

Biochemical and Molecular Characterization of Bangladeshi Wheat Varieties for Bread-Making Quality

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Abstract

Twenty-six wheat genotypes including 18 Bangladeshi varieties and 8 varieties/lines collected from different countries were evaluated for their bread-making quality. Total grain protein content was measured using Kjeldahl method. Presence of the high molecular weight glutenin subunits (HMW-GS) and their corresponding genes were characterized through SDS-PAGE and PCR based methods. Total protein content of 53.85% of the genotypes ranged between 12 and 14% which is considered as a suitable range of protein for making bread. At the Glu-A1 locus, Ax2* alleles were found with a frequency of 84.62%. At the Glu-B1 locus, 4 different alleles; Bx7, Bx7+By8, Bx7+By9 and Bx17+By18 were detected. At the Glu-D1 locus, PCR test result showed that 61.11% Bangladeshi wheat varieties contain Dx5+Dy10, regarded as the best allele for making bread. Four genotypes, Kalyansona, Sonora-64, Pavon-76 and BARI Gom 28, were found to have the highest quality score of 10. Among those, Kalyansona and BARI Gom 28 had the best HMW-GS combination of Ax2*, Bx17+By18, Dx5+Dy10 and Ax2*, Bx7+By8, Dx5+Dy10, respectively. This study will be important for specifying wheat genotypes for the food industry and further breeding for bread-making quality.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops in the world. It occupies 17 per cent of the total cultivated land and is the staple food for 35 per cent of the world's population (International Development Research Centre 2010). In Bangladesh, wheat is the second most important cereal crop after rice (Hossain and Teixeira 2013). It is the most preferred crop for making

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bread (Dewettinck et al. 2008). There are two types of wheat on the basis of endospermic texture, i.e. soft wheat and hard wheat. Hard wheat flours are suitable for making bread and soft wheat flours are suitable for making cookies and cakes (Dubcovsky et al. 1998).

Gluten, the main endosperm storage protein of wheat seeds is very important for determining the bread-making quality of flour. Compared to rye and barley, quality and concentration of gluten proteins is higher in wheat (Goesaert et al. 2005) making it the most common and suitable grain for baking bread. Gluten forms a continuous viscoelastic protein network when flour is mixed with water to form dough. Viscoelastic properties of gluten enable the wheat flour to be made into bread, cakes, biscuits and other food products. High molecular weight glutenin subunits (HMW-GS) is the most important group of gluten protein that determines the bread-making quality in wheat (*T. aestivum*) (Malik 2009). Dough elasticity is considered to be very important property of wheat for making bread. It is mainly determined by the composition of HMW-GS (Xu et al. 2008).

Generally, 3 - 5 HMW-GS alleles are expressed in each wheat cultivar. Allelic variations in HMW-GS and presence and absence of specific alleles play the critical role in determining bread-making quality. Presence of alleles Ax1, Ax2*, Bx17+By18, Bx14+By15 and Dy5+Dy10 in different combinations are considered as good for making quality breads. On the other hand, presence of null or Dx2+Dy12 showed negative impact on bread quality (Payne et al. 1987, Hamer et al. 1992, Gupta et al. 1996, Ahmad 2000).

It is generally assumed that Bangladeshi wheat varieties are not suitable for making bread. No information is available presently on the composition of HMW-GS in the Bangladeshi wheat varieties. Knowledge about the composition of HMW-GS is important for selecting varieties suitable for making good quality bread and breeding for improving wheat quality. Therefore, the present study was designed to identify HMW-GS of wheat varieties grown in Bangladesh along with some exotic genotypes in relation to the bread-making quality both at the protein and molecular levels using SDS-PAGE and PCR based techniques.

