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Investigating the role of high molecular weight glutenin subunits (HMW-GS) protein in end use quality of Indian flat breads

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Indian unleavened flat breads more commonly known as *chapati* are core to existence for two-third of its population. Glutenins and gliadins constitute gluten which gives extensibility and elasticity to the dough, traits which are of great importance in bread making. However, limited studies have been attempted to explore the relationship and functionality of the protein subunits with the quality of the unleavened flat breads. *Chapati* characterization and molecular analysis was carried on two set of genotypes firstly, different commercial wheat cultivars and genetic stocks for various quality parameters, secondly three back cross derived recombinant populations from the parents with different HMW-GS at *Glu 1B* locus to associate *chapati* quality with the glutenin subunits. Significant variation obtained in the genotypes revealed that tall varieties are distinct in quality followed by derivatives of C 306 and C 591 (7.8 for both DI 9 and DI 105). The SDS-PAGE analysis of the high molecular weight glutenin protein revealed that the subunit '20' at *Glu1B* locus is unique to tall traditional wheats. However, such investigations in the recombinant populations indicated complex inheritance of this trait.

Keywords: HMW-GS, SDS-PAGE, *gliadin, glutenins, Glu1B*, C 306

Introduction

Wheat (*Triticum aestivum*) is known to be a major source of energy and protein for human nutrition. Sixty five per cent of wheat grain is directly used as food, indicating its importance as main staple food. Wheat is consumed in many different forms such as bread, biscuits, noodles, macaroni, spaghetti, cakes, and pizzas etc. However, in Indian sub-continent and parts there of, wheat is mainly consumed in the form of *chapati* (unleavened flat breads), which is prepared from whole wheat meal (Rao *et al*, 1986)[1] Wheat protein is unique as it gives elasticity and extensibility to the wheat dough which determines the quality of the end product. These traits of wheat dough are due to the glutenins and the gliadins which in combination form the gluten. Wheat proteins are extensively studied due to the fact that there is a possible relationship between molecular size of wheat glutenin proteins and the bread making performance. Payne (1987)[2] devised a system of scoring wheat cultivars on the basis of the allelic subunit types of HMW glutenins for bread making quality. Similarly, Wrigley *et al* (1982)[3] reported the importance of gliadins in bread making quality of wheat. These and many more studies on wheat proteins have led to a better understanding of the genetics of the bread making quality of wheat. However, in spite of the predominant position of the *chapati* in the Indian diet, information on the relation of glutenin and gliadin with *chapati* making quality of wheat is poorly understood. Relatively small numbers of studies have been carried out to investigate and improve the quality of the *chapatis.* Most of these studies have involved the released varieties and the genetic stocks and were focused either on the technological aspects or on flour blends for enhancing the end use quality.

Variation in the glutenin subunits and their relation to the *chapati* making quality as so far attracted the use of released varieties in the present investigation. Very limited studies have tried to explore the relationship and functionality of the protein subunits with the quality of the unleavened flat breads. Some of the released varieties which are known to have best *chapati* quality are the traditional tall varieties or their derivatives. The advent of high yielding, input responsive semi-dwarf wheats in India has somehow led to the quality to be sidelined and hence this feature of the tall varieties got eroded from the breeding programmes. Varieties with good quality *chapatis*, including C 306 (best *chapati* quality variety), show the presence of HMW-GS '20' at *Glu* B1 locus

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(Sreeramulu *et al,* 2001)[4]. Misra (1998)[5] had earlier speculated that subunit '20' alone or in tandem with other subunits might provide some clue for screening breeding material for *chapati* quality. He also emphasized that varieties with high water absorption potential and its maximum retention need to be developed. Hemalatha *et al* (2013)[6] studied the effect of added arabinoxylans on the rheological properties of wheat dough, but could not come up with a concrete understanding of their role in *chapati* making quality. The role of high molecular weight dextrins in this process might provide some help in understanding the prolonged storage capacity of C 306. Ram (2003)[7] evaluated 172 wheat varieties including 25 durum wheat cultivars for HMW-GS composition using SDS-PAGE. The relationship between HMW-GS and sedimentation tests for dough strength was studied. The protein subunits 13 + 16 and 6 + 8 were found positively correlated with improved dough strength. Study by Srivastava *et al* (2003)[8] overall *Glu*-1 score with *chapati* quality in a set of 15 Indian wheats in order to predict the suitability of wheat for *chapati* making. The study clearly indicated that both quantitative and qualitative characters of the proteins influenced the *chapattis* making potential of the cultivars. Overall quality score of *chapati* was positively correlated with gluten content (r = 0.64), sedimentation value (r = 0.60) and *Glu*-1 score (0.58). The wheat varieties, with 5 + 10 glutenin subunit at *Glu* 1D, yielded excellent *chapatis.* The major limitations in the above studies were that, these were performed with different genotypes instead of populations/recombinant lines derived from parents with contrasting *chapati* quality traits. Das *et al* (2006)[9] evaluated selections differing in specific HMW subunits (5 + 10 & 2 + 12) of glutenins obtained through backcrossing for bread and *chapati* making quality. The HMW subunits 5 + 10 improved dough strength to give higher specific loaf volume and loaf that was more acceptable. The authors remarked that the significant differences were absent in baked *chapati*s indicating that individual high-molecular weight subunits/pairs are less likely to be a major factor in influencing *chapati* making quality.

