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Evaluation of Hexaploid Wheat Varieties for Making Bread by High Molecular Weight (HMW) and Low Molecular Weight (LMW) Analysis

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Abstract

Knowledge of composition of high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) and their associations with bread making and quality will contribute to genetically improving processing quality of bread wheats in Iraq. Six bread wheat varieties (Tammuz, Aras, Rabia, Cham 4, Cham 6 and Costantino) were conducted to detect the allelic variation at Glu-1 and Glu-3 loci by SDS-PAGE electrophoresis and to understand their effects on dough properties. Important methods applied for the breeding of bread-quality wheat (*Triticum aestivum* L.) consist of small-scale bread-quality tests for the determination of the grain protein content, SDS-sedimentation volume, thousand kernel weight and kernel diameter. The thousand kernel weight and SDS-sedimentation volume showed relatively higher significant among the varieties, whereas the flour yield showed no significance difference. The results of SDS-PAGE indicate that subunits/alleles 1 and null at Glu-A1, 7, 20 and 7+9 at Glu-B1, 2+12 and 5+10 at Glu-D1, allele a at Glu-A3, alleles d and m at Glu-B3 and alleles j and k at Glu-D3 in bread wheat varieties. The lowest frequency of subunit 7+9 was found in variety Tammuz. On the other hand, the variety Tammuz showed the highest value of score quality and the varieties Cham 4 and Cham 6 had the lowest value of score quality. Genetic diversity of wheat was evaluated by constructing the dendrogram for high molecular weight (HMW) and low molecular weight (LMW) gluten subunit bands.

الملخص

ان معرفة الغلوتينين ذات الاوزان الجزيئة الصغيرة والاوزان الجزيئة الكبيرة وارتباطاتها مع صناعة الرغيف ونوعيتة ستسهم في المعالجة وراثيا لتحسين نوعية الخبز والحنطة في العراق. و قد استخدمت طريقة الفصل الكهربائي لهلام كبريتات دوديكل الصوديوم متعدد الأكريلامايد لفصل جزيئات الغلوتينين ذات الوزن الجزيئي الصغير والوزن الجزيئي الكبير وللكشف عن تباين الأليلات فى المواقع Glu-1 و Glu-3 وفهم تأثيرها على صفات العجين في مختلف اصناف الحنطة الناعمة: تموز، آراس ، ربيعة، شام 4, شام 6 و كوستانتينو. ان أهم الأساليب المتبعة لدراسة نوعية الخبز والحنطة هي اختبارات الجودة لتحديد محتوى البروتين، حجم الترسبات للبروتين ، وزن الألف حبة, الطحين الناتج, وقطر الحبة. ان النتائج برزت فروق معنوية بين الاصناف من الحنطة بالنسبة لوزن الألف حبة وحجم الترسبات للبروتين، في حين لم تظهر اي الفروقات معنوية بين الاصناف بالنسبة للطحين الناتج. وتشير النتائج إلى وجود الأليلان 1 ولاغ (Null) في موقع Glu-A1 و الأليلات 7, 20 ,7+9 في موقع Glu-B1 و ألأليلات 2+12 و 5+10 في موقع Glu-D1 والأليل a في موقع Glu-A3 والأليلان d و m في موقع Glu-B3 والأليلان j و k في موقع Glu-D3. وان الغلوتينين ذو الأليل 7+9 عثر عليها فقط في الصنف تموز. و في المقابل، اظهر الصنف تموز أعلى قيمة للجودة في تصنيع الخبز في حين أعطت الاصناف شام 4 وشام 6 أدنى قيمة للجودة فى تصنيع الخبز. كما ان الاختلاف الوراثي للحنطة من حيث الغلوتينين درس عن طريق تكوين الرسم الشجري.

Keywords: Bread Wheat; Wheat Quality; HMW and LMW Of Glutenin; SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis).

1. Introduction

Wheat grain storage proteins are composed of 2 major fractions, gliadin and glutenin. Glutenin consists of both high-molecular-weight (HMW) and low-molecular weight (LMW) subunits. The HMW-glutenin subunits (HMW-GS) are encoded by Glu-A1, Glu-B1, and Glu- D1 on the long arm of chromosomes 1A, 1B and 1D, respectively (Payne et al., 1980). The LMW-glutenin subunits (LMW-GS) are encoded by Glu-A3, Glu-B3, and Glu-D3 on the short arm of these chromosomes (Gupta et al., 1990). Glutenin subunits were also classified into A (HMW-GS), B and C (LMW-GSs) subunits based on their mobility in SDS-PAGE analysis (Gupta et al., 1990). These glutenin subunits are polymerized by intermolecular disulfide bonds, which play a major role in the rheological properties of wheat flour doughs. It has been shown that allelic variations of HMW-GSs and LMW-GSs affect dough properties in various wheat cultivars (Gupta et al.,

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1991; Gupta et al., 1994; Khelifi et al., 1992; Nagamine et al., 2000; Payne et al., 1987a; Payne et al., 1987b). The HMW glutenins represents 5-10% of the total grain protein. The HMW glutenins are further subdivided into allelic pairs on 1B and 1D and a single subunit on 1A and each of these subunits influences wheat flour and dough quality. The role of individual LMW-GSs is, however, much less well characterized than that of HMW-GSs, because large numbers of the subunits display similar mobilities in SDS-PAGE analysis.