Materials and Methods

Twenty six wheat genotypes were selected for the study; 18 Bangladeshi wheat varieties: Kheri, Balaka, Ananda, Kanchan, Akbar, Barkat, Aghrani, Protiva, Sourav, Gaurav, Shatabdi, Sufi, Bijoy, Prodip, BARI Gom 25, BARI Gom 26, BARI Gom 27 and BARI Gom 28; two Indian varieties: Kalyansona and Sonalika; two varieties developed by CIMMYT (Mexico): Sonora- 64 and Pavon- 76; two British

varieties: Mulika and Paragon; two Australian lines developed by CSIRO: Westonia 5907, Westonia 5924.

Total nitrogen content of the wheat materials were determined by Kjeldahl method and the total amount of protein was estimated by multiplying the total nitrogen values with the conversion factor for wheat endosperm 5.7 (Morel and Bar-L'Helgouac'H 2000).

Protein extraction and SDS-PAGE analyses were done following the method described in the MASWHEAT (Marker Assisted Selection in Wheat) of UC Davis University, USA (<http://maswheat.ucdavis.edu/>). Proteins were extracted from 1-2 seeds of each wheat genotype. High molecular weight glutenin subunits were analysed by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) in 10% polyacrylamide gel (T=10%) with 1.28% cross-linker concentration (C=1.28%).

The electrophoresis was carried out in a Bio-Rad mini protein 3 system (Bio-Rad, USA) having gel size 8.3-7.3 cm. The samples were loaded at 25 μ l/lane. Electrophoretic separations were carried out at constant 200V for 1.5 hours. Following electrophoresis, gels were stained in a solution of methanol (400 ml), acetic acid (100 ml), Coomassie Blue R 250 (1 g) and distilled water (500 ml) for 16 hours followed by de-staining in tap water for 24 hrs with occasional shaking and change of water. The protein bands were imaged through gel documentation system (Alpha Inotech, USA). A molecular weight marker (10-200 KDa) was used for comparison (Wide Range Protein Molecular Weight Unstained Marker, Cat # BSM0661, Bio Basic Inc., Canada). The HMW-GSs individual or subunit pairs were used to predict the bread-making quality on the basis of the Glu-1 scoring system (Payne et al. 1987).

The 26 genotypes were grown in small plastic pots in the greenhouse. Young and tender leaves were harvested when they were 10 days old and 100 mg sample from each was used for DNA extraction. Genomic DNA was isolated by following the protocol of Genomic DNA Mini Kit (Plant) (Geneaid, Version: 04-24-13). Polymerase chain reaction (PCR) was carried out in a 20 μ l reaction volume containing 2 μ l 10 \times buffer (MgCl₂), 10 mM dNTPs (0.4 μ l), 5U/ μ l Taq DNA polymerase (Invitrogen) (0.5 μ l), 5 pmol/ μ l forward primer (2.0 μ l), 5 pmol/ μ l reverse primer (2.0 μ l), 50 ng/ μ l DNA template (2.0 μ l) and 11.1 μ l water. Sequences of PCR primers and fragment sizes are shown in Table 1. The PCR products were analyzed by electrophoresis in 1% agarose gel at 100V for 1 hr and visualized over ultraviolet light using a gel documentation system (Alpha Inotech, USA).

Table 1. PCR primers of the molecular markers used in the study.

Marker/ gene	Forward and reverse primers (5' - 3')	Allele	Fragment size (bp)	References
Ax2*	F: ATGACTAAGCGGTTGGTTCTT R: ACCTTGCTCCCCTTGCTTT	Glu-Ax2*	1319	Ma et al. (2003)
Ax1	R: ACCTTGCTCCCCTTGCTCCTG			
Bx7	F: ATGGCTAAGCGCCTGGTCTT R: TGCCTGGTCGACAATGCGTCGCTG	Glu-Bx7	2373	(Anderson and Greene 1989)
ZSBy8F5/ R5	F:TTAGCGCTAAGTGCCGTCT R:TTGTCCTATTTGCTGCCCTT	Glu-By8	527	Lei et al. (2006)
ZSBy9aF 1/R3	F:TTCTCTGCATCAGTCAGGA R:AGAGAAGCTGTGTAATGCC	Glu-By9	707/662	Lei et al. (2006)
BxFp	F: CGCAACAGCCAGGACAATT R: AGAGTTCTATCACTGCCTGGT	Glu-Bx17	675	Ma et al. (2003)
Dx5	F: CGTCCCTATAAAAGCCTAGC R: AGTATGAAACCTGCTGCGGAC	Glu-D1d	450	Ma et al. (2003)

