Characterization of Durum Wheat Genotypes by Seed Storage- Protein Electrophoresis

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Abstract:

Durum wheat grains from twenty five genotypes of 5x5 diallel crosses, which includes 20 crosses and 5 parents, were collected. Analyzing of seed storage –proteins for all genotypes done by sodium dodcyle sulphate polyacrylamide gel electrophoresis(SDS-PAGE). Evaluation of Genetic composition for HMW and LMW of glutenin and gliadin unit bands were done by comparing the results and number of bands and pair bands with the reference standards, results showed high rate of quality in addition to adaptation to local environment, results supported by measuring protein % and Ash% and color pigments.

Keywords: Durum wheat genotypes, SDS-PAGE, HMW, LMW, Color pigments.

Introduction:

Protein level and composition present in flour play a major role in quality. Each protein's structure is under genetic control and is strongly influenced by several environmental factors, So that breeders can select for varieties that have the best protein concentration and composition for high quality products. The types of proteins present also influence quality[1].

In durum wheat (*Triticum aestivum* L.), industrial quality is determined by genetic components and environmental conditions. Storage proteins like gliadins and glutenins are the most important genetic components, as they are responsible for visco-elasticity in dough.
The gliadin proteins are essential giving durum the ability to rise properly and fix shape on cooking. The evaluated germplasm showed gliadin γ-45 associated with good durum quality and γ-42 associated with bad durum quality. Glutenin is a protein best known for its role, along with gliadin, in the creation of gluten with its disulfide inter- and intra-molecule links. The tested germplasm showed high molecular weight glutenin subunit (HMWGs) allele 20, associated with poor quality as an example, most common wheat cultivars possess three to five HMW-GS, the low molecular weight-gluten subunits (LMW-GS) represents about one-third of the total seed protein and 60% of total gluten[2-4]. Studies of interaction between storage proteins and durum quality confirmed the positive effect in durum quality of 7+8 and 6+8 HMWGs alleles and A β-gli pattern. previous results confirm these alleles/patterns as useful markers to improve durum quality in breeding programmes [5,6].

The researchers discovered a correlation between the presence of certain HMW-GS and gluten strength (& some other quality parameters), measured by the SDS-sedimentation volume test [7]. Color is an important parameter involved in the definition of semolina and pasta quality, this character is mainly due to natural pigments (carotenoids) that are present at different levels in cereals and

cereal products, due to botanical origin, growing conditions, distribution in the kernel, and technological processes[8]. Flour whiteness and brightness showed a highly significant and positive correlation with starch content, the yespacness of the flour exhibited a highly significant positive correlation with kernel hardness, flour granulatin, protein content, ash content. The color of durum wheat is more or less yellow, or amber, and is caused by the presence of carotenoid pigments, mainly xanthophylls. The higher the color the better is durum suited for burghul, cous cous, semolina and pasta making and for the export market. The colors of semolina and flours are expressed using the L* a* b* color system (the three coordinates of CIELAB represent the lightness of the color). For semolina the higher the b* value the more yellowness[9].

There is another quality parameter related to pasta color, the color score, which combines L* and b* in one index. A spaghetti sample with a color score of 9.0 or higher is good. The range of the color score is 1 - 12.Color is an important parameter involved in the definition of semolina and pasta quality. This character is mainly due to natural pigments (carotenoids) that are present at different levels in cereals and cereal products, due to botanical origin, growing conditions, distribution in the kernel, and technological processes. In food industries, color measurements are usually performed by means of automatic instruments that are rapid and safe, as alternatives to the chemical extraction methods. Results have confirmed lutein and β-carotene as the main components mainly responsible for the yellow color in wheat grains[8].

Bright yellow colour of durum wheat products is the result of natural carotenoid pigment content and of their oxidative degradation by lipoxygenase (LOX) activity. The yellowness of the flour exhibited a highly significant positive correlation with kernel hardness, flour granulation, protein content, ash content and carotein content[9]. Although pigment content and LOX level are mainly varietal characteristics, they can be affected by environmental factors [10]. Protein content was investigated and the rate of its content of the flour ranged from 8.1% to 12.5%, and high temperature conditions may have important implications for wheat flour quality [11], The aim of the present study is to evaluate the genetic diversity in protein (Glutein and Gliadin) units in 25 local durum wheat produced by 5x5 full diallel crosses by using SDS-PAGE, and some other characters related to the quality.