While the HMW glutenins are the major determinants of bread quality, LMW glutenins and gliadins are also important. Genes encoding the LMW glutenins are present on the short arm of chromosome 1A, 1B, and 1D.The LMW glutenins are one-third of the total seed protein and 60% of the total glutenins. The HMW and the LMW glutenins form extensive disulphide linked polymers that influence the dough quality. The LMW glutenins form aggregates may be important for dough strength. The cysteine residues in the LMW structure helps to separate two different HMW polymer-building subunits. The chain extenders (having two or more cysteine residue) allow the formation of stronger dough's, while chain terminators have the opposite effect (Greenfield et al*.,* 1998; Masci et al., 1998). The chain extender proteins have increased strength and stability due to the longer repetitive domains. The polypeptides with single cysteine residue have decreased dough strength and stability as they act as chain terminators in the glutenin polymers. The reduction in proportion of LMW glutenins, results in dough properties shifting towards greater strength due to an increase in the HMW/LMW glutenin ratio (MacRitchie and Lafiandra 2001; Lawerence et al., 1998). The increase in the polymeric proteins results in a stronger dough strength that is good for bread quality. In contrast the dough mixing strength is reduced in deletion lines missing the HMW glutenins. An increase in the amount of polymeric protein and better flour performance has also been demonstrated (Ciaffi et al., 1995; Rogers et al., 1997; Lafiandra et al., 1998). The use of registered crop varieties makes their expeditious identification important; its significance is increased by the diversity of varieties in many important traits. Each variety is characterized by a specific set of traits that determine its use.

Gliadins and glutens are genetic markers allowing the expeditious and objective identification of a variety, determination of its genetic constitution, and determination of some important characteristics and traits. Genetic diversity is the basis for successful crop improvement and can be estimated by different methods such as morphological traits, end-use quality traits, and molecular markers (Fufa et al., 2005). The present study was undertaken to evaluate the quality and genetic diversity in gluten-subunits in six wheat varieties using SDS-PAGE.

2. Materials and Methods

2.1. Plant Sample

Grains of wheat varieties were collected from the department of Agriculture of Sulaimanyah. The bread wheat varieties (Tammuz, Aras, Rabia, Cham 4 and Cham 6) sown, grown and harvested in the same location. The origin of Tammuz, Aras and Rabia is Iraq while the origin of Cham 4 and Cham 6 is Syria (ICARDA). Costantino was used as a reference in this study. Cham 4 and Cham 6 were introduced to Iraq by FAO (Food and Agriculture Organization) since 1997.

2.2. SDS-PAGE Electrophoresis

2.2.1. HMW-GS Extraction

The grain protein HMW-GS was analyzed by using SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). The grains (30 gram) were ground to fine powder and 20 mg was weighed in 1.5 ml microtube. Three hundred microliter of protein extraction buffer [28.5% sample buffer (7% SDS, Tris-HCl 0.01 M (pH 6.8), 30% glycerol, 0.001% Coomassie bleu), 5% 2-mercaptoethanol] was added to each micro tube, kept 2 hr at room temperature (27°C) and centrifuged at 13000 rpm for 10 min. The supernatant contains 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 samples were heated at 80°C for 20 min and then 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 hours. After 18 hours, the gels were stained in 12.5% (w/v) trichloroacetic acid, 0.01% (w/v) Coomassie Brilliant Blue R250 and distained with distilled water (Akhtar et al., 1994).

2.2.2. LMW-GS Extraction

The flour (20 mg) was added to 500 µl 50% (v:v) propan-2-ol at 60°C for 30 min with agitation every 10 min at room temperature, then centrifuged at 13000 rpm for 10 min. The residue was washed with 500 µl 50% propan-2-ol for 30 min at 60 ºC. After centrifuging at 13000 rpm for 10 min, the supernatant was again discarded. This step to remove gliadins was repeated three times. Glutenin was then solubilized with 500 µl of solution [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 solution [50% (v:v) propan-2-ol, 0.08 M Tris-HCl (pH 8.5), 40 mM 4-vinylpyridine], and incubated for 3 hr 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, 0.02% Bromophenol blue or 0.001% Coomassie bleu]. Finally, 30 µl samples were loaded into the slots of SDS-PAGE (Cherdouh et al., 2005). 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 at15 mA/gel for 18 hours. After 18 hours, the gels were stained in 12.5% (w/v) trichloroacetic acid, 0.01% (w/v) Coomassie Brilliant Blue R250 and distained with distilled water (Akhtar et al., 1994).