Results and Discussion

Total protein content of the wheat genotypes studied varied between 11.08 and 13.7% (Table 2). Previous reports also showed that protein percentage among different Bangladeshi wheat varieties ranged between 11-14% (Kamal et al. 2003, Alam 2012, Hakim et al. 2012). Protein percentage ranging from 12-14% in wheat flour is desirable for making bread. Generally harder the wheat, higher the protein content in the flour and hard wheat flours are suitable for making bread (North American Millers' Association 2018). Total protein content of more than half of the genotypes (54.54%) ranged between 12-14% which is considered as a suitable range of protein for making bread.

Glutenin subunits having molecular weight of above 80 kDa are considered as HMW-GSs (Bietz and Wall, 1972). The SDS-PAGE results from the present study showed that molecular weight of all the identified bands ranged from 85-135 kDa (Fig. 1).

Three different types of Glu-A1 alleles were found in all 26 wheat genotypes: Ax1, Ax2* and the null allele. Ax2* was the most common allele found in almost 90% Bangladeshi wheat varieties and both of the Australian wheat varieties used in this experiment. In the Glu-1B locus, a total of four different alleles were found: Bx7, Bx7+By8, Bx7+By9 and Bx17+By18 (Table 3). Bx7 was a common

allele found in 90.9% of all the genotypes. In three genotypes (Lane 1, 3 and 5; Fig. 1), presence of both Bx7 and Bx17+By18 alleles were seen in SDS-PAGE. In Glu-1D loci three types of alleles were found: Dx5+Dy10, Dx12, Dx2+Dy12 (Table 3). Dx5+Dy10 and Dy12 alleles were found in 61.54 and 38.46% of the genotypes, respectively. Only one Bangladeshi wheat variety (BARI Gom 25) showed the presence of Dx2+Dy12 allele.