Materials and Methods:

Grains of twenty five durum wheat genotypes produced from 5x5 full diallel cross from Qlyasan- Agricultural research station of College of Agriculture, Univ. of Sulaimani. (table 1). The samples were prepared for later analysis in the ICARDA laboratories by the authors. The labor analysis includes identification of variability of seed-storage proteins by using SDS-PAGE and estimation of color of studied genotypes as well as seed protein% content and Ash %.

Table (1) Name of the samples and Symbols.

Symb	Sample name	Symb	Sample name
1	BakrajoXIraq7	14	Pansifle x Iraq-7
2	Iraq-7 x Bakrajo	15	Omrabi x Semito
3	Bakrajo x Omra	16	Semito x Omrabi
4	Omrabi x Bakrajo	17	Omrabi x Pansifle
5	Bakrajo x Semito	18	Pansifle x Omrabi
6	Semito x Bakrajo	19	Semito x Pansifle
7	Bakrajo x Pansifle	20	Pansifle x Semito
8	Pansifle x Bakrajo	21	Bakrajo-1
9	Iraq-7 x Omrabi	22	Iraq-7
10	Omrabi x Iraq-7	23	Omrabi
11	Iraq-7 x Semito	24	Semito
12	Semito x Iraq-7	25	Pansifle
13	Iraq-7 x Pansifle		

1. SDS-PAGE Electrophoresis Method:

Procedure of extraction

1- After grinding samples, 0.40 g was taken and transferred to a 1.5 ml microtube.

2- 1 ml of the clean solution (50%propanol-1)(n°2) was added and incubate it at 60°C for 30 min. With shacking each 10 min.

3- Centrifuge at maximum speediness for 10 min.

1- Adding 250 µl of reduction solution (50% propanol-1, 80mM Tris-HCl pH: 8.5, 2% DTT) (n°3) and incubated it at 60°C for 30mn. With regular shacking each 10mn.

4- Centrifuge at maximum speediness for 10mn.

5- Add 2.8 µl of 4-Vinylpiridine at each tube and incubate all at 60°C for 30mn without shacking.

6- For precipitation of glutenins, 1 ml of cold acetone (-20°C) was added to each tube and keep it at -20°C for 15mn or overnight Centrifuge, throw the supernatant, and the pellet was dried under fume-hood. Stored at -20°C. or resuspend it in the solution n°4.The supernatant contain dissolved extracted protein ready for experiment purposes. The subsequent steps which included preparing the gliadin gels (40% acrylamide), running (at 18°C) and staining were done according to the standard procedures.

2. Estimation of Color of Durum Wheat

Determination of β-carotene as the main components which mainly responsible for the yellow color in wheat grains was done by taking amounts of grain flour and added to glass eye of the instrument which was (CHROMA METER CR-400\ KONICA MINOLTA-JAPAN Made) (one by one and the glass was cleaned carefully after each sample). The ability of the index *b** to express natural dyeing was dependent on sample characteristics as demonstrated by the relationships found between this index and pigments.

3. Protein % and Ash%: Protein % and ash% measured by Infrared Grain Analyser. In Durum-Molecular Marker Assisted Lab.- ICARDA

Results and Discussions:

According to the result of the SDS-PAGE, in this study, SDS-PAGE of grain storage proteins was performed in order to analyze molecular weight of glutinin and glaidin subunits and investigate possible genetic diversity among different wheat varieties Fig. 1 and Fig.2, represents the electrophoresis of the glutenin and gliadin, as the major wheat protein content, results were compared with standards, which used as reference, the results of compareson with standards reveal heterogeneity among 25 genotypes, the electrophoresis result for glutenin showed several bands as HMW including the

bands number 5,7,8, 9,10, 17,18,20 which were common in all hybrids, especially the pair bands 5+10 and 17+18, because of the unique effect of them which may related to the existing or form of more additional systin, which is responsible of large size polymers, while Fig. 1 show similar results due to LMW bands in case of heterogenty among all genotypes.

