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Full Length Research Paper

Characterization and glutenin diversity in tetraploid wheat varieties in Sulaimanyah by wheat storage proteins

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Important methods applied for the breeding of bread-quality wheat (*Triticum durum* L.) consist of small-scale bread-quality tests for the determination of the grain protein content, SDS-sedimentation volume, thousand weight kernel and kernel diameter. Wheat grains of six varieties were analyzed. The thousand weight kernel, protein content, kernel diameter, SDS-sedimentation volume and SDS index showed a difference significant among the varieties, whereas the flour yield showed no difference significant. The quality score and the variability of seed storage-proteins were analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The high molecular weight (HMW) and low molecular weight (LMW) glutenin subunit band patterns for each variety were assigned the corresponding Payne numbers and theoretical quality scores based on those assignments. Three patterns of HMS-GS are designed: 6+8, 7+8 and 8+20. On the other hand, two patterns of LMW-GS were detected in the durum varieties. Genetic diversity of wheat was evaluated by constructing the dendrogram for high molecular weight (HMW) and low molecular weight (LMW) gluten subunit bands. It is concluded that seed storage protein profiles could be useful markers in the studies of genetic diversity and classification of adapted cultivars, thereby improving the efficiency of wheat breeding programs in cultivar development especially in a developing country.

Key word: Durum wheat, glutenin HMW-GS, glutenin LMW-GS, SDS-PAGE, wheat quality.

INTRODUCTION

The majority of the seed proteins are stored in the starchy endosperm in the form of prolamins, which are unique to cereal grains, and account for over half of the total seed nitrogen. The wheat prolamins are divided on the basis of function into two groups, the glutenins and gliadins, which together confer the properties of elasticity/ strength/and extensibility (viscosity). The prolamins of wheat are highly polymorphic polypeptide mixtures including more than 50 components with Mr values ranging from 30.000 to 90.000 (Payne et al., 1987). The gliadins are monomeric molecules (30 – 75 kDa) divided into several classes (α-, γ- and ω-gliadins). In contrast, the glutenins form large polymeric structures as a result of intermolecular disulfide bonds. The glutenins are divided into a low molecular weight (LMW = B subunits 66 - 33 kDa and C subunits 20 – 5 kDa) and high molecular weight (HMW = A subunits 100 – 140 kDa). The HMW glutenins, which represent approximately 0.5% of the total seed dry weight, have been studied extensively because of their effect on elasticity and hence the bread making quality of wheat dough. Extensive genetic and molecular analyses of wheat prolamin have established the chromosomal positions of the prolamin genes (Payne et al., 1987). The HMW genes (Glu-1) are located on the long arm of the homologous chromosomes 1A, 1B and 1D in the hexaploid wheat and 1A and 1B in tetraploid wheat, respectively. Two Glu 1A and Glu 1B loci are represented by 12 and 15 alleles. Each Glu 1 locus has 2 closely related genes which are most often marked as a x and y – type subunits, based on their electrophoretic mobility and isoelectric points. The HMW 1Bx, 1Dx and 1Dy are present in all cultivars, 1By and 1Ax, are present in some cultivars, but no cultivars contain 1Ay, because the gene is always silent. Similarly, the tetraploid durum wheat with the A and B genomes contain four HMW genes, of which no more than three

