Determination of relationship between HMW glutenin subunits and bread making quality in bread wheat

Maleki M, Fatehi F, Salavati A, Behamta MR

Plant Breeding Dept. Agricultural College, University of Tehran, Karaj, Iran

INTRODUCTION

The genetics and biochemistry of high-molecular-weight (HMW) glutenin subunits in wheat (Triticum aestivum) are now well understood by virtue of various studies (12, 16). Moreover, studies have revealed the correlation between certain HMW glutenin subunits and bread-making quality (16). HMW subunits are encoded at the Glu-1 loci of the group 1 chromosomes (1A, 1B, 1D), and each locus consists of two genes encoding an x-type and a y-type subunit (17). Because some genes are silent, wheat cultivars contain three, four or five subunits. Wheat storage proteins designated as prolamins consist of two protein classes: gliadins and glutenins. Gliadins are small, monomeric proteins comprising the α, β, γ and ω families. They make up 50% of prolamins (10). Glutenins consist of HMW (high molecular weight)and LMW (low molecular weight)glutenin subunits. Even though HMW glutenins constitute onlr 10% of the total storage proteins as compaired with 40% for LMW (10), it has been reported that HMW glutenin subunits have the largest effect on bread making quality (12). More recently, it has also been shown that loaf volume depends on the composition of the proteins. Glutenins and gliadins togethere represent ≈80% of the totall proteins in a typical wheat flour (4, 10). The contributions of gliadins and glutenins to dough properties have been long been recognized, and it has been suggested that the gliadins generally contribute to dough viscosity and glutenins contribute to dough elasticity (5). Mager effectson loaf quality have been demonstrated due to the high molecular weight glutenin subunits (HMW-GS) present (11), the glutenin-to-gliadin ratio (1, 6, 2), the molecular weight distribution(6, 3) and overall protein content (7). Each cultivar contains three to five HMW subunits that can be distinguished by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (8).

The research reported in this paper was carried out to identify combination of Glu-1 alleles and determination of relationship between HMW glutenin subunits and bread making quality by SDS-sedimentation test.

MATERIAL AND METHOD

Forty synthetic lines and forty Iranian cultivars were examined in this study. To determine the electrophoretic mobility of each HMW glutenin subunit by SDSPAGE, standards (Chinese Spring, Hirmand, Falat) that included the spectra of subunits expected in the lines were used. These lines were analyzed by SDS-PAGE, according to the procedure of Payne et al. (1979).

Gels were made with 7.5% (w/v) acrylamide and 0.2% (w/v) bisacrylamide and contained 1.5 M Tris-HCl, pH 8.8, and 0.27% SDS. The stacking gel contained 0.25 M Tris-HCl, pH 6.8. Wheat flour (10 mg) was suspended in 300 mL 0.25 M Tris-HCl buffer (pH 6.8) containing 2% (w/v) SDS, 10% (v/v) glycerol, and 5% 2-mercaptoethanol and was shaken for 2 h at room temperature. The suspension was heated at 95°C for 3 min. The top portion of the supernatant was collected after centrifugation for 3 min at 12 000 rpm, and a portion (30 µL) of the extract was loaded onto a gel slot. The electrode buffer was 0.025 M Trisglycine, pH 8.3, containing 0.1% (w/v) SDS. Electrophoresis was conducted at 10 mA constant current for 15 h until the tracking dye, bromophenol blue, reached the bottom of the gel. The gels were stained for several hours with Coomassie Blue R in aqueous ethanol and acetic acid. The system for numbering HMW glutenin subunit bands and that for allelic classification at Glu-A1, Glu-B1, and Glu-D1 loci, proposed by Payne and Lawrence (1983), were followed.

The SDS-Sedimentation test for carried out whit method of Quick and Donnelly (1983). Variance analysis of Glu-1 alleles, carried out by use of Factorial Design in base of Unbalance Completely Randomized Design. Comparisons of means of Glu-1 alleles carried out by Duncan's multiple range test. The Pearson correlation coefficient and Pearson correlation coefficient was used for simple correlation between Glu-1 alleles with each other and Glu-1 alleles with sedimentation volume, respectively. Analysis of regression was carried out by stepwise method; sedimentation volume and Glu-1 alleles was considered as dependent variant and independent variant respectively.

RESULT AND DISCUSSION

For synthetic lines and the Iranian wheat cultivars, 15 different alleles were identified, 3 corresponding to the Glu- A1 locus, 8 to Glu-B1, and 4 to Glu-D1 (Table 1). Each pattern included 3–5 bands of HMW glutenin subunits. It has been reported that HMW glutenin subunit composition is a useful system for wheat variety identification (10). The synthetic lines and the Iranian wheat cultivars in this study could be divided into 26 groups based on this parameter(Table 2).