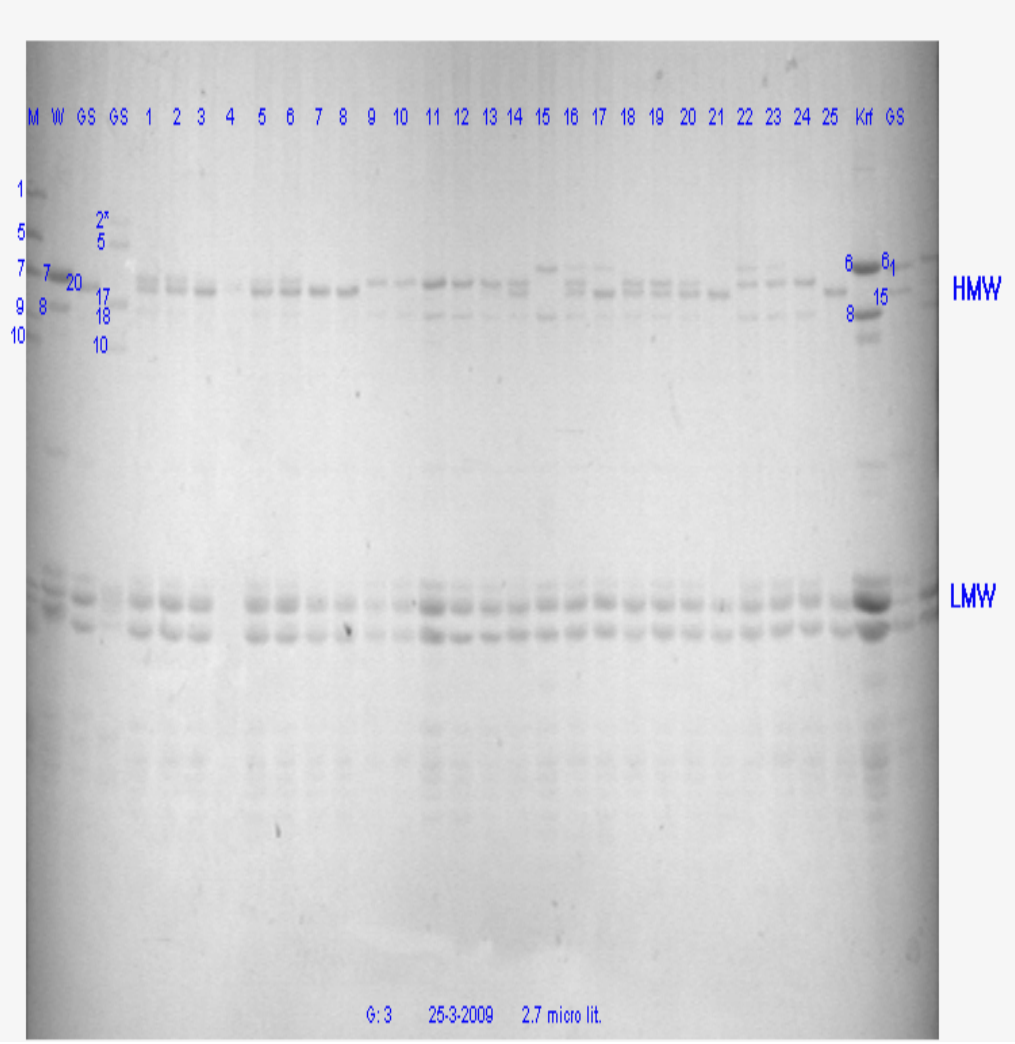

Fig.1 Glutenins (SDS). SDS-PAGE.

Fig. 2 show the results of electrophoresis protein analyzing for identifying gliadin content of the 25 studied genotypes as the second important protein content of durum wheat, showing the four special zones (α, β, ∞, γ) of gliadin in the diallel crosses (1-10) and reciprocal crosses (11-20) and parents (20-25), the Fig. of the gell indicate obviously to Omega zone with the bands 33, 34, 35, and 45, existing of band number 35 with 45 is very important as resulted from the electrophoresis of the standard variety Marcos (12). As shown from theFig.1 and Fig.2 this results was replicated obviously in all studied genotypes indicating to the high quality of both components HMW-GS and LMW-GS, and explain of the results by interpreting both of them(5), (6,4 and 3).

