread-making quality of wheat is generally very intricate, but is mainly controlled by its protein quality and protein content (Khalid et al., 2023). Based on HMW glutenin subunit composition and SDS- sedimentation value known for each genotype, Glu-1 quality score was assigned to each genotype by method described previously (Payne et al., 1987; Omogbolahan et al., 2025) except for the genotype Raj-3077, having subunit 11+18.

A conservative provisional score of 2 was assigned based on its reported association with moderate dough strength in earlier studies, therefore it was excluded from main comparative readings to avoid bias in estimating Glu-1 quality score. Clustering strength was also not driven by this genotype (Table 1 & Table 2).

Genotypes Bussard, C-591, C-306 and WH-533 were found to have the highest Glu-1 quality score of 9. So, these genotypes are referred as genetically favorable for bread-making characters, followed by HD-2009, HD-2204, WH-147, WH-283 which depicted Glu-1 quality score of 8, while the Glu-1 quality scores of other varieties fluctuated from 3-6 (Table 3).

To provide contextual validation for the Glu-1 quality scores and clustering patterns observed in the present study, previously reported functional quality associations of major HMW-GS combinations were compiled from the literature (Table 4). This comparison helps interpret the predictive relevance of glutenin subunit compositions identified herein in the absence of direct rheological measurements.

The consistency between literature-reported functional performance and the Glu-1 quality scores observed in the present study supports the use of HMW-GS profiling as a reliable predictive and prescreening tool for bread-making quality in wheat breeding programs.

Previous studies identified subunits 5+10 (Glu-D1), 1 and 2* (Glu-A1), and 7+8 (Glu-B1) to be linked with superior quality attributes (Bhagwat & Bhatia, 1993; Ivanov et al., 1998). 13 allelic variations were reported by Zhang et al. (2001) at Glu-D1, having 1.5+10 and 5+12 subunits showing quality potential comparable to 5+10. In this study (Table 3), 1 and 2* subunits of Glu-A1, 7+8 and 7+9 subunits of Glu-B1, and 5+10 subunits of Glu-D1 were linked to excellent bread-making quality.

The missing bands for the Glu-D1 could be due to two main reasons i.e. either they are 4x or 2x, having in their pedigree like Nap Hal, where there is a null allele at Glu-D1. However, the impact of non-null Glu-A1 alleles on durum wheat quality is unclear, with some studies suggesting no significant effect, while others indicate improved gluten strength and extensibility. Previous studies reported recurrent presence of 7+8 and 7+9 HMW glutenin subunits at Glu-B1 in European groups (Sontag-Strohm, 1996; Igrejas et al., 1999) and spring wheat cultivars (Tohver et al., 2001). Moreover, a strong positive effect of the 5+10 allele at Glu-D1 on wheat quality was demonstrated by Lukow et al. in 1989, with optimal combinations including 1 or 2* subunits in Glu-A1, 7+8 or 7+9 subunits in Glu-B1, and 5+10 subunits in Glu-D1 (Ivanov et al., 1998). Bread-making quality is principally determined by Glu-D1 (5+10), followed by Glu-A1 (1, 2*) alleles, while combinations like 5+10/2+12 are valuable in variable environments (Bedó et al., 1995).

Shitre et al. (2016) identified 10 alleles across loci (Glu-A1: null [48%], 1 [30%], 2* [22%]; Glu-B1: 17+18 [33%], 7+9 [27%], etc.; Glu-D1: 2+12 [60%], 5+10 [40%]), with quality scores ranging 4–10 (mean 6.95). Jang et al. (2021) found 22 HMW-GS alleles, with Glu-1 scores of 10 in 15.79% of genotypes featuring combinations like 2*/7+8/5+10. These subunit-HMW correlations enable SDS-PAGE-based screening for bread-making quality (Galova et al., 2002; Siddiqi et al., 2020).

Genetic similarity coefficients among the 26 genotypes ranged from 0.53 to 1.00. Cluster analysis (Fig. 1) revealed two major clusters: Cluster I (20 genotypes) with sub-clusters SG1a (4 genotypes) and SG1b (16 genotypes), and Cluster II (6 genotypes) with sub-clusters SG2a (4 genotypes) and SG2b (2 genotypes: C-306, C-591), indicating substantial genetic diversity.

