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SELECTION FOR QUALITY IN EARLY GENERATION BASED ON THE HMW SUBUNITS OF GLUTENIN IN BREADWHEAT

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ABSTRACT

The D genome encoded high molecular weight (HMW) subunits of glutenin 5 + 10 (Glu-1 score 4) from high grain-protein stock TW-1 and cv. Shera were transferred to cv. Kalyan Sona with allelic subunits 2 + 12 (Glu-1 score 2) using backcrosses. Selections in the BC_2F_5 and BC_1F_5 generations, homozygous for HMW subunits 5 + 10 or 2 + 12 were analysed for SDS sedimentation volume. Family means of selections carrying subunits 5 + 10 showed higher sedimentation volume than those with subunits 2 + 12. Thirteen selections were numerically superior to the recurrent parent Kalyan Sona in sedimentation volume, 12 of these carried subunits 5 + 10. These selections resembled Kalyan Sona in the A and B genome coded HMW subunits of glutenin, grain protein content, grain yield and plant type. Transfer of desirable HMW subunits of glutenin for modifying Glu-1 score and quality of cultivars using backcrossing and SDS-PAGE of half grains is demonstrated.

Key words: *Triticum aestivum*, wheat, glutenin, SDS-sedimentation test, SDS-polyacrylamide gel electrophoresis, quality.

Viscoelastic properties of wheat dough are the major determinant of bread making quality. The high molecular weight (HMW) subunits of glutenin contribute significantly to these properties. In a study of British wheats, 47-60% of the variation in bread making quality could be attributed to the variability in the HMW subunits of glutenin alone [1]. The HMW subunits of glutenin have been assigned scores according to their quality characteristics. The individual scores are added up to obtain overall quality score called the Glu-1 quality score, which is an indicator of bread making quality of the cultivar [1]. The HMW subunits 5 + 10 coded by the *Glu-D1d* locus on chromosome 1D are of greater significance for dough strength [2–5] and have been assigned the score of 4. Their allelic subunits 2 + 12 coded by *Glu-D1a*, are relatively less effective and are given the score of 2 [1]. Thus, substitution of subunits 5 + 10 in place of their alleles would result in higher Glu-1 score which, in turn, is expected to give better bread making quality. The SDS-sedimentation volume shows good

correlation with bread loaf volume [6]. Half seed analysis as a method for selection of specific HMW subunit patterns was proposed earlier [7]. The present paper reports selection based on HMW subunits of glutenin, using half seed analysis, in intercultivar crosses and associated changes in quality, as judged by SDS sedimentation volume.

MATERIALS AND METHODS

The high yielding, breadwheat cultivar Kalyan Sona having satisfactory quality for bread and chapati making was hybridized with a high grain protein stock TW-1. The A and D genomes encoded HMW subunits of these are shown according to their relative mobilities in Fig. 1.

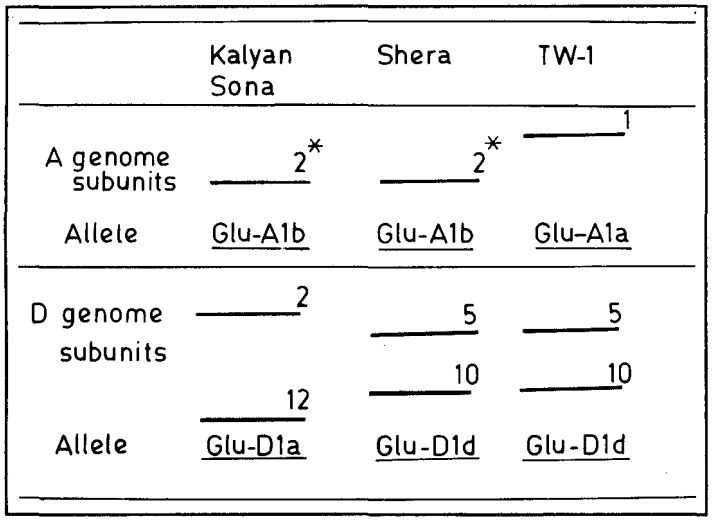

Fig. 1. Diagrammatic presentation of the A and D genome coded HMW subunits of glutenin of the wheat cvs. Kalyan Sona, Shera and TW-1. Relative mobilities of the subunits to be compared only within the genome.