The present investigation was carried out with an aim of understanding the role of HMW-GS and also exploring the functionality of HMW-GS *Glu 1B* 20 in the *chapati* making quality.

Materials and Methods

The research, conducted at Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India was largely built around following two types of plant materials:

i) Thirty three diverse wheat cultivars and genetic stocks for various quality parameters tested across two crop seasons;
ii) Three back cross derived recombinant populations from the parents with different HMW-GS at *Glu* 1B locus

The first set (Table 1) constituted of tall traditional cultivars of the pre-dwarfing era, their derivatives emanated from crosses with dwarf wheats, a set of present day cultivars excelling in yield along with one or two quality attribute such as, bread quality and grain appearance, stocks from the National Genetic Stocks Nursery (NGSN) which excelled in specific quality components and the commercial cultivars that

Table 1 — High molecular weight glutenin subunit composition and the *chapati* score of the genotypes evaluated

SNo	Genotype	Average *Chapati* score	*Glu* 1A	*Glu* 1D	*Glu* 1B
1	8A	7.9	N	2+12	20
2	9D	7.8	N	2+12	20
3	C 273	8.0	1	5+10	20
4	C 306	8.1	N	2+12	20
5	C 518	8.1	N	2+12	20
6	C 591	8.0	2*	2+12	20
7	WG 357	7.8	2*	2+12	20
8	PBW 343	7.6	1	5+10	7
9	PBW 534	7.4	2*	5+10	7
10	PBW 502	7.3	1	5+10	7
11	PBW 550	7.4	N	5+10	7
12	PBW 154	7.2	N	2+12	7+9
13	PBW 175	7.3	N	2+12	7+9
14	PBW 226	7.3	N	2+12	7+9
15	PBW 533	7.4	1	5+10	7
16	PBW 531	7.6	N	5+10	7
17	PBW 554	7.6	N	5+10	7
18	PBW 509	7.6	1	5+10	7+8
19	DI 105	7.8	1	2+12	20
20	DI 9	7.8	1	2+12	7+9
21	DBW 16	7.4	N	2+12	7+9
22	WH 423	7.7	1	5+10	8
23	WH 595	7.6	N	5+10	8
24	WH 712	7.4	N	5+10	8
25	WH 800	7.5	N	5+10	7
26	WH 1003	7.4	N	5+10	9
27	Lok 1	7.9	N	2+12	9
28	HI 1418	7.8	N	2+12	7+9
29	HI 1479	7.5	N	2+12	7+9
30	HD 2793	7.6	1	5+10	7+9
31	Pusa 5-3	7.2	1	5+10	7+9
32	K 0123	7.5	1	5+10	9
33	KYZ K2K-13	7.5	2*	5+10	7+8

rule the farmer's fields by virtue of yield but are average for quality traits. This set of diverse genotypes thus provides a quality spectrum of sufficient amplitude for deriving out any conclusions. This set was grown in replicated trials using standard agronomic practices with the aim of expressing the normal quality potential of the genotypes. The set used in first season was re-constituted in second year with some changes. Some genotypes with very low yield, red grains were replaced with wheats from the central zone of the country, which are known to have better quality attributes.