This suggested that these two genotypes are closely related with each other. Variety C 591 developed in 1935 at Layalpur now in Pakistan (Pal, 1966) and C 306 developed in 1966 at Hisar, Haryana, India (Yunus and Srivastava, 1994) are suitable for low input conditions. They are still considered to be the premium wheats in view of being best quality wheats for chapati making. The genotypes Bussard (high input variety) and WH-533 (suitable for water deficit condition) both have a quality score of 9 that falls in sub-cluster 2a and show high similarity index and hence resemblance for HMWs, while the genotypes C-591 (Quality score 9) and Bussard had low resemblance and clustered separately. All these four genotypes (Bussard, C 306, C 591 and WH 533) possessed desirable combination of Glu-1D (5+10), as well as Glu-1A (1) and Glu-1B (7+9, 20). Keeping in view the genetic polymorphism for HMW and quality scores (Goel et al., 2015, Nuttall et al., 2017), it would be possible to realize improvement in wheat quality through recombination breeding *vis-à-vis* sustainable wheat production in target environments (high/low input, water deficit) to support export-oriented agriculture.

While Glu-1 quality scores are not substitutes for direct rheological measurements, they have been shown to explain 33-50% of variation in bread-making quality and remain essential for early-generation screening (Michel et al., 2018).

The present clustering reflects genetic similarity in glutenin composition rather than absolute functional performance. Therefore, the identified superior genotypes represent promising candidates for further phenotypic validation under controlled baking and rheological assays.

Conclusions

In the present study, the electrophoretic patterns of glutenin protein profiles and Glu-1 quality scores of 26 wheat genotypes revealed that genotypes Bussard, C-591, C-306, and WH-533, with the highest Glu-1 quality score of 9, associated with superior bread-making potential due to the presence of favorable HMW glutenin subunits such as 5+10, 1, and 2*. Other genotypes, including HD-2009, HD-2204, WH-147, and WH-283, with quality scores of 8, also associated with good bread-making quality. This study emphasizes the importance of HMW glutenin subunit composition in predicting the bread-making quality. The results emphasize the utility of Glu-1 quality scores as a reliable selection criterion for prescreening wheat cultivars in breeding programs aimed at improving bread-making quality. The observed variation in glutenin subunit composition and corresponding quality scores highlights the genetic diversity among wheat varieties, which can be leveraged for targeted breeding to enhance wheat processing quality and end-use performance. However, future studies integrating SDS-sedimentation, solvent retention capacity, and gluten index measurements will be essential to fully validate the quality potential indicated by glutenin subunit composition.