Backcrossing and selection for HMW subunits. The F_1 and F_2 plants were allowed to self. The F_2 plants were harvested individually and were analysed by SDS–PAGE for their HMW subunit pattern, using half-seed analysis. The gel measured 17.7 x 13.8 cm x 0.7 mm. The running gel contained 0.13% bisacrylamide and 10% acrylamide, and the stacking gel contained 0.04% bisacrylamide and 3% acrylamide. The subunit 2* could be resolved only on 5% gels (0.26% bisacrylamide, 5% acrylamide). The grains were cut transversely to obtain 10–15 mg of endosperm portion. This was extracted with buffer containing 0.0625 M Tris-HCl, pH 6.8, 2% SDS (w/v), 5% 2-mercaptoethanol (v/v), 10% glycerol (v/v) and 0.001% Pyronin Y (w/v). The ratio of endosperm to buffer was 1 mg : 20 µl. The extraction procedure was the same as described in [2]. Samples were centrifuged in Eppendorf centrifuge when necessary and 15–20 µl of each sample was loaded per well. The 10% gels were run at 20–25 mA/gel till the dye front reached the end of the gel and the 5% gels were overrun for about 30 min at 30 mA/gel in constant current mode to obtain proper resolution.

The embryo halves corresponding to the heterozygous endosperms were germinated on moist cotton and transferred to pots after five days. These were backcrossed to the recurrent parent Kalyan Sona. This process was repeated for the BC_1F_1 and BC_2F_1 generations (Fig. 2). The BC_2F_1 and BC_2F_2 plants were allowed to self. In the BC_2F_3 generation, a few plants from each line were harvested individually and the rest of the line was bulk harvested. The single plant harvests were analysed for HMW subunits of glutenin [8]. Plants from the lines 43, 45, 47 and 49 were homozygous. These were carried forward to BC_2F_4 as plant-to-row progenies. The selections in the BC_2F_4 generation, originating from individual plants from the same line in the BC_2F_3, were treated together as a family.

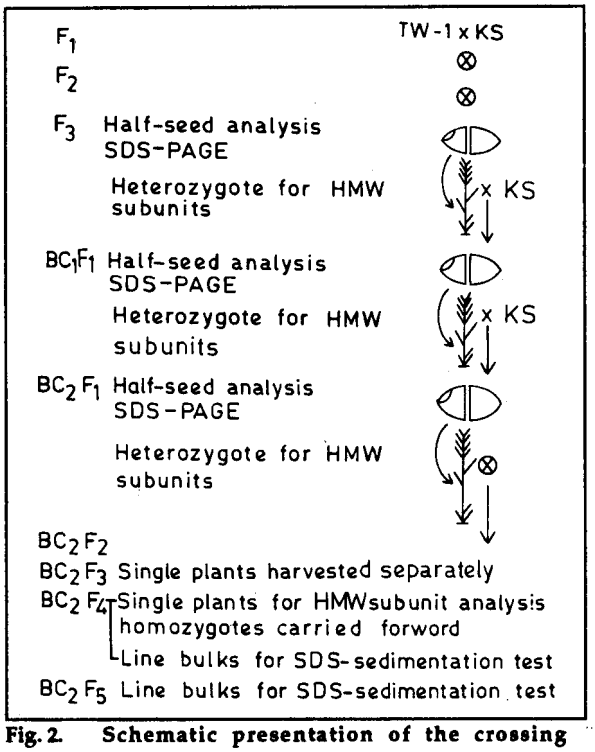

Fig. 2. Schematic presentation of the crossing programme.