The second set of the plant material used was the back cross derived recombinant populations derived from the parents differing in the glutenin subunits at *Glu* 1B and *Glu* 1D loci. The recipient parents were the high yielding genotypes PBW343 and PBW534. The donors for best *chapati* quality were the tall varieties viz. C273, C306 and C518. The five parental lines were included in the analysis and these also formed a part of first set, allowing their placement in the overall range of variation for various traits. Following three populations (BC_1F_5 generation) were used: C273/PBW343//PBW343, C306/PBW534//PBW534 and C518/PBW 343//PBW343

Molecular Characterization

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) for Glutenin Subunits

One dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Smith and Payne, 1984)[10] with little modifications was used to study the protein profiles of the varieties/lines.

Extraction of Proteins

Two to three seeds of each line were grinded using plier with smooth surface. The crushed mixture was transferred to an Eppendorf tube. The extraction solution was prepared by mixing 4 ml distilled water, 1.7 ml of 3X sample buffer and 0.3 ml of 2-mercaptoethanol. After adding extraction solution, the contents were mixed on vortex mixture for 3 - 4 minutes. The samples were kept at room temperature for about 1 hour.

Preparation of Separating and Stacking Gels

To prepare separating gel, 7.25 ml of 35% acrylamide, 2.08 ml of 2% bisacrylamide, 5.1 ml distilled water and 9.4 ml Tris (pH 8.8) were mixed in a beaker. 0.25 ml of SDS, 0.94 ml of freshly prepared 1% ammonium persulphate and 30 µl of N,N,N,N' tetramethylenediamine (TEMED) were added and the solution was thoroughly mixed. The stacking gel was prepared by mixing 1.75 ml of 35% acrylamide, 0.75 ml of 2% bisacrylamide, 8.0 ml of distilled water and 2.5 ml of Tris (pH 6.8). 0.20 ml of SDS, 0.94 ml of freshly prepared 1% ammonium persulphate and 40 µl of N,N,N',N'tetramethylenediamine (TEMED) were added and the solution was thoroughly mixed. The protein samples were placed in boiling water for 10 - 12 minutes. The tubes were then centrifuged at 4000 rpm for 10 minutes. The supernatant so obtained was used for loading gel and run as per established procedure. Gel staining was done with methanol: GAA:distilled water and 0.5 g Coomassie Brilliant Blue-R250 dye. The gel was kept in the staining solution overnight. Gel destaining was done for 10 - 12 hour in a solution containing methanol GAA distilled water. The gels were analyzed under white light in gel documentation system. The band numbering was done according to Payne and Lawrence (1983)[11].

Chapati Making Characteristics

The whole meal was produced by grinding the grains in a laboratory stone grinder (*chakki*). The gap between the two stone discs was adjusted so as to pass the meal through 40 microns mesh sieve. The 50 g whole meal (*atta*) and optimum quantity of water were mixed mechanically for 2 min using Swanson mixer. The dough was evaluated for stickiness while rounding it up manually and kept in the humidity cabinet maintained at 30°C and 80% R H for 30 min The dough was sheeted to 2 mm thickness with the rolling pin and *chapatis* of 15 cm diameter were cut using appropriate die. *Chapatis* were baked on an automatic *roti-maker* having thermostatically controlled constant temperature for 20 sec on one side and for 40 sec on other. Finally, it was puffed for 10 sec by turning the *chapati* and bringing the upper plate of the *roti-maker* in contact with the *chapatis*. *Chapatis* were cooled to room temperature in the humidity cabinet and evaluated by a panel of trained judges using the evaluation characteristics for scoring viz., dough stickiness (5), puffing of *chapati* (5), color of *chapati* (5), texture of *chapati* (5), taste of *chapati* (5), flavor of *chapati* (5) and texture of *chapati* after 2 hrs (5). The total score was finally calculated out of a maximum of ten.

Results

The results of present study are presented according to the plant material used. Firstly, results from the set of genotypes were analyzed and then conclusions were

derived based on the data from the second set of plant material involving the recombinant populations.

