Indian J. Genet., **64**(2): 108-111 (2004)

High molecular weight glutenin subunits variation in relation to biscuit making quality in wheat (*Triticum aestivum* L. em. Thell.)

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(Received: February 2004; Revised: April 2004; Accepted: April 2004)

Abstract

The SDS-PAGE of 30 wheat genotypes demonstrated the presence of 1, 2* and null HMW-GS at Glu-1A locus, 7+8, 7+9, 14+15 and 17+18 at Glu-1B and 2+12 and 5+10 HMW-GS at Glu-1D locus. The two durum wheats PDW 215 and WH 896 lacked all the HMW-glutenin subunits, *viz.*, 1, 2*, null, 7+8, 7+9, 14+15, 17+18, 2+12 and 5+10. The 30 wheat genotypes displayed polymorphism for HMW-GS at Glu-1A, Glu-1B and Glu-1D loci. The diversity of the Genotypes for HMW-GS was also apparent from the dendrogram drawn using UPGMA cluster analysis. The wheat genotypes Raj 3765, UP 2576 and UP 2530 having cookie spread ratio of 6.1, 7.0 and 10.3, respectively carried 2+12 subunits at Glu-1D suggesting a relationship between better biscuit making quality and HMW-GS 2+12. HMW-GS 5+10 was shown to be associated with inferior biscuit making quality in PBW 175, PBW 343, UP 2567, UP 2569, UP 2425, UP 2562 and PBW 373. However, this relationship did not hold true in few cases possibly due to influence of other factors.

Key words: Wheat, HMW-GS, SDS-PAGE, quality breeding

Introduction

Having made on unprecedented growth in the production and productivity level of wheat in India from 6.4 million tonnes out of 9.8 million hectares with a productivity of 6.5 q/ha in 1950 to a record 75.57 million tonnes from 28 million hectares with a productivity of 26.28 q/ha [1], the focus of the research needs to be oriented towards quality breeding in a systematic and phased manner. With the rapid urbanization, changing food habits and the increasing scope of export, the demand of wheat with specific quality, for example biscuit making is likely to go up.

The quality of wheat grain is largely determined by the quality and quantity of starch and non-enzymatic storage proteins which constitute the gluten. The physicochemical characteristics of the wheat flour are determined mainly by the protein content, gluten strength and starch damage. These quality traits in turn decide the suitability of particular wheat variety for bread, biscuit and *chapati* making. The appropriate values for various quality parameters related to biscuit making are 10.0 % for protein, 30-40 ml for sedimentation value which is directly related to gluten strength and 23 for water absorption ratio [2-4]. Further, wheat gluten is sub-divisible into two major protein types, *viz.*, gliadin and glutenin. When glutenins are fractionated on SDS-PAGE, they form 2 groups, *viz.*, high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS). There is wide variation in wheat varieties for electrophoretic patterns of both HMW-GS and LMW-GS [5, 6]. Therefore, this investigation on characterization of 30 relatively new varieties/genotypes of wheat using SDS-PAGE and finding association of HMW-GS (high molecular weight - glutenin subunits) with biscuit making quality was undertaken to provide a base for quality breeding in wheat. Further, diversity analysis with respect to SDS-PAGE pattern of seed proteins was ascertained through dendrogram analysis.

Materials and methods

The experimental materials constituted 30 wheat genotypes of which all were hexaploid bread wheat except PDW 215 and WH 896 which were tetraploid durum wheats (Table 1). These genotypes were evaluated in a randomized block design using four replications where sowing was done on November 17, 2000 (normal sowing date). Each genotype was represented by four rows, 2.5 m long each, spaced 23 cm apart. Plant to plant distance within row was about 10 cm. The distance was maintained by thick sowing followed by thinning. Standard cultural practices were followed to raise the crop. For SDS-PAGE and cookie spread ratio, composite samples from two central rows were utilized.

The sodium dodecyl sulphate polyacrylamde gel electrophoresis (SDS-PAGF) was based on Laemmli [7] and as modified by Lawrence and Shepherd [8].