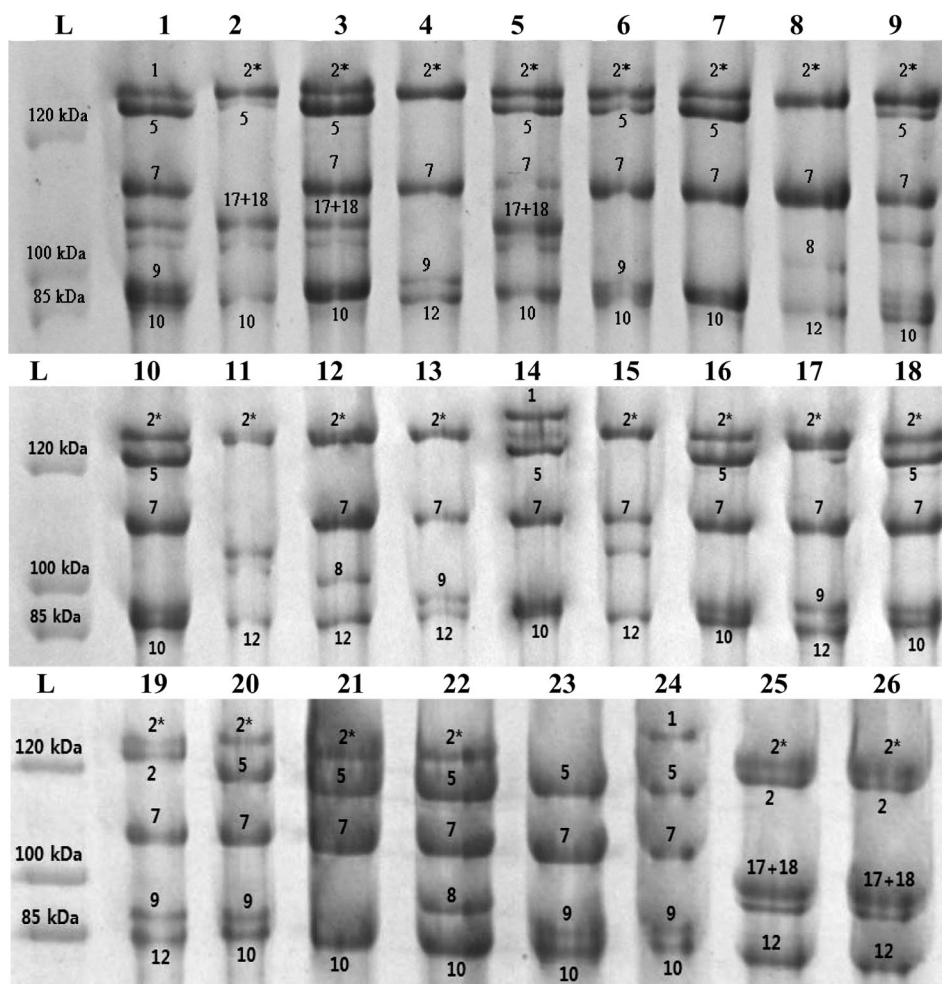


Fig. 1. SDS-PAGE of major allelic forms of HMW subunits present in wheat genotypes 1-26. [1 = Kheri; 2 = Kalyansona; 3 = Sonora-64; 4 = Sonalika; 5 = Pavon-76; 6 = Balaka; 7 = Ananda; 8 = Kanchan; 9 = Akbar; 10 = Barkat; 11 = Aghrani; 12 = Protiva; 13 = Sourav; 14 = Gaurav; 15 = Shatabdi; 16 = Sufi; 17 = Bijoy; 18 = Prodip; 19 = BARI Gom 25; 20 = BARI Gom 26; 21 = BARI Gom 27; 22 = BARI Gom 28; 23 = Mulika; 24 = Paragon; 25 = Westonia 5907; 26 = Westonia 5924].

Table 2. Total protein percentage of the wheat genotypes under study.

Name of the varieties and lines	Protein (%)	Name of the varieties and lines	Protein (%)
1. Kheri	12.24	14. Gaurav	12.24
2. Kalyansona	11.89	15. Shatabdi	11.43
3. Sonora- 64	11.25	16. Sufi	13.06
4. Sonalika	13.06	17. Bijoy	12.24
5. Pavon- 76	11.25	18. Prodip	12.71
6. Balaka	11.43	19. BARI Gom 25	13.53
7. Ananda	11.89	20. BARI Gom 26	11.08
8. Kanchan	12.24	21. BARI Gom 27	11.75
9. Akbar	13.06	22. BARI Gom 28	11.75
10. Barkat	12.42	23. Mulika	9.97
11. Aghrani	12.24	24. Paragon	14.69
12. Protiva	11.89	25. Westonia 5907	11.25
13. Sourav	13.70	26. Westonia 5924	12.42

PCR test results showed that Ax2* and Bx7 alleles were present in 84.62% and 84.62% of the genotypes studied which is nearly matched the results obtained by SDS-PAGE analysis. Although the presence of By8 and By9 alleles were observed in SDS-PAGE, no bands were obtained by PCR using By8 and By9 specific primers. Bx17 specific primers amplified the expected band from five genotypes. PCR test was done to confirm the presence of Bx7 and Bx17+18 alleles in same varieties. Two genotypes (Sonora-64 and Pavon-76) showed amplification for both Bx7 and Bx17+By18 in PCR (Fig. 2C, D, lane 3 and 5). Presence of the Dx5 allele was found in 61.54% of the genotypes evaluated which is consistent with the results obtained from the SDS-PAGE analysis except for 2 genotypes, Balaka and Kanchan (Figs 1 and 2E, lane 6 and 8).