Observation from the Set of Cultivars and Genetic Stocks

Significant genotypic differences over years were observed for *chapati* score of the genotypes. The mean *chapati* score (Table 1) ranged from 7.2 in 'Pusa 5-3' to 8.1 in case of 'C 306' during the first year of the trial. The *chapati* score showed variation between the groups of genotypes, with the tall wheats of pre-dwarfing era excelling over other groups. The tall varieties not only established themselves as a distinct group with highest *chapati* score but the next numerical best score (7.8 for both DI 9 and DI 105) were also observed in the derivatives of C 306 and C 591. The commercial high yielding cultivars gave intermediate *chapati* scores, significantly inferior to the best tall wheat i.e., C 306. Similar was the case of genetic stocks and other varieties which showed *chapati* scores well below the best entry i.e., C 306. Stocks excelling in one or more quality component (e.g. K 0123, WH 595, Pusa 5-3 for high protein content and WH 712, WH 800 for high sedimentation value) did not yield good *chapaties*. The advanced lines (PBW 531, PBW 534, PBW 554) used in the set performed almost similar to the commercially released varieties. On the whole the genetic stocks DI 9, DI 105 and WH 423 with *chapati* scores of 7.8, 7.8 and 7.7, respectively were adjudged to be at par with the best cultivar C 306. In the second year, significant genotypic differences were again observed for *chapati* score. The tall varieties were also found significantly superior to all other groups during second year, thus confirming their status as best *chapati* wheats. The commercial wheat group gave intermediate score. DBW 16 (having a perfect '*Glu*' score of 10) and PBW 533 were included in the set as they are identified for their excellent bread making properties. These varieties did not perform well for *chapati* making giving a score of 7.4 only. Among the released varieties known for *chapati* quality, Lok 1 (*chapati* score 7.9) performed better than others in the group though slightly lower than C 306. Thus Lok 1 was able to express good *chapati* quality outside Central Zone also, where it is recommended for cultivation. Similarly, HI 1418 also registered a *chapati* quality score of 7.8, at par with C 306. PBW 154 (7.2), PBW 175 (7.3) and PBW 226 (7.3) fell into next group with respect to *chapati* quality. These genotypes are regarded to be superior for *chapati* quality among released semi-dwarf varieties. The chapatti score of PBW 175 is attributable to C-306 in its parentage.

The high molecular weight (HMW) glutenin subunits of all the genotypes were analyzed using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 1). The genotypes along with different HMW subunits are given in Table 1. Also the average *chapati* score of the genotypes carrying different HMW-GS is given in Table 5a.

Glu 1A

Out of a total of 33 cultivars and genetic stocks studied for two years in the study, 18 (53%) genotypes were observed to have 'Null' allele at *Glu* 1A locus. 11 (32%) genotypes had subunit '1' at this locus, with remaining 5 (15%) genotypes having '2^{*}' glutenin subunit.

Glu 1D

The *Glu* 1D locus was observed to have either '5 + 10' or '2 + 12' glutenin subunit among all the genotypes studied. Fifteen (42%) genotypes showed presence of '2 + 12' subunit and 19 (58%) genotypes were observed to have '5 + 10' subunit. The good *chapati* quality wheats, such as C 306, C 591, C 518, Lok 1, PBW 175, PBW 226 were found to have '2 + 12' subunit at this locus. The presence of this subunit '2 + 12' which represents weaker gluten, in superior *chapati* quality wheats gives indication that stronger gluten may not be required to produce good *chapati*s. Other than above mentioned cultivars, the '2 + 12' subunit was observed to be present in 8A and 9D, the probable donors of superior *chapati* quality to the C-series and in the cultivars such as WG 357 and PBW 175, which have C varieties in their parentage. The commercial wheat variety DBW 16, the genetic stock DI 105 (dwarf version of C 591) and the advanced lines such as HI 1418 and HI 1479 also showed '2 + 12' subunit at *Glu* 1D locus.

Glu 1B

Much of the variation in the high molecular glutenin subunits was however observed at *Glu* 1B locus. Various subunits observed at this locus in the cultivars under study were '7 + 9' (30%), '20' (23%), '7' (23%), '9' (<1%), '7+8' (9%) and '8' (9%). The tall wheat group which excelled in *chapati* quality had '20' subunit at *Glu* 1B locus. This subunit was observed to be specific to the group except that DI