are active. Results reported so far have shown that the non-expression of the 1Ay gene can be caused by nucleotide changes in the pro motor region (Forde et al., 1985) or by the presence of a transposon-like insertion in the encoding region (Harbred et al., 1987). About 80 percent of the durum lines do not have HMW-glutenin subunit encoded by Glu-A1 locus (Branlard et al., 1989). Due to lack of Glu-A1 locus specific HMW-glutenin subunits and absence of D genome, bread making quality of *T. durum* has been found to be very poor. Diploid and tetraploid wild progenitors of wheat, however, have been found to contain both Ax and Ay subunits coded by Glu-A1 (Waines and Payne, 1987). The Glu-A1 locus is less polymorphic than the Glu-B1 and Glu-D1 loci. Glu-A1x locus is characterized by three main alleles, namely a, b and c (Payne and Lawrence, 1980). The alleles a and b code for glutenin subunits 1 and 2*, respectively while c is a null allele (Thompson et al., 1983). In addition to three major alleles at Glu-A1x locus, SDS-PAGE analysis of primitive cultivars and races of durum and bread wheat have revealed a few additional allelic variants at this locus (Thompson et al., 1983; Margiotta et al., 1993). All of the new allelic variants have electrophoretic mobility similar to subunits 2* and 1 except the subunit 2.1* (Tahir et al., 1996), which has much reduced mobility. Based on worldwide observations relating to the correlation between HMW-patterns and wheat quality, Payne et al. (1987) proposed the use of the “Glu-1 quality score” to predict the baking quality of wheat varieties. According to Payne et al. (1981), and Branlard and Dardevet (1985), subunits 1 and 2* at the Glu- A1 locus make similar contributions to good quality. Also a rye – adjusted Glu-1 quality score has been calculated for cultivars containing the 1B/1R rye translocation. Genes encoding LMW (Gli – 1), γ- and ω-gliadins are grouped at loci on the short arms of chromosomes 1A, 1B and 1D. The genes for α-gliadins (Gli-2) are found on the short arms of chromosomes 6A, 6B and 6D and respectively 1A, 1B and 6A and 6B of tetraploid wheat (Vasil and Anderson, 1997). The genes controlling of the B-subunits of glutenin, the major group of LMW glutenins, have been mapped on the short arm of chromosome 1A and 1B (Payne et al., 1987). The close relationship between LMW glutenin and gliadin genes in durum wheat has been object of several studies. Firstly the B-LMW subunits coded by genes at the Glu-3 loci have been linked only with ω and γ-gliadins coded at the Gli- 1 loci on the short arm of chromosomes 1A and 1B (Payne et al., 1987; Pogna et al., 1990). Subsequently, improved prolamin extraction (Singh et al., 1991) and the evidence obtained for the presence of additional loci coding these glutenins, led to identification of Glu-B2 (Liu and Shepherd, 1995) located between Glu-B1 and Gli-B1 loci in chromosome 1BS tightly linked to the Gli-B3 locus. Nieto-Taladriz et al. (1997) identified eight allelic variants in Glu-A3, nine at Glu-B3 and two at Glu-B2 loci. Several other loci controlling minor gliadin and glutenin components have also been detected on chromosome arm 1BS as a Gli-B5 (Mazza et al., 1996) and Glu-4 (Liu and Shepherd, 1995).

MATERIALS AND METHODS

Plant sample

Grains of five wheat varieties were collected from the Department of Agriculture, University of Sulaimanyah, Bakrajo, Sulaimanyah, Iraq.

Measurements

Thousand grains were weighed using an analytical balance (AND HR-60). Whole grains were photographed and image analysis was used to measure length and width of grains by using the Image J software

SDS-PAGE electrophoresis

HMW-GS extraction

The seed protein HMW-GS was analyzed by using SDS-PAGE. The grains were ground to fine powder and 20 mg was weighed in 1.5 ml microtube. 300 ml protein extraction buffer [28.5% sample buffer (7% SDS, Tris-HCl 0.01 M, pH 6.8, 30% glycerol, 0.001% comassie blue) and 5% 2-mercaptoethanol] was added to each micro tube, kept 2 h at room temperature (27°C) and centrifuged at 13000 rpm for 10 min. The supernatant contain dissolved extracted protein HMW-GS ready for experiment purposes, which could be kept for longer time at 4°C. Before the loading of samples on the SDS-PAGE gel, the sample were heated at 80°C for 20 min and then the samples loaded on SDS-PAGE. The gel consisted of a 15% separating gel, pH 8.4, beneath a 3% stacking gel, pH 6.8. Electrophoresis was carried out at room temperature using a home-made vertical electrophoresis apparatus, and the running was performed at 15 mA/gel for 18 h. After 18 h, the gels were stained in 12.5% (w/v) trichloroacetic acid, 0.01% (w/v) Coomasie Brilliant Blue R250 and distained with distilled water