2.2.3. Two Dimensional Gels A-PAGE X SDS-PAGE

The two dimensional Acid-PAGE x SDS-PAGE was performed by the protocol as described by Pagne et al., 1984. After the first dimension A-PAGE (Acid

polyacrylamide gel electrophoresis), the gels were cut into single strips and incubated for 15 min in 0.0625 M Tris-HCl (pH 6.8), 2% (w/v) SDS, 40% (w/v) glycerol. The strips were then loaded onto a SDS-PAGE gel prepared as described above. Gels were run at 40mA/gels at room temperature and stopped 30 min after the tracking dye had reached the bottom of the gel. They were stained as described above.

2.3. 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 38-12 (AACC 2000) and reported as protein by N*6.25. (American Association of Cereal Chemists. 2000 approved method of the AACC, 9th ed. Method 38-12).

2.4. SDS-Test

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

2.5. Measurement of Quality Traits

- Thousand Kernel Weight: We prepared a 300 gram of wheat grain by removing all dockage, shrunken and broken seeds, and other foreign material. The samples (300 gram /variety) divided into five lots of 60 gram and the number of seeds was calculated for each lot.
- Length And Large Kernel Diameter: the diameter of kernel was calculated by taking several photos of five lots of each variety (10 gram/lot). The diameter was determinate by Image-J software.
- Flour Yield: five lots of 60 gram of each variety were weighted before and after grinding. The flour yield was measured by the difference between the weight of kernel before grinding and the weight of flour yield.

2.6. 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. LSD (least significant difference) test was carried out using a statistical package SPSS-PC, version 15. UPGM (Unweighted Pair Group Method with Arithmetic) used for construct the dendrogram.

3. Results and Discussion

3.1. Characterization of Wheat Varieties by Quality Evaluation

Thousand kernel weight, length diameter kernel, larger diameter kernel, protein content, SDS-Test and SDS index showed significant differences, while flour yield was not significant (Table 1). Costantino gave the lowest thousand kernel weight and Aras gave the highest value. In terms of kernel quality, inverse relationships have been reported between the kernel size and protein content (O'Brien and Ronalds, 1984). The highest value for length kernel diameter was that of Tammuz, Aras and Rabia while Cham 4, Costantino and Cham 6 showed lower value for length kernel diameter (Table 1). The varieties Cham 4, Cham 6 and Tammuz had the highest values of larger kernel diameter and Aras, Costantino and Rabia had the lowest values. The flour protein content ranged from 8.14 to 9.24 and Costantino showed the lowest value. According to the SDS-test, the varieties were divided into five groups. The SDS-test of Tammuz was highly significant than the rest of varieties. The variety Cham 4 had the lowest value of SDS-test suggesting poor insoluble protein content. The SDS-sedimentation volume correlated with the amount of total HMWG subunits and individual HMWG subunits. Some subunits were positively correlated, and the others were negatively correlated with the sedimentation volume (Seilmeier et al., 1991). Carrillo et al. (1990) reported that HMWG subunits had additives and epistatic effects on the SDS-sedimentation volume. Rharrabti et al. (2003) studied the relationship between some quality traits and yield of durum under different conditions.

3.2. Characterization of Wheat Varieties by SDS-PAGE

3.2.1. Allelic Variation of HMW-GS Subunits at Glu-1

The wheat varieties analyzed showed four different HMW glutenin banding patterns (Table 2). The frequency of occurrence of HMW glutenin subunits with composition of 1 (50%), 7+9 (16.67%), 7 (50%), 20 (33.33%), 5+10 (33.33%) and 2+10 (66.67%). Subunit 20 had different effects on the functional properties in three subunits at the Glu-D1 allele. Subunit 20 with a background of subunits 2+12 had a smaller negative effect than subunits 5+10 and subunits 2.2+12 (Kanenori et al., 2003). Subunits 17+18 had higher values of functional properties than subunits 7+9 with a background of subunits 2.2+12 (Kanenori et al., 2003). On the other hand, based on the analysis of various cultivars from several countries, Moonen et al., (1983) reported that the Glu-A1b alleles exerted stronger effects on the SDS sedimentation than Glu-A1a.