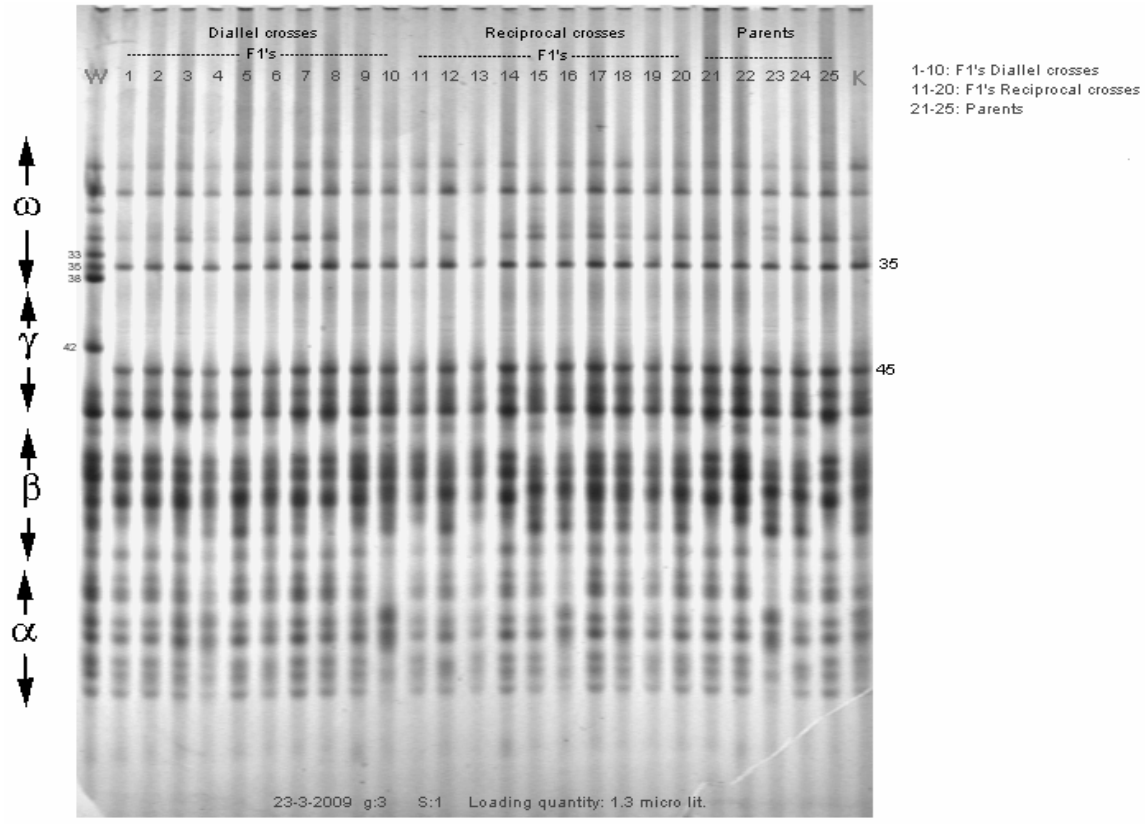

Fig.2 (Gliadin (SDS). SDS-PAGE.

Table 1 indicate to Protein content and Ash as percentage of the parents and durum hybrids, the percentage of protein content of parents was between 14.4 and 16.2 to the Iraq-7 and Semito respectively, while the range of hybrids was between 15.5 and 18.2 to Bakrajo x Simeto and Bakrajo x Iraq-7 respectively, and the range of Ash percentage for parents was between 3.2 to 3.4 for Iraq-7 and Simeto, and 3.3 to 3.6 for the hybrids pansifel x Iraq-7 and Bakrajo x Simeto respectively, results of the present study exceeded some previous study (11). Table 2 show the color space system L*a* b* of the parents and hybrids too, relating to L*a* b* which indicate to Flour color and pigment density of the studied genotypes, there are no significant differences among hybrids and among parents too, but the results reveal high carotene content in the color of studied durum genotypes, the value of b* ranged from 14.6 to 17.1, showing good quality of durum and positive relation between Yellowness of the flour and protein content and Ash content and Carotein content, but no correlation was found between the colour and the amount of total protein. The result of two previous reports (13, 9) confirms our observation that there were no correlation between the color and the amount of the total protein as shown in table 2.

Table (1) Protein% and Ash% content of studied genotypes.