LMW-GS extraction

20 mg of flour was extracted three times with 50% (v:v) propan-2-ol at 60°C for 30 min with agitation every 10 min. Glutenin was then solubilized with 50% (v:v) propan-2-ol, 0.08 M Tris-Hcl (pH 8.5), 20 mM dithiothretol at 60°C for 30 min. The supernatant was diluted with 1 volume of 50% (v:v) propan-2-ol, 0.08 M Tris-HCl (pH 8.5), 40 mM 4-vinylpyridine, and incubated for 3 h at 60°C. Glutenin was precipitated by 1 ml acetone and the dried pellet was solubilized in 200 µl of buffer (7% SDS, Tris-HCl 0.01 M, pH 6.8, 30% glycerol, 0.001% comassie bleu). Finally, 30 µl samples were loaded into the slots of SDS-PAGE.

Quantification of protein

Percentage of nitrogen was determined on 0.25 g of flour by the Dumas combustion method using a nitrogen analyzer according to Approved Method 46-30 (AACC, 1995) and reported as protein by N*6.25 (American Association of Cereal Chemists, 1995; approved method of the AACC, 9th ed. Method 46-30).

Table 1. Kernel weight, kernel diameter, flour yield, flour protein content, SDS-sedimentation volume and SDS index of wheat varieties.

Varieties	TKW (g)	LEKD (mm)	LAKD (mm)	FY (%)	PC (%)	SDS-test (ml)	SDSi
Bakrajo 1	40.94 a	7.04 a	2.79 a	71.30 a	9.51 a	24 a	2.52 a
Ovanto	48.15 b	6.65 b	2.77 a	71.73 a	9.33 a	28 b	3.00 b
Cemmitto	55.41 c	7.17 a	2.27 b	71.70 a	9.35 a	22 c	2.36 ac
Creso Italian	52.43 d	7.20 a	3.24 c	71.14 a	13.59 b	30 d	2.21 c
Creso kurde	57.53 e	6.85 ab	3.07 c	70.93 a	9.45 a	22 c	2.33 ac
Acsad 65	39.54 f	6.44 b	2.78 a	71.17 a	9.36 a	27 b	2.98 b
LSD	1.29	0.28	0.29	1.64	0.48	1.45	0.17

TKW = Thousand kernel weight; LEKD = length kernel diameter; LAKD = large kernel diameter; FY = flour yield; PC = protein content; SDS-test = SDS-sedimentation test;
SDSi = SDS-sedimentation index; LSD = least significant difference.

SDS-test

5 ml of distilled water was put in the cylinder and 0.5 g of flour and added to the cylinder. The cylinder was closed and shaken 15 times (one per second) at the first and second minute; the cylinder was shaken again 15 times (one per second). At the completion of 3 min and 45 s, the cylinder again was shaken 15 times (one per second) and 5 ml of SDS/ lactic acid [20 g of SDS in 1 L of distilled water and 20 ml of mix (10 ml lactic acid 88% and 80 ml of distilled water) was add to SDS solution] added to cylinder and the cylinder was shaken 4 times. At the completion 6, 8 and 10 min, the cylinder was shaken again 4 times. The volume of sediment was read at the completion of 25 min. The value obtained is multiplied by 10 to obtain a value of sedimentation volume compared with 100 ml of solution

Data analysis

Electrophoregrams for each variety were scored and the presence (1) or absence (0) of each band noted. Presence and absence of bands were entered in a binary data matrix. All analysis was carried out using a statistical package SPSS-PC, version 15, by using the Dice similarity. UPGM used for construct the dendrogram.