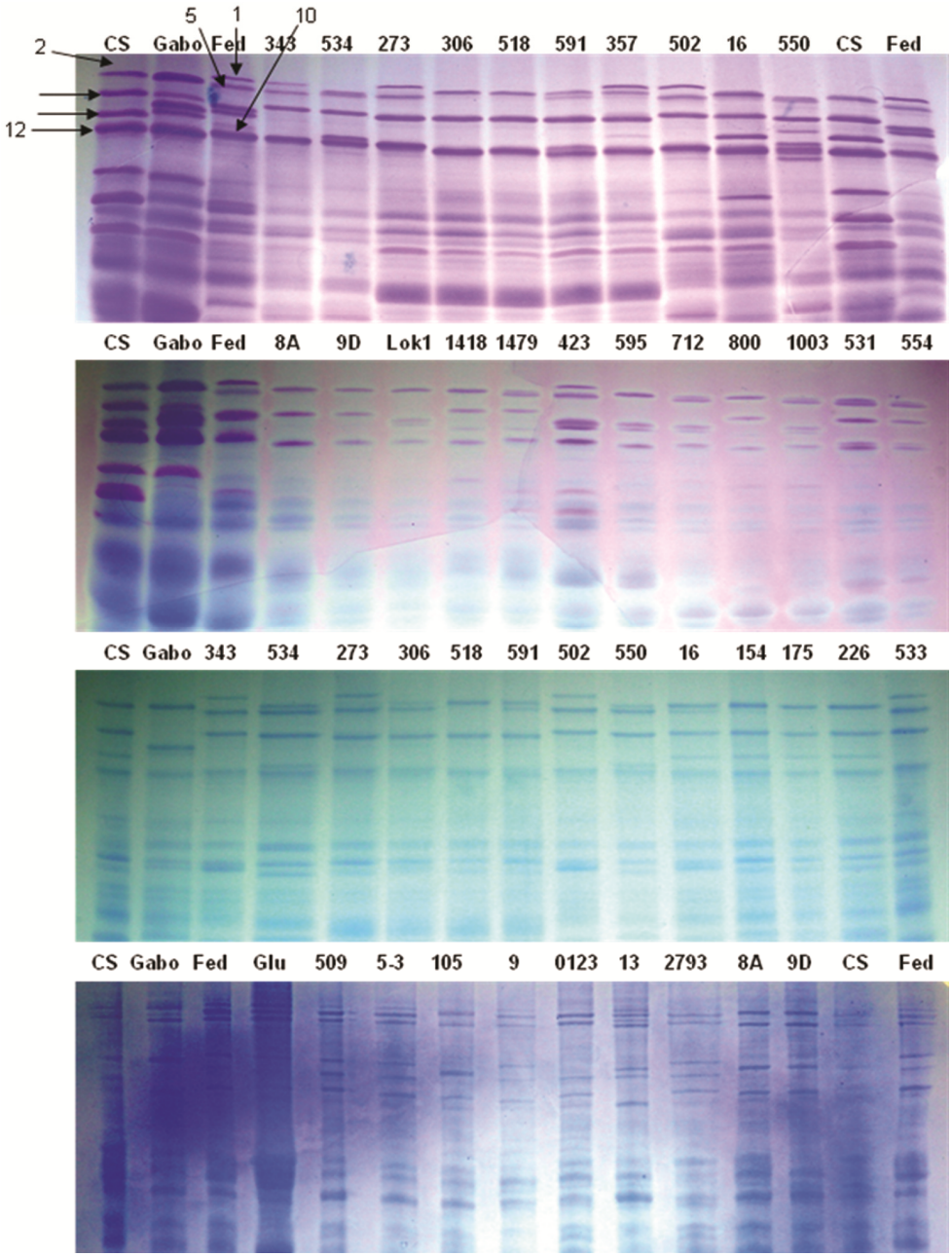

Fig. 1 — HMW-GS protein profile of the cultivars and genetic stock.

105 (dwarf version of C 591) also had this subunit. No other genotype in the set was found to have this subunit thus indicating that this subunit may play an important role in the superior *chapati* quality of tall C-series varieties.

Observation on Back Cross Derived Recombinant Populations (BC-RILs)

The BC-RIL populations, under study provide an opportunity for a real assessment of the role of *Glu* 1B subunit '20' in *chapati* making quality.

Significant genotypic differences were observed for *chapati* score in all the three populations *viz:* (C273/PBW343//PBW343), (C306/PBW534//PBW534) and (C518/PBW343//PBW343). The data on *chapati* score for the populations is given in Table 2, 3 and 4, respectively. Populations 'C273/PBW343//PBW343' and 'C518/PBW343//PBW343' showed normal distribution of lines for the trait, whereas in population 'C306/PBW534//PBW534' transgression of *chapati* score was observed on inferior side. In

Table 2 —High molecular weight glutenin subunit at *Glu 1B* locus of the lines in population C273/PBW343//PBW343