The varieties Tammuz, Aras and Costantino showed the presence of allele 1 at the locus Glu-A1. On the other hand, the varieties Rabia, Cham 4 and Cham 6 revealed the absence of allele at the locus Glu-A1 (Table 2). All varieties showed the presence of alleles at locus Glu-B1. At Glu-B1, there are three types of alleles a, c, e (7, 7+9 and 20) (Tables 2 and 3). The SDS-PAGE analysis showed two types of alleles at locus Glu-D1: a, d (5+10 and 2+12) (Tables 2 and 3). Figure 2 showed the two dimensional A-PAGE x SDS-PAGE maps of reduced and alkylated glutenin from wheat varieties. The HMW-GS of glutenin were identified easily (Figure 2). The results confirmed the significantly beneficial effects of the Glu-D1d on the dough and gluten strength (Tadashi et al., 2006). Kanenori et al. (2003) showed that the Glu-A1a and Glu-A1b alleles exerted similar effects on the gluten score.

Table 1. Thousand kernel weight, length kernel diameter, large 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
Tammuz	40.80 ab	6.25 a	3.14 a	74.82 a	9.15 a	40 a	4.37 a
Aras	41.82 a	6.17 a	2.47 b	74.07 a	9.09 a	35 b	3.85 b
Rabia	35.62 cd	6.11 a	2.07 c	73.64 a	9.03 a	22 e	2.43 d
Costantino	34.61 d	5.52 b	2.14 c	74.92 a	8.14 b	30 c	3.68 b
Cham 4	36.34 c	5.54 b	3.19 a	74.36 a	9.24 a	24 d	2.59 cd
Cham 6	39.67 b	5.40 b	3.16 a	74.52 a	9.09 a	25 d	2.75 c
LSD	1.32	0.33	0.25	1.87	0.35	2.72	0.28

TKW: Thousand kernel weight, LESD: Length kernel diameter, LASD: Large kernel diameter, FY: Flour yield, PC: Protein content, SDS-test: Sedimentation Test, SDSi: Sedimentation Index.Values shown by the same letter are not significantly different (p=0.05) by LSD test.

Table 2. HMW subunits composition and quality score of wheat varieties.

Varieties	Glu:A1	Glu:B1	Glu:D1	Quality Score
Tammuz	1	7+9	5+10	14
Aras	1	20	5+10	10
Rabia	Null	20	2+12	5
Costantino	1	7	2+12	7
Cham 4	Null	20	2+12	5
Cham 6	Null	20	2+12	5

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

Varieties	Glu:A1	Glu:B1	Glu:D1
Tammuz	a	c	d
Aras	a	e	d
Rabia	-	e	a
Costantino	a	a	a
Cham 4	-	a	a
Cham 6	-	a	a

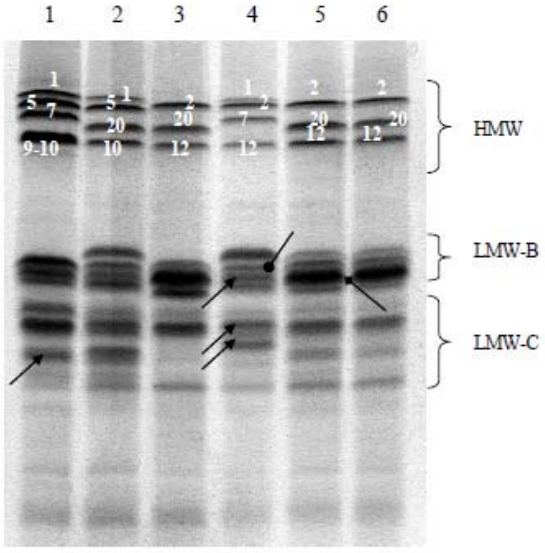

Figure 1. SDS-PAGE gel showing the HMW and LMW-GS glutenins of bread wheat varieties.
1: Tammuz, 2: Aras, 3: Rabia, 4: Costantino, 5: Cham 4, 6: Cham 6. Arrow head: alleles encoded by locus Glu-D3, arrow square: alleles encoded by Gl-B3, arrow circle: alleles encoded by Glu-A3.

From the electrophoretic spectra the individual HMW glutenin subunits were determined and so Glu-quality score was calculated (Table 2). The highest value of Glu-score was achieved by the variety Tammuz. On the other hand, Cham 4 and Cham 6 showed the lowest value of score quality (Table 2). These results show that the variety Tammuz contains more of insoluble protein than others of varieties. The couple of subunits 5+10 is the good marker for breading-making quality and the subunit 20 is the marker for weak wheat quality (Payne and Lawrence 1983, Payne et al., 1987a). HMW-GS may be used as a molecular marker of bread-making quality of wheat. The verified correlations between bread-making quality and specific HMW subunit of glutenin can be utilized by wheat breeders using SDS-PAGE of proteins as a screening test for the prediction of breading-making quality of wheat.