RESULTS AND DISCUSSION

Characterization of wheat varieties by quality evaluation

Quality traits of wheat varieties were determined as shown in Table 1. There were significant differences among varieties for thousand kernel weight, kernel diameter, SDS-Test, and SDS index. The thousand kernel weight significantly higher than all others parameters. Variety Creso Kurde produced the highest kernel weight, while the variety Acsad 65 produced the lowest one. Significantly length was measured for Bakrajo 1, Cemmitto and Creso Italian than Creso Kurde, Ovanto and Acsad 65. On the other hand, the difference significant was found for larger kernel diameter. The highest value for larger kernel diameter was that of Creso Italian and Creso Kurde, while the Cemmitto showed lower value for larger kernel diameter (Table 1). The highest value for length kernel diameter was that of Cemmitto, Bakrajo 1 and Creso Italian while the Ovanto and Acsad 65 showed lower value for length kernel diameter (Table 1). As far as flour yield concern, the difference significant was not shown among the varieties. A difference significant was found among the varieties for protein content. The sedimentation test is an important and quick indicator, which can be determined with different modifications. In the evaluated samples of durum wheat, the SDS-Test values varied between 22-30 ml. The highest level of SDS-sedimentation values was found in Creso Italian and the lowest levels od SDS-sedimentation was detected in Cemmitto and Creso Kurde. On the other hand, the highest value of SDS-sedimentation index (SDSi) was found in Acsad 65 and Ovanto and the lowest value was found in Creso Italian.

The SDS-sedimentation volume correlated highly with the insoluble protein content, loaf volume, and dough strength (Axford et al., 1979; Blackman and Gill, 1980; Moonen et al., 1982). The varieties which contain allele 20 had the lowest volume, suggesting poor insoluble protein content.

Allelic variation of HMW-GS and LMW-GS

Wheat seed storage protein, composed of HMW-GS and LMW-GS, is one of the main contributors to wheat flour quality that affects food preference. In the present study, emphasis was placed on the diversity of the durum wheat in glutenin subunits. SDS-PAGE electrophoretic pattern of endosperm proteins (HMW and LMW-glutenins) of *T. durum* are shown in Figure 1 and Table 2. On the basis of the different mobility and corresponding banding patterns presence or absence, we designated the allelic variation of durum wheat. The glutenin subunits pattern was determined in 20 mg of flour/ variety. Creso Italian was used as references to determine the relative motilities of these subunits. In the analysis of 6 varieties of durum wheat a total of 3 different HMW-GS glutenin subunit patterns were detected (Figure 1). Each variety possessed 2 subunit bands. Region of migration corres-