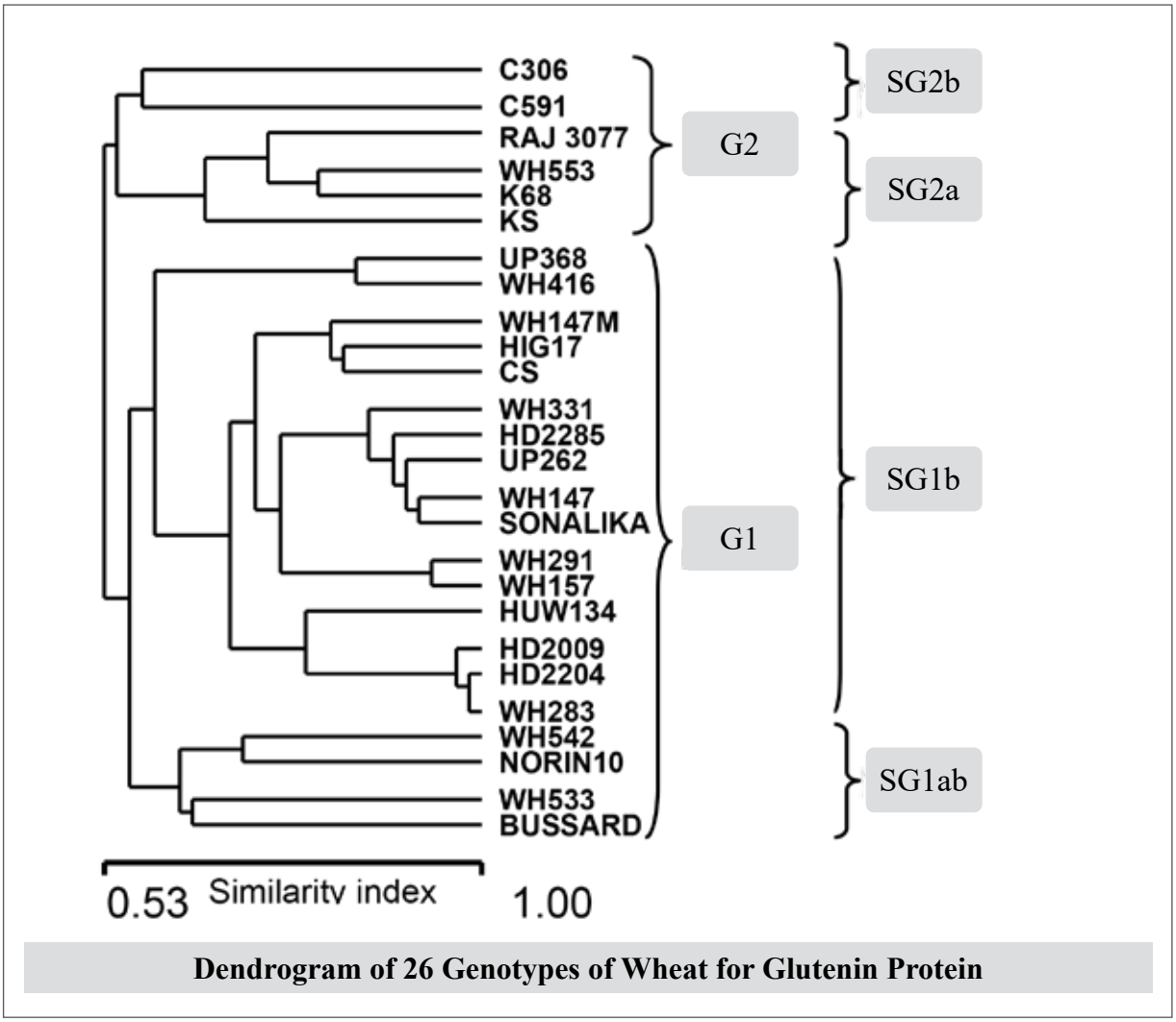

Figure 1. Dendrogram depicting genetic similarity among 26 wheat genotypes based on high molecular weight (HMW) glutenin subunit composition (Glu-1 loci).

Table 1. Profiles of high and low molecular weight glutenin protein subunits in bread wheat.

No.	Genotypes	Glu-A1	Glu- B1	Glu-D1
1	Bussard	1	7+9	5+10
2	C306	1	20	5+10
3	C591	1	20	5+10
4	CS	N	7+8	2+12
5	HD 2009	2*	7+8	2+12
6	HD 2204	2*	7+8	2+12
7	HD 2285	2*	7+8	
8	HIG 17	N	7+8	
9	HUW 134	N	7+8	2+12
10	K 68	N	17+18	
11	KS	N	17+18	2+12
12	Norin 10	N	7+9	2+12
13	Raj 3077	N	11+18	
14	Sonalika	2*	7+9	
15	UP 262	2*	7+8	
16	UP 368	2*	13+16	
17	WH 147	2*	7+8	2+12
18	WH 147M	N	7+8	
19	WH 157	2*	7+9	
20	WH 283	2*	7+8	
21	WH 291	2*	7	
22	WH 331	2*	7+8	
23	WH 416	2*	13+16	
24	WH 533	1	7+9	5+10
25	WH 542	N	7+9	
26	WH 553	N	17+18	

Table 2. SDS-sedimentation test-based bread-making quality scores allocated to HMW glutenin subunits (single and pairs).

Score	Glu-A1	Glu-B1	Glu-D1
4 (good)	-	-	5+10
3	1	17+18	-
3	2*	7+8	-
3	-	13+16	-
2	-	7+9	2+12
2	-	-	3+12
1 (poor)	Null	7	4+12
1	-	6+8	2 + 10
1	-	20	-

Table 3. HMW glutenin subunit composition and Glu-1 quality score.

No.	Genotype	Subunits	Total
1	Bussard	3 + 2 + 4	9
2	C-306	3 + 2 + 4	9
3	C-591	3 + 2 + 4	9
4	CS	1 + 3 + 2	6
5	HD 2009	3 + 3 + 2	8
6	HD 2204	3 + 3 + 2	8
7	HD 2285	3 + 3 + 0	6
8	HIG 17	1 + 3 + 0	4
9	HUW 134	1 + 3 + 2	6
10	K 68	1 + 3 + 0	4
11	KS	1 + 3 + 2	6
12	Norin 10	1 + 2 + 2	5
13	Raj 3077	1 + 2 + 0	3
14	Sonalika	3 + 2 + 0	5
15	UP 262	3 + 3 +0	6
16	UP 368	3 + 3 + 0	6
17	WH 147	3 + 3 + 2	8
18	WH 147M	1 + 3 + 0	4
19	WH 157	3 + 2 + 0	5
20	WH 283	3 + 3 + 2	8
21	WH 291	3 + 1 + 0	4
22	WH 331	3 + 3 + 0	6
23	WH 416	3 + 3 + 0	6
24	WH 533	3 + 2 + 4	9
25	WH 542	1 + 2 +0	3
26	WH 553	1 + 3 + 0	4

Table 4. Literature-reported functional quality associations of major HMW-GS combinations identified in this study

HMW-GS Combination	Reported SDS / Dough Strength	Literature Source
1 / 7+9 / 5+10	High	Payne et al., 1987; Lukow et al., 1989
2* / 7+8 / 2+12	Moderate	Bedó et al., 1995
Null / 7 / 2+12	Low	Jain et al., 2002

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