In SDS-PAGE, Ax1 allele in 'Kheri' (Fig. 1; lane 1) looks different than the Ax1 allele found in Gaurav and Paragon (Fig. 1; lane 14 and 24), but repeated PCR analyses with Ax1 specific primers showed positive results confirming the presence of Ax1 allele in Kheri. In Mulika in SDS-PAGE no allele was found in "A" locus (null allele) (Fig. 1; lane 23) but later PCR analysis showed positive result for Ax1 allele (Fig. 3; lane 23). This might happen when the gene is present but not expressed into a protein. Presence of Ax2* in the combination with Dx5+Dy10 are normally associated with superior bread quality (Payne et al. 1987, Dong et al. 2009). Almost half (46.15%) of the wheat genotypes from the present study have this combination and can be considered as suitable for making good quality bread. These genotypes can also be used in breeding programmes for incorporating any of these alleles into new wheat varieties.

Table 3. Presence of HMW glutenin subunits detected by SDS-PAGE and PCR based analyses from 26 wheat genotypes under study.

Sl. No.	Genotypes	HMW-GS/alleles*						Quality score**
		SDS-PAGE Locus			PCR Locus			
		Glu-A1	Glu-B1	Glu-D1	Glu-A1	Glu-B1	Glu-D1	
1	Kheri	Ax1	Bx7+By9	Dx5+Dy10	Ax1	Bx7	Dx5	9
2	Kalyansona	Ax2*	Bx17+By18	Dx5+Dy10	Ax2*	Bx17+By18	Dx5	10
3	Sonora- 64	Ax2*	Bx7/Bx17+By18	Dx5+Dy10	Ax2*	Bx7/Bx17+By18	Dx5	8/10
4	Sonalika	Ax2*	Bx7+By9	Dy12	Ax2*	Bx7	-	>5
5	Pavon- 76	Ax2*	Bx7/Bx17+By18	Dx5+Dy10	Ax2*	Bx7/Bx17+by18	Dx5	8/10
6	Balaka	Ax2*	Bx7+By9	Dx5+Dy10	Ax2*	Bx7	-	9
7	Ananda	Ax2*	Bx7	Dx5+Dy10	Ax2*	Bx7	Dx5	8
8	Kanchan	Ax2*	Bx7+By8	Dy12	Ax2*	Bx7	Dx5	>6
9	Akbar	Ax2*	Bx7	Dx5+Dy10	Ax2*	Bx7	Dx5	8
10	Barkat	Ax2*	Bx7	Dx5+Dy10	Ax2*	Bx7	Dx5	8
11	Aghrani	Ax2*	-	Dx12	Ax2*	-	-	>3
12	Protiva	Ax2*	Bx7+By8	Dy12	Ax2*	Bx7	-	>6
13	Sourav	Ax2*	Bx7+By9	Dy12	Ax2*	Bx7	-	>5
14	Gaurav	Ax1	Bx7	Dx5+Dy10	Ax1	Bx7	Dx5	8
15	Shatabdi	Ax2*	Bx7	Dy12	Ax2*	Bx7	-	>4
16	Sufi	Ax2*	Bx7	Dx5+Dy10	Ax2*	Bx7	Dx5	8
17	Bijoy	Ax2*	Bx7+By9	Dy12	Ax2*	Bx7	-	>5
18	Proddip	Ax2*	Bx7	Dx5+Dy10	Ax2*	Bx7	Dx5	8
19	BARI Gom 25	Ax2*	Bx7+By9	Dx2+Dy12	Ax2*	Bx7	-	7
20	BARI Gom 26	Ax2*	Bx7+By9	Dx5+Dy10	Ax2*	Bx7	Dx5	9
21	BARI Gom 27	Ax2*	Bx7	Dx5+Dy10	Ax2*	Bx7	Dx5	8
22	BARI Gom 28	Ax2*	Bx7+By8	Dx5+Dy10	Ax2*	Bx7	Dx5	10
23	Mulika	Null	Bx7+By9	Dx5+Dy10	Ax1	Bx7	Dx5	7
24	Paragon	Ax1	Bx7+By9	Dx5+Dy10	Ax1	Bx7	Dx5	9
25	Westonia 5907	Ax2*	Bx17+By18	Dx2+Dy12	Ax2*	Bx17+By18	-	8
26	Westonia 5924	Ax2*	Bx17+By18	Dx2+Dy12	Ax2*	Bx17+By18	-	8