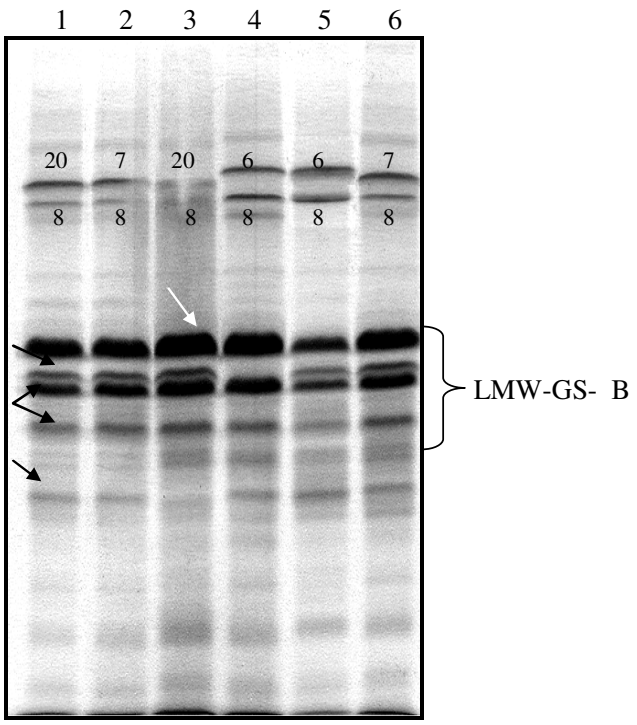

Figure 1. SDS-PAGE showing the LMW-GS Glutenins of durum wheat varieties. HMW glutenin subunits were designated according to Payne and Lawrance (1983). LMW were designated by Ruiz and Carrillo (1993) and Nieto-Taladriz et al. (1997). 1 = Bakrajo 1, 2 = Ovanto, 3 = Cemmitto, 4 = Creso Italian, 5 = Creso kurd, 6 = Acsad 65. Black arrow: Glu-B3, white arrow: Glu-A3.

Table 2. HMW-GS glutenin subunit compositions and quality scores for durum wheat varieties.

Varieties	Glu:A1	Glu:B1	Quality score
Bakrajo 1	Null	20+8	1
Ovanto	Null	7+8	4
Cemmitto	Null	20+8	1
Creso Italian	Null	6+8	2
Creso kurde	Null	6+8	2
Acsad 65	Null	7+8	4

HMW-GS and wheat quality are numbered according to Pagne and Lawrence (1983).

ponding to the various type of gluten proteins are indicated in the Figure 1 and Table 2 and Payne numbers are shown to the electrophoretic pattern.

The HMW-GS are ranked as follows: 7+8 is considered the best allele, 6+8 slightly less good than 7+8 and a pattern with a band designated 20+8 is considered least good (Pogna et al., 1990; Carillo et al., 1990). The wheat varieties Creso Kurde, Creso Italian and Bakrajo 1, Cemmitto were uniform in HMW-GS, having the combination 6+8 and 20+8. But the two varieties Ovanto and Acsad 65 have the 7+8 allele. In tetraploid wheat such as

Table 3. Allele's composition at the Glu-1 loci of durum wheat varieties.

Vari eties	Glu:A1	Glu:B1
Bakrajo 1	a	e
Ovanto	a	b
Cemmitto	a	e
Creso Italian	a	d
Creso kurde	a	d
Acsad 65	a	b

The alleles are designed according to Payne and Lawrence (1983).

durum wheat which no D genome, 1D-coded subunits will obviously be missing. We observed that the genes (Glu-A1) locate on chromosome 1A is silent is said to be null for all varieties. In contrast to the Glu-A1, the genes locate on chromosome 1B are expressed. The variety Acsad 65 and Ovanto have the highest quality score and the variety Cemmitto and Bakrajo 1 have the lowest quality score (Table 2). These results showed that the varieties Ovanto and Acsad 65 are the best varieties for making the paste. Table 3 summarizes the allelic composition at the Glu-A1 and Glu-B1. The table showed three different alleles b, d and e at locus Glu-B1.

In this study, SDS-PAGE of grain storage proteins HMW-GS and LMW-GS was performed in order to analyze molecular weight of gluten subunits and investigate genetic diversity among different wheat varieties (Figure 2). The electrophorogram showing proteins banding pattern of different wheat varieties are given in Figure 1. A total of 15 bands were obtained among which 8 bands were shown variation but the other bands common in all varieties. We studied the LMW-GS composition of the six wheat varieties by SDS-PAGE analysis (Figure 1). The different LMW-glutenin B subunit banding patterns were identified with 3-4 subunits each (Figure 1). The analyses of LMW-GS subunits led to the identification of four alleles at Glu-B3 and one allele at Glu-A3. The patterns were characterized by the absence of the strong staining in some variety and showed the difference in the all mobility of subunits. Table 4 showed the small difference among the wheat varieties for LMW-GS subunits. The Variation has been found among the six durum varieties analyzed. A total of three different HMW patterns and two different LMW patterns were detected. Four alleles at locus Glu-B3 was found in all varieties without Creso Italian which showed three alleles in this locus. This difference may be coming from the different resource. Several studies have demonstrated that LMW glutenin subunits are responsible for durum wheat quality (Payne et al., 1984; Pogna et al., 1990; Ruiz and Carrillo, 1993). The studies classified durum wheat into several models, LMW-1 and LMW-2 (Pagne et al., 1984). LMW-1^{-}, LMW-2^{-} and LMW-2^{*} (Carrillo et al., 1990) based only on the B-LMW glutenin subunits of slower mobility. Thus LMW-1 and LMW-1^{-} have been related to poor quality