Entry No.	*Glu 1B* locus	*Chapati* score	Entry No.	*Glu 1B* locus	*Chapati* score
1	7	7.6	36	20	7.4
2	7	7.7	37	7	7.5
3	7	7.6	38	20	7.4
4	20	7.7	39	20	7.5
5	7	7.7	40	20	7.6
6	7	7.6	41	7	7.6
7	20	7.5	42	7	7.6
8	H	7.7	43	7	7.5
9	-	7.6	44	7	7.5
10	20	7.2	45	7	7.8
11	7	7.6	46	H	7.4
12	7	7.8	47	H	7.1
13	7	7.6	48	H	7.7
14	7	7.3	49	7	7.5
15	H	7.6	50	20	7.3
16	H	7.5	51	7	7.5
17	-	7.6	52	7	7.6
18	20	7.7	53	7	7.3
19	20	7.4	54	20	7.1
20	20	7.5	55	7	7.3
21	7	7.7	56	H	7.0
22	7	7.7	57	7	7.2
23	7	7.9	58	7	7.3
24	20	7.5	59	7	7.5
25	20	7.6	60	7	7.6
26	7	7.6	61	20	7.5
27	7	7.7	62	7	7.4
28	7	7.5	63	7	7.7
29	7	7.7	64	20	7.5
30	7	7.5	65	20	7.6
31	H	7.6	66	7	7.5
32	H	7.6	67	7	7.5
33	20	7.5	68	7	7.3
34	20	7.4	69	7	7.5
35	7	7.5	70	7	7.5

Table 3 —High molecular weight glutenin subunit at *Glu 1B* locus of the lines in population C306/PBW534//PBW534

Entry No.	*Glu 1B* locus	*Chapati* score	Entry No.	*Glu 1B* locus	*Chapati* score
1	20	7.7	36	7	7.3
2	20	8.0	37	7	6.9
3	7	7.9	38	7	6.9
4	7	7.9	39	7	6.9
5	7	7.9	40	20	7.1
6	7	7.9	41	7	7.3
7	20	7.4	42	7	7.2
8	20	7.7	43	7	7.0
9	20	7.6	44	20	7.2
10	7	7.8	45	20	7.0
11	H	7.9	46	7	7.3
12	20	7.7	47	7	7.1
13	20	7.9	48	7	7.4
14	20	7.8	49	7	7.3
15	20	7.9	50	20	7.5
16	7	7.9	51	7	7.1
17	7	7.9	52	H	7.0
18	H	7.8	53	7	7.4
19	7	7.7	54	H	7.0
20	20	8.0	55	7	7.2
21	7	7.6	56	20	7.2
22	H	7.5	57	20	7.1
23	7	7.4	58	7	7.1
24	7	7.0	59	H	7.0
25	7	7.2	60	20	7.0
26	20	7.0	61	7	7.2
27	20	7.2	62	7	7.0
28	20	7.2	63	20	7.0
29	20	7.0	64	20	6.9
30	H	6.9	65	20	7.1
31	7	7.0	66	20	7.2
32	20	7.3	67	7	7.2
33	20	7.2	68	7	7.2
34	20	7.2	69	7	7.0
35	7	7.1	70	7	7.2

population 'C273/PBW343//PBW343' the parental values of *chapati* score observed were 8.2 and 7.7 for C 273 and PBW 343, respectively. None of the genotypes were able to surpass C 273 for *chapati* score, whereas 56 out of 70 lines gave a score inferior to PBW 343. For 14 genotypes, the score ranged between the parental values. In case of population 'C306/PBW534//PBW534' the parental values observed for *chapati* score were 8.2 and 7.4 for C 306 and PBW 534, respectively. Twenty two genotypes out of 70 ranged between the parental values whereas remaining 48 gave a score inferior to PBW 534. Similarly, in population, 'C518/PBW343//PBW343' none out of 80 lines were found to outscore C 518 whereas 71 genotypes gave a score inferior to PBW 343. Only 9 genotypes gave a *chapati* score in parental range.