Table 1. HMW-glutenin subunits, Glu-1 score and cookie spread ratio of 30 wheat genotypes

S. No.	Genotype	HMW			Glu-1 score	Cookie spread ratio
		Glu-1A	Glu-1B	Glu-1D		
1.	C 306	2*	14+15	2+12	10	5.2
2.	UP 2539	2*	17+18	2+12	8	5.0
3.	PBW 175	1	7+ 9	5+10	9	4.9
4.	UP 2526	2*	7+ 8	2+ 12	8	4.9
5.	UP 262	Null	7+ 9	2+12	5	5.0
6.	PBW 343	1	7+ 9	5+10	9	5.1
7.	UP 2567	1	7+ 8	5+10	7	5.6
8.	RAJ 3765	2*	7+ 9	2+12	8	6.1
9.	PBW 299	Null	7+8	2+12	6	4.9
10.	UP 2575	1	17+18	2+12	5	5.1
11.	WH 896		Durum			5.4
12.	UP 2574	Null	7+ 9	2+12	5	4.8
13.	UP 2382	2*	7+ 9	2+12	7	5.6
14.	UP 2559	Null	7+ 9	2+12	5	5.3
15.	UP 2569	1	7+ 9	5+10	9	5.5
16.	UP 2425	2*	7+ 8	5+10	10	5.4
17.	UP 2562	Null	17+18	5+10	8	5.5
18.	Sonalika	2*	7+ 9	2+12	7	5.2
19.	UP 2530	2*	7+ 8	2+12	8	10.3
20.	UP 2576	Null	17+18	2+12	6	7.0
21.	UP 2577	2*	17+18	2+12	8	5.1
22.	RAJ 3077	2*	17+18	2+12	8	4.8
23.	UP 2506	2*	7+8	2+12	8	5.4
24.	PBW 373	1	7+ 9	5+10	9	5.6
25.	UP 2579	Null	7+ 8	2+12	6	4.8
26.	UP 2513	Null	17+18	2+12	6	5.2
27.	UP 2338	2*	17+18	2+12	8	4.5
28.	PDW 215		Durum			5.4
29.	UP 2528	2*	7+ 8	2+12	8	4.7
30.	UP 2113	2*	7+ 9	2+12	7	5.3

Cookie spread ratio was determined following the procedure as described by Mishra *et al.* [9]. The numbers assigned to high molecular weight glutenin subunits were identified for each genotype according to the numbering system as suggested by Payne and Lawerence [10].

Gel photographs were performed with a Gel Doc (Gene Genius Bioimaging System). Bands were recorded as presence with a score of 1 or absence with a score of 0. Grouping was carried out by unweighted pair-group method using arithmetic averages (UPGMA) cluster analysis using standard software package.

Results and discussion

The HMW-GS, glu-1 score and cookie spread ratio of 30 wheat genotypes are given in Table 1. It can be seen from the results that Glu-1A locus was characterized by 1, 2* and null glutenin subunit phenotypes. At Glu-1B locus, 7+8, 7+9 and 14+15 and 17+18 HMW-GS were present. The Glu-1D locus showed the existence of 2+12 and 5+10 HMW-GS. Two durum wheats, WH 896 and PDW 215 were devoid of these HMW glutenin subunits.

There was polymorphism for HMW-GS at Glu-1A, Glu-1B and Glu-1D loci in 28 wheat genotypes excluding two durum cultivars i.e. PDW 215 and WH 896 which were devoid of genome D and thus failed to show HMW glutenin subunits. A critical look at Table 1 further showed that 50 % of the genotypes had 2* subunits at Glu-1A locus and 28.57 % of the genotypes carried null units. The remaining 21.43% genotypes showed the presence of subunit 1 at Glu-1A locus. There was polymorphism at Glu-1B locus where 39.29 % of the genotypes carried 7+9 subunits and 28.57 % genotypes included 7+8 and 17+18 subunits each. The remaining 3.57 % genotypes contained 14+15 subunits. At Glu-1D locus, only two subunits 5+10 and 2+12 were present in 25 and 75 % of the genotypes, respectively. The diversity of the genotypes with respect to HMW-GS was reflected in the dendrogram of the wheat genotypes on the basis of UPGMA cluster analysis (Fig. 1).