Grain protein content and SDS-sedimentation test. Wholemeal from the BC_2F_4 line bulks and BC_2F_5 line bulks were analyzed for SDS sedimentation volume [6]. Grain protein was estimated from wholemeal on Technicon Autoanalyzer [9].

Cross between Shera and Kalyan Sona. In another set cv. Shera (HD 1925) was crossed with cv. Kalyan Sona and the latter used as the recurrent parent. The BC_1F_1 seeds heterozygous for B and D genome subunits were selected and grown. The resulting plants were allowed to self. The BC_1F_2 plants were harvested individually and carried forward as plant-to-row progenies. From the BC_1F_3 lines a few phenotypically promising plants were harvested individually and carried forward as plant-to-row progenies, the BC_1F_4 lines were harvested individually and the seeds were analysed for HMW subunits of glutenin and SDS sedimentation value as described for the previous cross. In this study selections in BC_1F_4 generation resembling Kalyan Sona for the A and B genome subunits but differing for the D genome subunits were considered.

The statistical analyses of SDS sedimentation volume means were as described in [10]. For the TW-1 x Kalyan Sona data, single classification ANOVA with unequal sample sizes was used while for the Shera x Kalyan Sona data, analysis of variance for two groups with equal sample size was used.

RESULTS

The results of SDS–PAGE and SDS-sedimentation volume of the BC_2F_4 lines and mean values for the BC_2F_5 families are presented in Table 1. In the BC_2F_4 lines, three subunit combinations were available for comparison 1, 5 + 10 (lines 43, 47); 2*, 5 + 10 (line 49) and 1, 2 + 12 (line 45), each resulting from two backcrosses to Kalyan Sona (2*, 2 + 12). Five grains

Table 1. A and D genome subunit composition, SDS-sedimentation volume, grain protein percent and grain yield of selections in BC_2F_4 and BC_2F_5 generation in the cross between cvs. TW-1 and Kalyan Sona of wheat

Family No.	No. of selections in family	HMW subunit		Sedimentation volume		Grain protein (%)	Grain yield (g/m^2)
		A genome	D genome	ml in BC_2F_4	ml in BC_2F_5		
43	5	1	5 + 10	70.0	79.8 ± 1.7[a]	15.12 ± 0.33	331.6 ± 14.5
45	8	1	2 + 12	65.0	59.1 ± 1.0[b]	14.32 ± 0.38	305.3 ± 5.3
47	3	1	5 + 10	67.5	67.5 ± 3.2[c]	14.16 ± 0.20	320.1 ± 11.3
49	7	2*	5 + 10	61.0	65.6 ± 1.8[c]	14.10 ± 0.16	312.7 ± 6.4
TW-1+		1	5 + 10	80.0		16.44	228.2
Kalyan Sona+		2*	2 + 12	68.0		15.50	344.0

+Mean of three samples.

SDS-sedimentation volume means with different superscripts (a, b, c) differ significantly at 5% level.

from each of the BC_2F_5 harvests were pooled and analysed on SDS-PAGE, these confirmed the subunit composition observed in the BC_2F_4 seeds (Fig. 3). As expected, protein profiles of the selections showed greater resemblance to the recurrent parent Kalyan Sona than the donor parent TW-1. Higher SDS-sedimentation volume was observed in the selection 43 (1, 5 + 10) in the BC_2F_4. The mean SDS-sedimentation volume of its progeny was also the highest in the BC_2F_5 and considerably higher than the recurrent parent Kalyan Sona. BC_2F_4 selections 47 (1, 5 + 10), 49 (2*, 5 + 10) and their progenies in BC_2F_5, however showed lower SDS-sedimentation volume than the line 43 or its progeny, respectively. The BC_2F_4 selection 45 showed relatively low SDS-sedimentation volume. Its progeny mean in BC_2F_5 was distinctly lower than all other families (Table 1). Similarly in the BC_2F_5 generation, derived from the cross (Shera x Kalyan Sona) x Kalyan Sona, families having subunits 5 + 10 showed higher mean SDS-sedimentation volume than those with subunits 2 + 12 (Table 2).