Discussion

Major differences in the glutenin subunits of the parents involved were identified at *Glu* 1B and *Glu* 1D loci (Fig. 2). For *Glu* 1D locus the donors were having subunit '2 + 12' except C 273 which had '5 + 10' whereas both the recipients i.e. PBW 343 and PBW 534 had '5 + 10' subunit. Since glutenin subunit '20' at *Glu* 1B locus has been documented to be associated with superior *chapati* quality of C series varieties, the recombinant populations represented themselves as an important experimental material to study the importance of this subunit, as the parents of all the three recombinant populations differed for this subunit. All the three populations show segregation for this *Glu* 1B allele (Table 5b). While the three C-series varieties carry subunit '20', other parents, i.e. PBW 343 and PBW 534 show the presence of subunit

Table 4 —High molecular weight glutenin subunit at *Glu 1B* locus of the lines in population C518/PBW 343//PBW343

Entry No.	*Glu 1B* locus	*Chapati* score	Entry No.	*Glu 1B* locus	*Chapati* score
1	-	7.6	41	7	7.6
2	H	7.5	42	H	7.5
3	H	7.2	43	7	7.6
4	7	7.4	44	7	7.6
5	20	7.2	45	7	7.4
6	-	7.5	46	20	7.4
7	7	7.7	47	7	7.6
8	-	7.8	48	7	7.5
9	H	7.4	49	7	7.5
10	-	7.4	50	20	7.5
11	7	7.4	51	7	7.4
12	20	7.5	52	7	7.2
13	20	7.4	53	7	7.2
14	20	7.4	54	H	7.0
15	H	7.5	55	20	7.3
16	-	7.6	56	7	7.4
17	20	7.4	57	7	7.5
18	7	7.4	58	7	7.5
19	7	7.5	59	7	7.2
20	20	7.6	60	7	7.2
21	20	7.5	61	7	7.5
22	20	7.7	62	7	7.3
23	20	7.6	63	20	7.3
24	H	7.6	64	20	7.1
25	20	7.4	65	20	7.4
26	20	7.7	66	7	7.3
27	H	7.5	67	7	7.4
28	7	7.7	68	7	7.1
29	7	7.4	69	20	6.8
30	7	7.4	70	20	6.9
31	7	7.8	71	7	7.0
32	20	7.6	72	7	7.3
33	20	7.8	73	7	7.3
34	20	7.8	74	7	7.1
35	7	7.3	75	7	7.4
36	7	7.6	76	20	7.3
37	7	7.7	77	20	7.2
38	20	7.5	78	20	7.3
39	7	7.5	79	20	7.1
40	7	7.5	80	20	7.1

'7'. The Table 2, 3 and 4 present the HMW-GS at *Glu1B* locus in the lines from recombinant populations 'C273/PBW343//PBW343', 'C306/PBW534//PBW534' and 'C518/PBW 343//PBW343', respectively.

The aim of the present study was to investigate the role of HMW-GS in determining the *chapati* making properties in bread wheat. The strategy aimed at using two different sets of plant materials (homozygous genotypes and recombinant populations) to associate *chapati* quality with the glutenin subunits. Significant variation obtained in the genotypes revealed that the cultivars such as C 306 remain unsurpassed for *chapati* quality in the Indian wheat programme to date. The mean *chapati* score showed variation

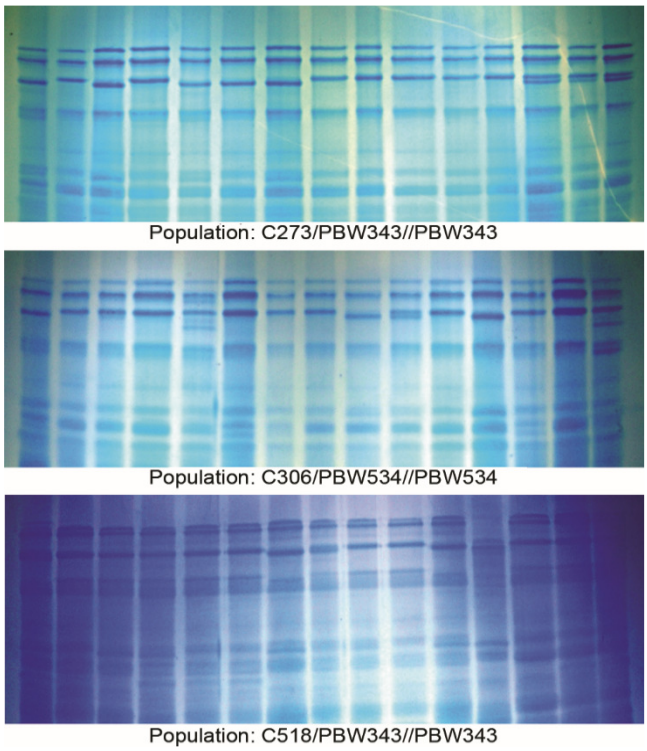

Fig. — 2 HMW-GS protein profiles of some lines from the recombinant populations.