Several electrophoretic techniques have been applied to separate the subunits of glutenin in bread wheat cultivars. The method proposed by Gupta and Shepherd (1990) consists of a two-step, one-dimensional fractionation by SDS-PAGE in which glutenin is reduced before being loaded onto a gradient gel for the second step. A great number of bread wheat cultivars have been analyzed by this method. Three different groups of electrophoretic patterns were identified and attributed to genomes A, B and D. A similar approach was proposed by Khelifi and Branlard (1992) and Redealli et al., (1995). Redealli (1995) showed the fraction of HMW-GS and LMW-GS of glutenin by one and two dimensional gels. The storage proteins of hexaploid wheat are important nutritionally but, above all, because of the unique cohesive-elastic properties they bestow on dough made from wheat flours. The HMW subunits of glutenin are considered to be the most important components with respect to the baking quality. Correlations have been established between particular HMW glutenin subunits and bread-making quality of wheat (Payne et al., 1987a; Kriac et al., 1997).

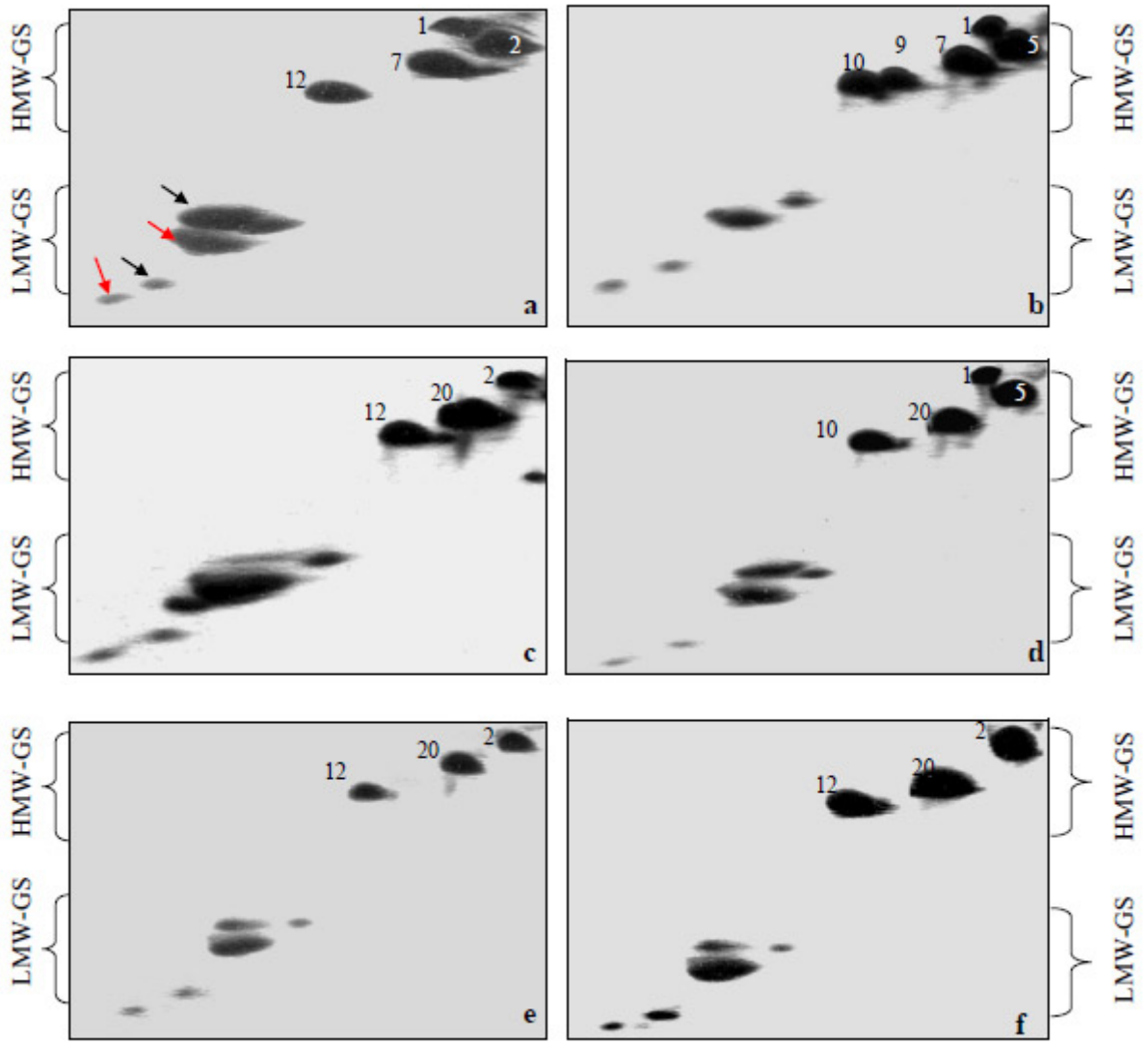

Figure 2. Two-dimensional A-PAGE x SDS-PAGE fractionation of HMW and LMW-GS from bread wheat varieties. a: Costantino, b: Tammuz, c: Rabia, d: Aras, e: Cham 6, f: Cham 4.

3.2.2. Allelic Variation of LMW-GS Subunits at Glu-3

Low molecular weight glutenins are important because the variation in LMW-B glutenin decides the end use quality. We studied the LMW-GS composition of the six wheat varieties by SDS-PAGE analysis. SDS-PAGE profiles of LMW glutenins showed five different LMW glutenin subunits at Glu-3 loci (Figure 1, Table 4). The differences between the patterns were for mobility of bands. The LMW-5 is the most frequent type and the rest of them are present only in one variety. We detected the B and C subunit of LMW-GS in all varieties (Figure 1). The SDS-PAGE gel also showed the C subunit of LMW-GS with a higher mobility than B subunit of LMW-GS (Figure 1). Each variety had 5-6 bands of LMW glutenin B and C subunits coded Glu-A3, Glu-B3 and Glu-D3 loci (Table 4). The same variations were detected in the LMW-Gs among the varieties. According to the profile of LMW subunit B, the varieties divided into four groups: group 1 contains Tammuz, group 2 includes Rabia, group 3 contains Aras and Costantino, group 4 