The positive effect of 5+10 and 7+8 subunits has been showed in different studies (3, 9, 13). The frequency of these two subunits in studied lines and cultivars was more than other subunits. Common combinations of HMW subunits were null-7+9-5+10, 2*-7+8-2+12 and null-7+8-2+12 that observed in 10, 7, 7 lines and cultivars respectively. Combinations of 2*-7+8-5+10, 2*-17+18-5+10, 2*-13+16-5+10 and 1-13+16-5+10 that received qualitative score of 10 were observed in 5, 3, 4 and 1 lines, respectively. Variance analysis of Glu-1 showed that Glu-B1 and Glu-D1 alleles had significant effect whereas Glu-A1 didn't show significant effect on sediment altitude.

Comparisons of means of allele in Glu-A1, Glu-B1 and Glu-D1 indicated that 2* subunit in Glu-A1 had highest means and significant difference with null and 1 alleles, While there wasn't significant difference between null and 1 alleles. In Glu-B1, 17 + 18, 13 + 16 and 7 + 8 alleles showed highest means while in Glu-D1, highest means were belong to 5+10 and 2+12 alleles. The results of simple correlation between HMW and sedimentation volume indicated that in probability level of 1% null allele Glu-A1 has a negative correlation (r=0/387). 2* subunits in this loci along with 5+10subunits in Glu-B1 and 17 + 18 subunits in Glu-D1 showed a significant and positive correlation with sedimentation volume in probability level 1%. With regard to the results of step wise regression analysis, it was indicated that in first step 5+10 subunits entered to the model with a responsibility of 15.4% of variation in sedimentation volume. In second and third steps, 17+18 subunits and 7+8 subunits entered to the model that was responsible for 5.8% and 5% of variation in sedimentation volume respectively. In final step, null subunits entered to the model. In general, alleles of 2* of Glu-A1, 5+10 of Glu-D1 and 17+18 of Glu-B1 showed maximum effects on sedimentation volume based on regression analysis. This model is responsible for only 31.2% of variation (Table 3).

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Table1- Glu-1 alleles and their frequency

Glu-1 loci	Glu-A1			Glu-B1									Glu-D1		
Glu-1 alleles	null	1	2*	17+18	14+15	13+19	13+16	7+9	7+8	7	6+8	5+10	2***+12	2+12	12
Frequency	35	12	33	15	1	1	7	16	36	2	2	39	7	33	1
Partial Frequency	43.75	15	41.25	18.75	1.25	1.25	8.75	20	45	2.5	2.5	48.75	8.75	41.25	1.25

Table 2- Combination of Glu-1 alleles and quality score for lines and cultivars

Combination of Glu-1 alleles	Percentage frequency	Number of cultivar	Quality score
2*, 17+18, 5+10	5	4	10
Null, 17+18, 5+10	2.5	2	8
2*, 7+9, 5+10	3.75	3	9
Null, 7+8, 2***+12	7.5	6	nd
2*, 7+8, 2***+12	1.25	1	nd
Null, 7+9, 2+12	2.5	2	5
2*, 7+8, 2+12	8.75	7	8
Null, 7+9, 5+10	12.5	9	7
2*, 7+8, 5+10	6.25	5	10
Null, 7+8, 2+12	8.75	7	6
Null, 7+8, 5+10	5	4	8
2*, 13+16, 5+10	5	4	10
1, 7+8, 2+12	5	4	8
1, 6+8, 2+12	1.25	1	6
1, 17+18, 2+12	3.75	3	8
2*, 13+16, 2+12	2.5	2	8
Null, 6+8, 5+10	1.25	1	6
1, 7+9, 5+10	2.5	2	9
2*, 7, 5+10	2.5	2	8
Null, 7+8, 12	1.25	1	nd
Null, 17+18, 2+12	1.25	1	6
Null, 14+15, 5+10	1.25	1	7
2*, 17+18, 2+12	6.25	5	8
1, 13+16, 5+10	1.25	1	10
1, 7+8, 5+10	1.25	1	10
Null, 13+19, 2+12	1.25	1	nd

Table 3- Regression analysis of the accepted alleles that can be used to predict sedimentation volume

Glu-1 loci	Alleles	Coefficient of regression (*B*)	Standard Error (SE)	Partial R^2	Cumulative R^2	F
Glu-D1	5+10	9.255	1.188	0.154	0.154	19.905**
Glu-B1	17+18	7.963	1.796	0.058	0.212	15.990**
Glu-B1	7+8	5.653	1.603	0.050	0.262	13.487**
Glu-A1	null	-3.461	1.124	0.052	0.314	17.716**