Table 5a — The average *chapati* score of the genotypes carrying different HMW-GS

Glu 1A locus			*Glu 1B* locus						*Glu 1D* locus	
Null	1	2*	7	8	9	7+8	7+9	20	5+10	2+12
7.60 ± 0.064	7.59 ± 0.066	7.20 ± 0.433	7.47 ± 0.039	7.70 ± 0.104	7.60 ± 0.150	7.55 ± 0.050	7.24 ± 0.228	7.94 ± 0.046	7.40 ± 0.124	7.71 ± 0.023

Table 5b — The average *chapati* score of different recombinant lines in three populations carrying different HMW-GS

C273/PBW343//PBW343		C306/PBW534//PBW534		C518/PBW 343//PBW343	
Subunit 7	Subunit 20	Subunit 7	Subunit 20	Subunit 7	Subunit 20
7.55 ± 0.025	7.47 ± 0.035	7.33 ± 0.057	7.36 ± 0.066	7.42 ± 0.030	7.39 ± 0.047

between the groups of genotypes, with the tall wheats over other groups. The tall varieties not only established themselves as a distinct group with highest *chapati* score but the next numerical best score (7.8 for both DI 9 and DI 105) also went to the derivatives of C 306 and C 591. A high *chapati* score (7.9) was obtained by Lok 1, indicating its superior *chapati* quality. This trait however seems to be derived from another tall traditional variety NP 4 which is one of its parents. The genetic stocks, on the other hand failed to give good *chapatis* thus emphasizing the fact that good *chapati* quality resulted from a combination of different component traits and not because of one or two traits. The advanced breeding lines and the commercially grown varieties were intermediate in performance for the *chapati* score. As has been evident from most of the studies on wheat quality, the *chapati* making trait has eroded with efforts in increasing the wheat yields. Different studies in *chapati* quality (Revanappa, 2009; Yadav, 2010)[12,13] have also indicated similar finding, however the studies were mainly based on released cultivars. Earlier studies (Misra 1998, Sreeramulu *et al,* 2004)[4,5] have given an indication of importance of subunit '20' at *Glu* 1B locus in determining superior *chapati* quality of C 306. '7 + 9' subunit at *Glu* 1B locus was also found to be associated with good *chapati* quality genotypes such as Lok 1 and PBW 175. The frequency of presence of this subunit in the set of genotypes also refers to its importance in determining the quality of wheat varieties, as the set was not a random collection but genotypes known to excel in one or other component of quality were assembled. The SDS-PAGE analysis of the high molecular weight glutenin protein profiles of the genotypes revealed that the subunit '20' at *Glu 1B* locus is unique to tall traditional wheats.

The recombinant populations were derived using three donor parents (C 273, C 306 and C 518) and two recipients (PBW 343 and PBW 534). These parents differed for the HMW-GS at *Glu*-B1 and *Glu*-D1 loci. The results showed differences in HMW-GS profile at all the 3 major loci: *Glu*-A1, *Glu*-B1 and *Glu*-D1 of wheat varieties PBW 343 and C 306. For *Glu* 1D locus the donors were having subunit '2 + 12' except C 273 which had '5 + 10' whereas both the recipients i.e. PBW 343 and PBW 534 had '5 + 10'. Earlier reports have also suggested the differences between the genotypes for glutenin and gliadin subunits. An acid-PAGE analysis of gliadins from 159 Indian wheat cultivars developed during last five decades was accomplished by Ram *et al* (2005)[14]. The study indicated that both the cultivars PBW 343 and C 306 differed in their gliadin profiles. However, in all the three populations, the better parental level (C series varieties) was not recovered indicating complex control of *chapati* quality. Based on a study on popular bread wheat cultivars, Mohan *et al* (2013)[15] have also advocated a complex structure of the grain quality for end use products such as *chapati.* This absence of superior transgressive segregants was also indicative of fact that positive alleles governing *chapati* quality are largely concentrated in one parent. The *Glu*-B1 subunit 20 did not show any association with *chapati* quality in the recombinant populations as was evinced from the results obtained on the set of genotypes. This could be attributed to the quantitative nature of wheat quality trait. Many smaller affect glutenin and gliadin subunits may be involved in expressing good *chapati* making quality. The other subunits were found to be equally distributed and thus need to be investigated in details.

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