No.	N. Prot. %	N. Ash %	No.	N. Prot. %	N. Ash %
1	18.2	3.6	16	15.9	3.4
2	17.4	3.6	17	16.6	3.4
3	16.8	3.4	18	17.1	3.5
4	15.7	3.4	19	16.7	3.5
5	15.5	3.4	20	16.6	3.4
6	15.9	3.5	21	15	3.3
7	17	3.4	22	14.4	3.2
8	17.3	3.5	23	14.9	3.3
9	16.3	3.4	24	16.2	3.4
10	16.2	3.4	25	15.8	3.3
11	16.8	3.4	Min	14.42	3.22
12	16.5	3.4	Max	18.23	3.63
13	16.8	3.4	Mean	16.31	3.41
14	16.1	3.3	STDS	0.84	0.09
15	16	3.4			

Table (2) Color indicator of st udied genotypes

Symbol	L*	a*	b*	Symbol	L*	a*	b*
1	85.3	0.9	15	15	86.4	0.5	14.6
2	85.1	0.6	16.9	16	86	0.8	14.8
3	84.9	0.9	15.6	17	85.6	0.6	15.5
4	85	1	15.5	18	85.5	0.8	15.1
5	84.9	0.8	15.9	19	86.2	0.5	14.8
6	85.2	0.9	15.6	20	84.7	0.9	15.5
7	84.7	0.8	16.2	21	85.8	0.3	16.1
8	84.6	0.9	16.5	22	85.7	0.1	16.6
9	85.6	0.7	16.2	23	85.9	0.6	14.7
10	85.2	1	15.8	24	84.5	1.1	15.4
11	85.6	0.5	16.5	25	85.3	0.7	15.6
12	84.3	1.2	15.7	Min	84.17	0.09	14.62
13	84.9	0.8	17.1	Max	86.41	1.18	17.05
14	84.2	0.5	16.5	Mean	85.25	0.72	15.74
				STDS	0.59	0.24	0.7

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هەڵسەنگاندنی 25 پێکهاتەی بۆماوەیی لە گەنمی زبر کە بە هۆی دوانە لێکدانی ئەلیلی 5×5 بەرهەم هێنرابوو بە ڕێگای گواستنەوەی کارەبایی بۆ پرۆتینە ئامارکراوەکانی تۆو

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پوختـــــــــە

تۆوی 25 پێکهاتەی بۆماوەیی گەنمی زبر، کە لە لێکدانی 5×5 ی دوانە ئەلیلی یەوە بەرهەم هێنرابوو، کە 20 لێکدراو و5 باوانی لە خۆ گرتبوو، ئاماده کراو شیکاری پرۆتینە ئامارکراوەکان کرا بە بەکارهێنانی ڕێگای گواستنەوەی کارەبایی SDS-PAGE و هەڵسەنگاندنی پێکهاتەی بۆماوەیی بۆ مۆلیکیولە بەرزە پێکهاتەکان و مۆلیکیولە نزمە پێکهاتەکان کرا بۆ گلۆتینین و کلایەدین، و ئەنجامەکان بە نمونە ستاندەردەکان پێوانە کرا، لە ئەنجاما پلەیەکی بەرزی جۆرایەتی و توانای خۆ گونجاندن لە گەڵ ژینگەی خۆجێتی دەرکەوت، بۆ دڵنیا بوونی ئەنجامەکان پێکهاتەی پرۆتینی و خۆڵەمێش و ڕەنگی ئارد ئەنجامدرا.

تقييم 25 تركيب وراثي من الحنطة الخشنة تم انتاجها بواسطة التهجين التبادلي الكامل 5×5 بطريقة الترحيل الكهربائي للبروتينات المخزونة في البذور

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الخلاصـــــــة

تم تجميع بذور الحنطة الخشنة من 25 تركيبا وراثيا ناتجة من التهجين التبادلي 5*5 و يتضمن 20 هجينا و خمسة اباء، تم تحليل البروتينات المخزونة في البذور بواسطة طريقة الترحيل الكهربائي على هلام البولي اكريلامايد و بوجود مادة الSDS (SDS-PAGE)، تم تقييم التركيب الوراثي للجزيئات العالية التركيب و الجزيئات المنخفضة الوزن الجزيئي للكلوتينن و الكلايدين، و تم مقارنة النتائج بالنماذج الستاندرد، حيث ظهرت درجة عالية من الجودة و القابلية للتكيف للبيئة المحلية، و قد تم تدعيم النتائج بقياس المحتوى البروتينى و الرماد وكذلك قياس الوان الطحين.