includes Cham 4 and Cham 6. On the other hand, the profile of LMW subunit C divides the varieties into five groups: group 1= Tammuz, group 2= Aras, group 3= Rabia, group 4= Costantino, group 5= Cham 4 and Cham 6. Several LMW subunits in the SDS-PAGE patterns are not attributed to any specific Glu-3 locus because of the overlapping between polypeptides encoded by different alleles (Pogna et al., 1995). Low

Table 4. Allele's composition at the Gli-3 loci and LMW pattern of bread wheat varieties.

Varieties	Glu:A3	Glu:B3	Glu:D3	LMW pattern
Tammuz	a	m	k	LMW-1
Aras	a	m	k	LMW-1
Rabia	a	d	j	LMW-3
Costantino	a	m	k	LMW-4
Cham 4	a	d	j	LMW-5
Cham 6	a	d	j	LMW-5

molecular glutenin subunits are important in determining the dough viscoelastic properties of hexaploid and tetraploid wheat flours (Pogna et al., 1990). However, the basis of differences in effects of different low molecular weight subunits alleles on dough properties is still largely unknown. Gupta and MacRitchie (1991) have shown that the Glu-A3m produces no major B subunit, whereas allele Glu-A3a codes for the major B subunit. This allelic difference was found to be responsible for variation in both the size distribution of the glutenin polymers and dough strength.

3.3. Genetic Diversity among The Varieties

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 Figures 1. A total of 18 bands were obtained among which 12 bands were show variation but the other bands common in all varieties (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 (Figure 3). 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 Alignment indicated that the phylogenetic tree was divided into three parts: group 1= Cham 4, Cham 6, Rabia; group 2= Tammuz, Aras and group 3= Costantino. The varieties Cham 4 and Cham 6 showed more similarity than others varieties. On the other hand, Costantino showed more distance with the others varieties. Genetic diversity of European spelts wheat was evaluated by constructing the dendrogram for HMW and LMW glutenin subunit bands (Xueli et al., 2005). Fufa et al. (2005) reported that the genetic diversity estimates based on seed storage protein were lowest because they were the major determinants of end-use quality, which is a highly selected trait.

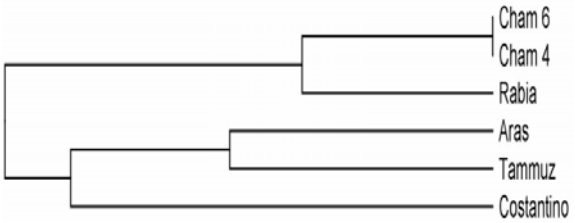

Figure 3. Dendrogram of bread wheat varieties showing the relationship among the varieties based on SDS-PAGE-HMW and LMW-GS.