The BC_2F_5 families 43, 47, 49 from the cross TW-1 x Kalyan Sona and BC_1F_5 families 40 and 41a from the cross Shera x Kalyan Sona resembled the recurrent parent Kalyan Sona in

Table 2. D genome subunit composition, SDS-sedimentation volume, grain protein % and grain yield of selections in BC_1F_5 generation from the cross between cvs. Shera and Kalyan Sona of wheat

No. of selections	HMW subunit D genome	SDS-sedimentation volume (ml)	Grain protein (%)	Grain yield (g/m^2)
6	5 + 10	71.2 ± 1.3^a	14.05 ± 0.17	367.8 ± 14.2
6	2 + 12	61.3 ± 2.5^b	14.41 ± 0.11	311.5 ± 10.8
Shera*	5 + 10	61.0	15.41	239.6
Kalyan Sona*	2 + 12	68.0	15.47	352.4

*Mean of three samples.

SDS-sedimentation means with different superscripts (a, b) differ significantly at 5% level.

appearance flowering and maturity time, grain protein content and grain yield. These, however, differed from Kalyan Sona, BC_2F_5 family 45 and BC_1F_5 families 33, 34, 35 and 41b in having higher Glu-1 score due to subunits 5 + 10.

DISCUSSION

Selection for quality in early generation till recently relied on protein percentage, Pelshenke value and SDS-sedimentation volume. The HMW subunits of glutenin provide a new selection criterion based on half-seed analysis of the progeny from selected F_2 plants. The present experiments were carried out to select for the HMW subunits of glutenin in intervarietal crosses, and the resulting changes in quality parameters as estimated by SDS-sedimentation volume were followed.

Comparison of D genome subunits. The selections with specific subunit combinations provide comparison between the D genome subunits 5 + 10 and 2 + 12. Backcrossing twice to the recurrent parent is expected to reduce the variation due to other grain proteins. Selections in BC_2F_5 generation with subunits 5 + 10 had higher mean SDS-sedimentation volume than those with subunits 2 + 12 despite comparable protein content. The variation among selections with subunits 5 + 10 and lower SDS-sedimentation volume of the selections with subunits 2 + 12, than Kalyan Sona, may be due to segregation for other proteins related to poor quality.

These results demonstrate that using this procedure Glu-1 score of a cultivar could be improved. The selections with higher Glu-1 score were either similar to or better than the recurrent parent Kalyan Sona. The moderate quality of Kalyan Sona despite subunits 2 + 12 indicates that it has other proteins contributing to quality. Segregation for these may have contributed to the variability among selections with subunits 5 + 10. In BC_2F_5, one out of

three families selected for subunits 5 + 10 had higher SDS-sedimentation volume than Kalyan Sona. Overall, 12 out of the 13 selections with higher SDS-sedimentation volume carried subunits 5 + 10. The HMW subunits of glutenin thus provide a qualitative selection criterion in early generation for improvement of Glu-1 score and quality. Selection for subunits with high, medium or low Glu-1 scores would help in selecting for varieties with high, moderate or low dough strength important in bread, chapati and biscuit making, respectively. Usefulness of HMW glutenin loci in the development of new wheat varieties with specific desired characteristics has been emphasized [11]. Manipulation of HMW subunits of glutenin is reported to change bread making quality independent of the grain protein content [5]. In the present experiment the high SDS-sedimentation volume of the family 43 and low SDS-sedimentation volume of the family 45 appeared to be independent of grain protein content. Similarly, in the cross Shera x Kalyan Sona, the 5 + 10 carriers had higher mean SDS-sedimentation volume compared to 2 + 12 carriers despite similar protein content. Selection on the basis of HMW subunits of glutenin would modify the present emphasis on high grain protein content which is usually associated with lower grain yield at equal nitrogen levels [12]. The half-grain analysis and the Glu-1 score would also be useful in altering the HMW subunit composition and consequently quality of an otherwise desirable cultivar by backcrossing.

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