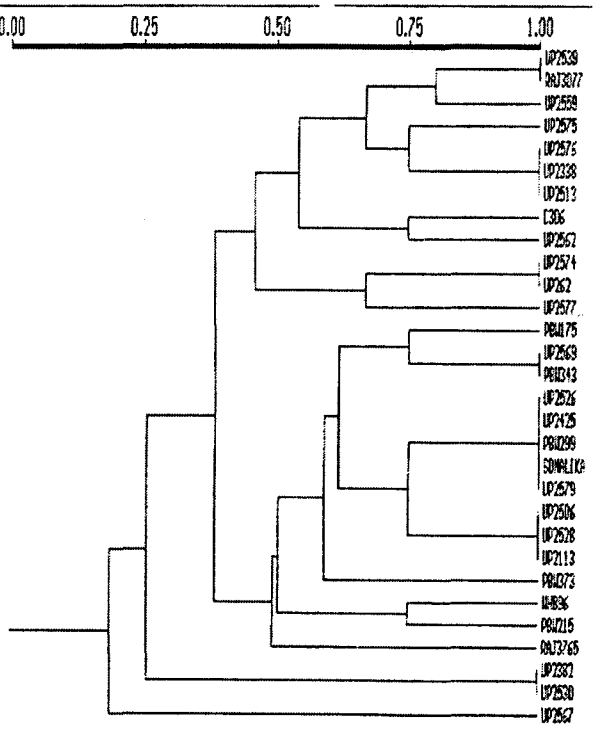

Fig. 1. Dengrogram of 30 wheat genotypes

There were seven small similarity groups within which the genotypes were related. On a broader scale there were three dissimilar groups where UP 2539, Raj 3077, UP 2559, UP 2575, UP 2576, UP 2338, UP 2513, C 306, UP 2562, UP 2574, UP 262 and UP

2577 constituted the biggest single group. The next group included PBW 175, UP 2569, PBW 343, UP 2526, UP 2554, PBW 299, Sonalika, UP 2579, UP 2506, UP 2528, UP 2113, PBW 373, WH 896, PDW 215 and Raj 3765. The third group included UP 2382, UP 2530 and UP 2567. The polymorphism for HMW-GS has been widely reported in wheat including wild species such as *Aegilops*, *T tauschii*, einkorn species and emmer species as summarized by Gianibelli *et al.* [11].

Incidentally, Gupta and Singh [12] used HMW-GS to identify certain wheat genotypes where HMW-GS composition of wheat varieties UP 2338, UP 2382, PBW 343, Raj 3077, Raj 3065, PBW 373 and Sonalika was exactly as observed in the present investigation.

A perusal of Table 1 showing the relationship of HMW-GS and cookie spread ratio indicated that the genotypes with 6.1, 7.0 and 10.3 cookie spread ratio (Raj 3765, UP 2576 and UP 2530, respectively) suitable for biscuit making contained 2+12 subunits at Glu-1D. The genotypes with the low cookie spread ratio (PBW 175, PBW 343, UP 2567, UP 2569, UP 2425, UP 2562, PBW 373) which were considered to be inferior for biscuit making and superior for bread making contained the subunits 5+10 at Glu-1D focus. The relationship of HMW-GS to bread-making quality and glutenin elasticity is well established [13]. Several HMW-GS have been closely associated with bread making quality. Payne *et al.* [5] analyzed progenies of crosses between common wheat cultivars for SDS sedimentation volume and subunit composition. They showed that certain allelic subunits impart differential effects on gluten quality. One example is the allelic variation at the Glu-1D locus of bread wheats where alternative pairs of subunits 5+10 (associated with good quality) and subunits 2+12 (associated with weaker dough quality) were identified. Such results have been confirmed in laboratories elsewhere, for example Branlard and Dardevet [14] reported that the alveograph parameters W (gluten strength) and P (tenacity), and the Zeleny sedimentation values are correlated positively with subunits 7+9 and 5+10, and negatively with bands 2+12, whereas subunit 1 is correlated to W and subunits 2* and 17+18 with G (extensibility). Based on analyses of large number of cultivars, a scoring system for HMW-GS has been developed [13] in which individual subunits are graded with numbers based on quality evaluations. A given cultivar can then be assigned a Glu-1 score which is the sum of contributions of each of the three HMW-GS loci. The HMW-GS score has more influence in some sets of wheat than in others [15]. This is likely to be due to the complex interaction of factors that define wheat quality. Nevertheless, reference to HMW-GS composition has proved valuable in the segregation of lines in the process of breeding for specific quality targets [16, 17].