In conclusion, the results showed that there was the significant difference among the varieties for some of quality traits tested. The electrophorogram revealed that HMW subunit 7+9 which related with the strong quality of wheat for baking is less frequent in the varieties tested. In contrast, HMW subunits 2+12 and 20 which related with the weak quality for making of bread is more frequent in the varieties tested. The LMW-B subunits revealed more high level of polymorphisms than LMW-C subunits. Identification of glutenin subunits in bread wheat varieties in Iraq may be useful for selection aims in breeding programs to determine the relationship between gluten visco-elastic properties.

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References

Akhtar H and Odean ML.1994. Characterization of the 1B/1R translocation in wheat using water extractable protein concentrate. Euphytica. 78: 109-113.

American Association of Cereal Chemists. 2000. Approved methods of the AACC, 10th ed. Method 38–12A Wet gluten, dry gluten, water-binding capacity, and gluten index. The American Association of Cereal Chemists, Inc., St. Paul, MN, USA.

Carrillo JM, Rousset M, Qualset CO & Kasadora DD. 1990. Use of recombinant inbred lines of wheat for study of associations of high-molecular-weight glutenin subunit alleles to quantitative traits 1. Grain yield and quality prediction tests. Theor. Appl. Genet. 79: 321–330.

Cherdouh A, Khelifi D, Carrillo JM, Nieto-Taladriz MT. 2005. The high and low molecular weight glutenin subunit polymorphism of Algerian durum wheat landraces and old cultivars. Plant breeding. 4: 338 – 342.

Ciaffi M, Margiotta B, Colaprico G, De-Stafani E, Sgrulletta D, and Lafiandra D. 1995. Effect of high temperatures during grain filling on the amount of insoluble proteins in durum wheat. J. Cereal Sci. 49: 285-296.

Fufa H, Baenziger PS, Beecher I, Dweikat V, Graybosch RA, Eskridge KM. 2005. Comparison of phenotypic and molecular marker-based classifications of hard red winter wheat cultivars. Euphytica. 145: 133-146.

Greenfield JJA, Ross-Murphy SB, Tamas L, Bekes F, Halford NG, Tatham AS & Shwery PR. 1998. Rheological properties of monomeric and polymeric forms of C-hordein, sulphur-poor prolamins of barley. J. Cereal Sci. 27: 233-236.

Gupta RB & Shepherd KW. 1990. Two-step one dimensional SDS-PAGE analysis of LMW subunits of glutelin. Variation and genetic control of the subunits in hexaploid wheat. Theor. Appl. Genet. 80: 65–74.

Gupta RB, Bekes F & Wrigley CW. 1991. Prediction of physical dough properties from glutenin subunit composition in bread wheats. Cereal Chem. 68: 328–333.

Gupta RB and MacRitchie F. 1991. A rapid on-step one dimensional SDS-PAGE procedure for analysis of subunit composition of glutenin in wheat. J. Cereal Sci. 14: 105-109.

Gupta RB, Paul GB, Cornish GA, Palmer FB & Rathjen AJ. 1994. Allelic variation at glutenin subunit and gliadin loci, *Glu-1*, *Glu-3* and *Gli-1*, of common wheats. 1. Its additive and interaction effects on dough properties. J. Cereal Sci. 19: 9–17.

Gupta RB, MacRitchie F. 1994. Allelic variation at glutenin subunit and gliadin loci, *Glu-1, Glu-3* and *Gli-1* of common wheats. II. Biochemical basis of the allelic effects on dough properties. J. Cereal Sci. 19 :19–29.

Kanenori T, Zenta N, Wakako F, Tatsuo K & Hiroaki Y. 2003. Difference in Combination between *Glu-B1* and *Glu-D1* Alleles in

Bread-Making Quality Using Near-Isogenic Lines. Food Sci. Technol. Res. 9 (1): 67–72.

Karic J, Gregova E, Tisova V. 1997. Genetic variability at the Glu-1 loci in old and modern wheats (*Triticum aestivum* L.) cultivated in Slovakia. Genet. Res. Crop Evol. 44: 301–306.

Khelifi D & Branlard G. 1992. The effects of HMW and LMW subunits of glutenin and of gliadins on the technological quality of progeny from four crosses between poor bread making quality and strong wheat cultivars. J. Cereal Sci .16: 195–209.

Lafiandra D, Masci S, Margiotta B & De Ambrogio E. 1998. Development of durum and bread wheat with increased number of High Molecular Weight glutenin subunits. Pages 261-264. in. Proc 9th International Wheat Genetics Symposium, Vol. 4. Grain Quality. A.E. Slinkard. ed. University of Saskatchewan: Saskatoon, Canada.

Lawerence GJ, MacRitchie F & Wrigley CW. 1998. Dough and baking quality of wheat lines deficient in glutenin subunits controlled by Glu-A1, Glu-B1 and Glu-D1 loci. J. Cereal Sci. **7**: 109-112.