* Detected HMW-GS/alleles are mentioned below each HMW glutenin locus of the wheat genome ('-' = where the correct allele could not be determined).

**Total of quality scores (Payne et al. 1987) assigned to individual HMW glutenin subunits or subunit pairs (4 for Dx5+Dy10, 3 for Ax1, Ax2*, Bx7+By8, Bx17+By18, 2 for Bx7+By9, Dx2+Dy12, 1 for Null, Bx7, Bx6+By8).

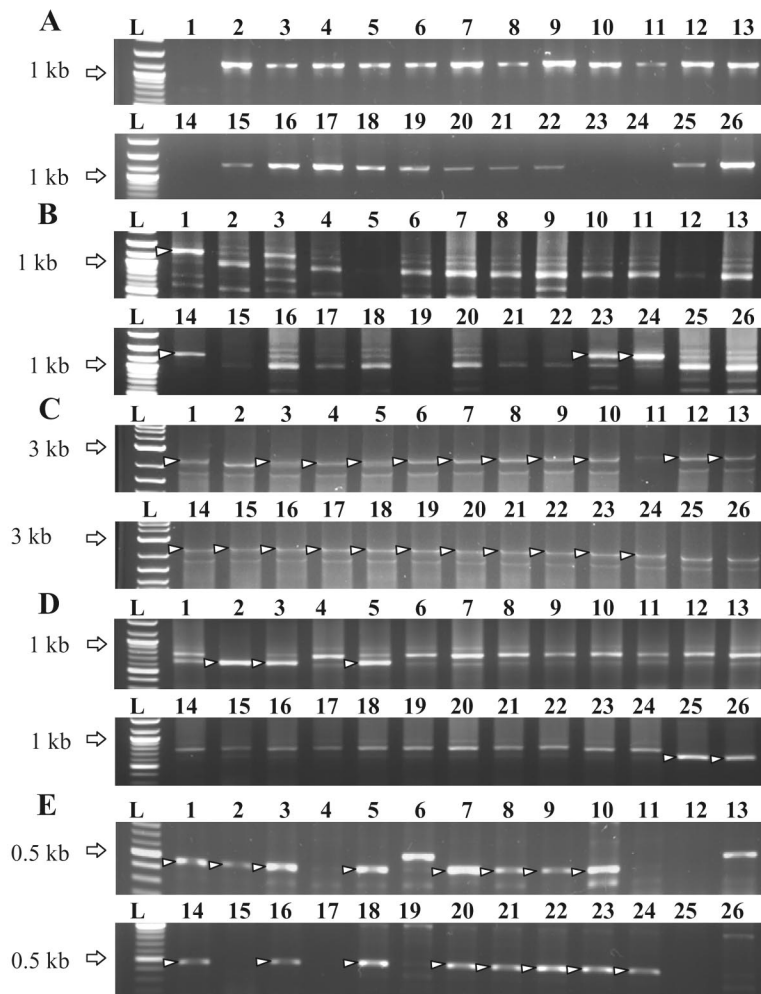


Fig. 2. PCR amplification of different HMW-GS alleles from 26 wheat genotypes. (A) Ax2* allele; (B) Ax1 allele; (C) Bx7 allele; (D) Bx17 allele and (E) Dx5 allele [Bands for the respective alleles are arrowed where applicable; lanes 1= Kheri; 2=Kalyansona; 3=Sonora- 64; 4=Sonalika; 5=Pavon-76; 6=Balaka; 7=Ananda; 8=Kanchan; 9=Akbar; 10=Barkat; 11=Aghrani; 12=Protiva; 13=Sourav; 14=Gaurav; 15=Shatabdi; 16=Sufi; 17=Bijoy; 18=Prodip; 19=BARI Gom25; 20=BARI Gom 26; 21=BARI Gom 27; 22=BARI Gom 28; 23=Mulika; 24=Paragon; 25=Westonia 5907; 26=Westonia 5924; L = DNA ladder].