The Glu-1 scores obtained are shown in Table 1. As per this table, the lines with 2+12 subunits had a Glu-1 score ranging from 5 to 10. Normally the lilies with 2+12 subunits are supposed to have weak gluten and thus their Glu-1 score should have been at lower side. However, as observed by MacRitchie *et al.* [15] this situation is unlikely to occur in some cases because of involvement of other factors like LMW-GS, gliadins and abiotic stresses. Further, it is also to be kept in mind that sometimes one aspect is overlooked when using this scoring system is that subunits with the same electrophoretic mobility in SDS-PAGE differ in some other features like small differences in protein sequences and surface hydrophobicity. For example, after the Glu-1 score was established, Sutton [18] found differences in retention time for subunit 8 in some cultivars when subjected to RP-HPLC. He concluded that two different subunit 8 were involved (8 and 8*). Also, different electrophoretic mobilities were reported for subunit 7 (7 and 7*). Thus, four different alleles, instead of just one are expected for this pair (7+8, 7*+8, 7+8*, 7*+8*) as reported by Marchylo *et al.* [19]. Interestingly, there are contrasting effects and, hence, the score originally given to the pair 7+8 is sometimes misleading [11].

On the basis of cookie spread ratio, UP 2338 (4.5) was found to be inferior for biscuit making and UP 2530 (10.3) as the most suitable cultivar for biscuit making, although both had similar Glu-1 score of 8. Other genotypes with relatively high cookie spread ratio and suitable for better cookie making were UP 2576 (7.0), Raj 3765 (6.1), UP 2382, UP 2576 and PBW 373 (each with 5.6). All these lines shown to be superior for biscuit making carried 2+12 subunits at Glu-1D suggesting a relationship between better biscuit making quality and HMW-GS, 2+12.

However, it can be mentioned that several wheat genotypes with 2+12 HMW-GS at Glu-1D which should have given good cookie spread ratio, failed to do so, for example, UP 2526, UP 262, PBW 299, UP 2574, Raj 3077, UP 2579 and UP 2338 where cookie spread ratio was equal to or less than 5.0. Thus presence of 2+12 HMW-GS should be taken as a broad indicator of better biscuit making quality while screening large number of germplasm/breeding lines of wheat under quality breeding. Once the lines are narrowed down, actual test for cookie spread ratio must be done.

HMW-GS 5+10 was shown to be associated with inferior biscuit making better bread making in PBW 175, PBW 343 (newly released established variety), UP 2567, UP 2569, UP 2425 (recently released variety from Pantnagar suitable for late sowing), UP 2562 and

PBW 373 (recently released sister line of PBW 343, suitable for late sowing). On overall basis, the results indicated a good scope of wheat breeding specifically for bread and biscuit making using the lines from the present investigation with two diagonally opposed objectives firstly to increase protein and sedimentation value with HMW-GS 5+10 for better bread-making and secondly with low protein and sedimentation value and HMW-GS 2+12 for better biscuit making. However, in view of the fact that in few cases the relationship of HMW-GS 2+12 with better biscuit making quality and that of HMW-GS 5+10 with better bread making quality did not hold true due to involvement of other factors, the use of these subunits as selection parameter for quality improvement cannot be generalized. Instead, these could be used to narrow down large number of segregating/germplasm/breeding lines of wheat in a wheat breeding programme aimed at improving biscuit /bread making quality.

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