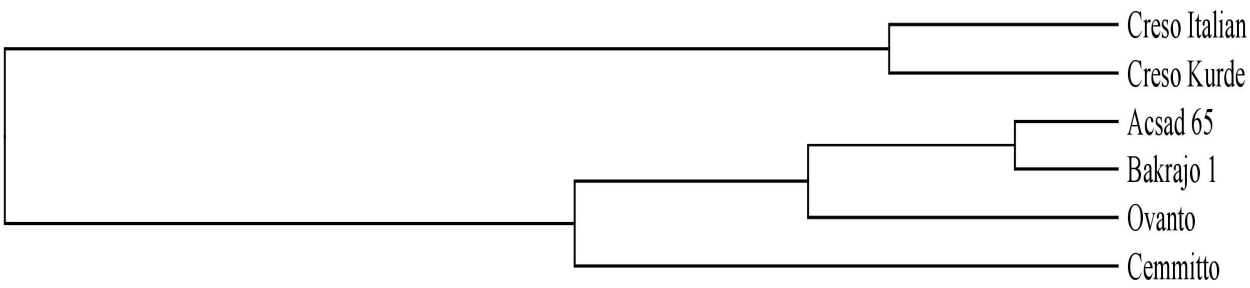

Figure 2. Dendrogram of durum wheat varieties showing the similarity among the varieties based on SDS-PAGE- HMW-GS and LMW-GS.

Table 4. Allele's composition at the Glu-3 loci of durum wheat varieties.

Varieties	Glu:A3	Glu:B3	LMW pattern
Bakrajo 1	a	b	LMW-1
Ovanto	a	b	LMW-1
Cemmitto	a	b	LMW-1
Creso Italian	a	c	LMW-2
Creso kurde	a	b	LMW-1
Acsad 65	a	b	LMW-1

The alleles are designed according to Ruiz et al. (1993) and Nieto-Taladriz et al. (1997).

and LMW-2 and LMW-2^{-} to good quality (Carrillo et al., 1990; Pogna et al., 1990). The relationship between some Glu-B3 variants is determined by one and two dimensional SDS-PAGE procedure (Gupta and Shepherd, 1990). Ciaffi et al. (1992) showed the relationship between the protein storage LMW of Creso and pasta making. The authors used the SDS-test, HMW and LMW-GS analysis for determining the relationship.

Genetic diversity of glutenin subunits

In this study SDS-PAGE of grain storage proteins HMW and LMW-GS was performed in order to analyze molecular weight of gluten subunits and investigate genetic diversity among different wheat varieties. The electrophorogram showing proteins banding pattern of different wheat varieties are given in Figure 1. To investigate evolutionary relationships among the bread wheat varieties according to HMW and LMW glutenin subunits, phylogenetic trees were drawn from the alignment of these varieties based on both HMW and LMW-GS. Cluster analysis of wheat grain storage proteins was performed on the results of SDS-PAGE using the software UPGMA to find out the diversity among the given wheat varieties. The results of cluster analysis are given in the dendrogram on the bases of matrix Dice (Figure 2). The diagram revealed four main groups: group 1= Creso Kurde and Creso Italian, group 2= Acsad 65 and Bakrajo 1, group 3= Ovanto , group 4= Cemmitto. The varieties Creso Kurde and Creso Italian and Acsad 65 and Bakrajo 1 showed more similarity than others varieties. On the other hand, Cemmitto showed more distance from the others varieties (Figure 2).

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