Marchylo et al. 1992 and Butow et al. 2003 stated that Bx7 enhanced dough quality, although quality score of Bx7 is very low (1) according to (Payne et al. 1987). Most of the (94.44%) Bangladeshi genotypes possess Bx7 allele that can contribute in enhancing dough quality. Presence of allelic pair Bx17+By18 in wheat varieties has also been reported to be good for bread-making quality (Xu

et al. 2008, Dong et al. 2009, Liang et al. 2010) having a high quality score 3 (Payne et al. 1987). Kalyansona, Sonora-64, Pavon-76, Westonia-5907 and Westonia 5924 having the above allelic combination could be considered as good bread-making wheat genotypes. However, Sonora-64 and Pavon-76 showed presence of both for Bx7 and Bx17+By18 alleles both in SDS-PAGE and PCR analysis (Table 3).

The Glu-D1 locus has the largest effect on bread-making quality (Krystkowiak et al. 2017). The combination of Dx5 with Dy10 is associated with good bread quality (Payne et al. 1981, Payne et al. 1987, Popineau et al. 1994, Cong et al. 2007) and the quality score for this allele is 4, which is the highest score among all the HMW-GSs (Payne et al. 1987). Therefore, 61.54% of the genotypes evaluated in the present experiment having Dx5+Dy10 allelic pairs could be considered of very good bread-making quality. On the other hand, allelic pair Dx2+Dy12 is associated with poor bread quality and has been assigned low quality score (2). Only BARI Gom 25 a Bangladeshi wheat variety showed positive result for Dx2+Dy12. However, in 'A' locus, Ax2* allele has been found in this variety which is usually associated with high bread-making quality. Further bread baking tests might be able to show actual quality status of this variety.

Thirteen different allelic combinations were detected in the present study with a total of 14 Glu-1 loci. The most common allelic combination (2*, 7 and 5+10) was found in 23.08% of the genotypes. Both Ax2*, Bx17+By18, Dx5+Dy10 and Ax2*, Bx7+By9, Dy12 were the second most frequent allelic combinations and were detected in 11.54% of the genotypes. The overall quality scores displayed a range from >3 to 10, however generally good quality score of eight was more frequent (42.31). The highest quality scores of 10 and 9 were observed in 15.38 and 15.38% of the genotypes, respectively. Both Sonora-64 and Pavon-76 have been scored 8 and 10 since both of them showed presence of Bx7 and Bx17+By18 alleles in SDS-PAGE and PCR. Kalyansona and BARI Gom 28 had the highest quality score 10 and the best HMW-GS combination of Ax2*, Bx17+By18, Dx5+Dy10 and Ax2*, Bx7+By8, Dx5+Dy10, respectively (Table 3). However, in PCR no result was obtained for By8 and By9.

On the basis of allelic compositions and gluten quality scores assigned according to Payne et al. (1987), one Bangladeshi variety BARI Gom 28 has very good bread-making alleles and can be used for producing high quality bread. Other Bangladeshi wheat genotypes e.g., Balaka, Ananda, Akbar, Barkat, Gaurav, Sufi, Prodip, BARI Gom 26 and BARI Gom 27 are also of good bread-making quality as these varieties have important bread quality determining alleles: Ax1, Ax2* and Dx5+Dy10, thereby their greater utility in bread-making

quality improvement breeding is suggested. Results presented here are based on laboratory analyses of protein and DNA markers only. Further investigation by rheological studies of the dough properties and actual baking experiments will help to confirm the findings of this study.

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