MacRitchie F and Lafiandra D. 2001. The use of near-isogenic wheat lines to determine protein composition-functionality relationship. Cereal Chem. 78: 501-506.

Masci S, D'Ovidio R, Lafiandra D & Kasarda DD. 1998. Characterization of a low-molecular-weight glutenin subunit gene from bread wheat and the corresponding protein that represents a major subunit of the glutenin polymer. Plant Physiol. 118:1147-1158.

Moonen JHE, Scheepstra A & Graveland A. 1983. The positive effects of the high molecular weight subunits 3 + 10 and 2* of glutenin on the bread making quality of wheat cultivars. Euphytica. 32: 735–742.

Nagamine T, Kai Y, Takayama T, Yanagisawa T. & Taya. 2000. Allelic variations of glutenin subunit loci Glu-1 and Glu-3 in southern Japanese wheat and their effects on dough and gluten properties. Cereal Sci. 32: 123–135.

O'Brien L & Ronalds JA. 1984. Yield and quality interrelationships amongst random F_3 lines and their implications for wheat breeding. Australa. J. of Agri. Res. 35(4): 443 – 451.

Payne PI, Law CN, Mudd EE. 1980. Control by homoeologous Group 1 chromosome of the high-molecular-weight subunits of glutenin, a major protein of wheat endosperm. Theor. Appl. Genet. 58: 113-120.

Payne PI & Lawerence GJ. 1983. Catalogue of alleles for the complex gene loci, Glu-A1, Glu-B1, Glu-D1 which code for high-molecular-weight subunits of glutenin in hexaploid wheat. Cereal Res. Comm. 11: 29-35.

Payne PI, Jackson EA, Holt LM, Law CN. 1984. Wheat storage proteins: Their genetics and their potential for manipulation by plant breeding. Philos. T. Roy Soc B. 304:359-371.

Payne PI, Nightingale MA, Krattiger AF & Holt LM. 1987a. The relationship between HMW gutenin subunit composition and the bread-making quality of British-grown wheat varieties. J. Sci. Food Agr. 40: 51–65.

Payne PI, Seekings JA, Worland MG, Jarvis M, Holt L. 1987b. Allelic variations of glutenin subunits and gliadins and its effect on breadmaking quality in wheat: Analysis of F5 progeny from Chinese Spring X Chinese Spring (Hope 1A). J. Cereal Sci. 6: 103--118.

Pogna NE, Redaelli R, Vaccino P, Biancardi AM & Peruffo ADB. 1995. Production and genetic characterisation of near-isogenic lines in the bread wheat cultivar Alpe. Theor Appl. Genet. 90:650-658.

Pogna PE, Autran JC, Mellini F, Lafiandra D & Feillet P.1990. Chromosome 1B-encoded gliadins and glutenin subunits in durum wheat: genetics and relationship to glutenin strength. J. Cereal Sci. 11: 15–34.

Redaelli R, Morel MH, Autran JC & Pogna NE.1995. Genetic analysis of low M_r glutenin subunits fractionated by two-dimensional electrophoresis (A-PAGE × SDS-PAGE). J. Cereal Sci. 21:5-13.

Rharrabti Y, Villegas D, Royo C, Martos-Núñez V & García del Moral LF. 2003. Durum wheat quality in Mediterranean environments: II. Influence of climatic variables and relationships between quality parametersField. Crops Res. 133-140.

Rogers WJ, Miller TE, Payne PI, Seekings JA, Holt LM & Law CN. 1997. Introduction to bread wheat (*Triticum aestivum* L.) and assessment for bread making quality of alleles from T. boeticum Boiss. Ssp. Thaoudar at Glu-A1 encoding two high-molecular weight subunits of glutenin. Euphytica. 93: 19-29.

Seilmeier W, Belitz HD. and &Weiser H. 1991. Separation and quantitative determination of high-molecular-weight subunits of glutenin from different wheat varieties and genetic variants of the vari ety Sicco. Z. Lebensm Unters Forsch. 192: 124–129.

Tadashi T, Shojiro I and Tatsuya M. Ikeda. 2006. Effects of High-molecular-weight and Low-molecular-weight Glutenin Subunit Alleles on Common Wheat Flour Quality. Breeding Sci. 56 (2): 131-136.

Xueli A, Qiaoyun L, Yueming Y, Yinghua X, Hsam SLK, Zeller FJ. 2005. Genetic diversity of European spelt wheat (*Triticum aestivum* ssp. *spelta* L. em. Thell.) Revealed by glutenin subunit variations at the *Glu-1* and *Glu-3* loci. Euphytica. 146(3): 193-201.