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Detection of genes associated with qualitative characteristics of gluten

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Abstract. The research was aimed at analyzing allelic variants of protein in wheat varieties used in Iraqi bakery and evaluating these varieties via genetic source using grain quality selection. Variety tests were carried out at field experimental station of Russian State Agrarian University — Moscow Timiryazev Agricultural Academy. The analysis of wheat grain quality was made after harvesting in mid August. Allele state of genes controlling the quality of gluten in wheat grain was determined using the PCR method. Samples of Iraqi wheat varieties 12 (soft wheat) and One (durum wheat) are characterized by considerable variation of gluten content and quality. The five varieties whose genotype include an allelic variant of high molecular weight glutenins Glu-D1 5+10 and subunit Glu-A1-2* (Fateh, Tamuz-3, Abigh-reb-3, Iraq and Maxibak) were also studied. The highest gluten content was from 31.5 % in Iraq to 35.3 % in Fateh variety, while the gluten quality was not below the second group. Five varieties — Farah, Al-Murug, Sham-6, Tahadi and Sabirbeg — had unusual combination of the allelic state of Glu-D1 2+12 (which is usually associated with low gluten quality) with a 2* subunit for the Glu-A1 locus, which determines the possibility of improving gluten quality to the wheat varieties.

Key words: soft wheat, grain quality, protein content, gluten content, allelic, glutenins condition, baking qualities

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Introduction

Republic of Iraq has long been known among wheat producers, since the time of Hammurabi and its Babylonian Empire. "Mesopotamia" (the land lies between two Rivers — the Tigris and the Euphrates) was the most productive part of the world in terms of wheat yields. However, The Republic of Iraq was compelled to import wheat grains from other countries in quantity of half its need (41—50 %). Over the last thirty years, wheat grain demand in Iraq is approximately 4.6 million tons per year according to FAO (2014). Wheat production in Iraq declined yearly compared with the other countries. As a result, Iraq ranked 38th place among producing countries with an annual wheat production 2.8 millon tones according to FAO (2014). Therefore, issue of finding genetic originals with high productivity depends on selection of varieties by determinate gluten parameter in food industry.

Protein content is one of the most significant indices of wheat grain quality. The protein problem is directly related to the grain quality issue. The main aspects of the protein problem are protein status and quality as the structural basis for gluten. The most significant technological features of flour and the fact that proteins are the primary nutrient elements of bread, bakery and pasta. Bread is foundation of human nutrition, nutritional value of which mainly relies on flour type and raw dough structure. Content of protein, minerals, and vitamins declines with a reduction in the output of flour in it. Baking properties of flour depend on complexes of protein-proteinase and amylase-carbohydrate.

The protein-protein complex involves protein flour, proteolytic enzymes, as well as proteolysis activators and inhibitors. The complex's state is the primary factor determining the flour's "strength". The quantity and quality of gluten as well as structural and chemical characteristics of the experiment were assessed. Proteins were divided into fractions according to their capacity to dissolve in different solvents: soluble globulins (9.4 %), water-soluble-albumins (16.2 %), alcohol-soluble-prolamin (wheat-gliadins) (34.2 %) and alkali-soluble-glutelins (in wheat-glutenins) (37.6 %). Number on the fractional composition was given for baking flour of the highest grade. Albumins and globulins play an important role in the process of plant growth [1]. DNA marking methods are particularly important in selection of grain quality, making it possible to speed up process of selecting genotypes with outstanding grain technological features, which is mainly due to protein quality. Water insoluble protein fractions, so-called spare proteins-gliadins and glutenins play the primary technological role in bakery manufacturing during dough kneading. Glutenin is the basis, and gliadin is its gluing origin. Analysis of the regularities of genetically determined variability of these proteins is of great importance for the creation of varieties with high grain quality [2-5].

Glutenins have a significant effect on wheat's baking features as they determine gluten elasticity, the most significant being the elevated molecular weight proteins. Thus, the glutenin composition, in particular of the high molecular weight fraction, determines strength of gluten, and its elasticity.

The grain endosperm texture is the most significant wheat quality feature. High quality soft wheat has a tough grain structure with endosperm. In the 1990s protein (friabiline) used as a softness marker was discovered to consist of puroindolines and a family of softness proteins (GSP-1) [6]. As surface-active proteins, puroindolins

interact with lipids of starch grain membranes, forming a layer between them and grain's protein matrix, thus, protecting starch grains from destruction during grinding [7]. Temirbekova et al. found that seed protein content of Moskovskaya 39 was high (15.84 %) in hot and 16.60 % in dry conditions [8]. The ordinary cultivar contained grain protein of 14.1—17.0 %, gluten content of 25.0—38.2 %. The puroindolin content in wheat seeds was 0.07—0.10 % of dry matter. It belongs to the albumin class but is easily dissolved in water only after starch granules are released from the membranes' long-lasting lipid-protein complex [9].

The major determinants of wheat quality are endosperm texture and protein content. Endosperm texture has a profound effect on milling, baking and end-use quality. A varietal character, endosperm hardness, is also influenced by environment. It is controlled by the hardness (Ha) locus on the short arm of 5D chromosome. Grain hardness is mainly influenced by various physical and chemical factors like protein, virtuousness, kernel size, water-soluble pentagons, moisture content and lipids [10]. SDS electrophoresis separation revealed two isoforms of this protein-puroindolin a (PINA) and puroindolin b (PINB) very close to electrophoretic mobility. The genes encoding each of these proteins were fully linked together in chromosome 5DS [11]. PINA and PINB gene collaboration guarantees that soft or hard endosperm texture is formed [7, 12]. Variations of PINA or PINB can modify grain hardness significantly due to tryptophanrich domain related to PINB (allele Pin b-D1b). The amino acid glycine is changed.

Materials and methods

12 Iraqi varieties of soft wheat (*Triticum aestivum L.*) — Fateh, Almurug, Alrasheed, Ibaa-99, Sham-6, Tamuz-3, Abighreb-3, Iraq, Ibaa-95, Tahadi, Maxibak, Sabirbeg and one durum wheat Farah were studied at field experimental station of Russian State Agrarian University — Moscow Timiryazev Agricultural Academy in 2016—2017. After harvesting wheat grain quality was analyzed in the laboratory of grain technology in Nemchinovka Federal Research Center. Wet gluten was estimated with a device INDEX GLUTEN GLUTOMATIC. Dry gluten was calculated by placing wet clot in an oven at 100 °C for 24 hours.

The allelic state of Glu-D locus high-molecular gluten controlling gluten quality in wheat grain was determined by using the PCR technique of polymerase chain reaction. The presence of high-molecular gluten in locus with a combination of 5+10 or 2+12 subunits was determined using the primers: Dx5F and DxF at the Center of Molecular Biotechnology in Russian State Agrarian University — Moscow Timiryazev Agricultural Academy.

DNA was isolated by the CTAB method as follows:

1. Place young seedlings in eppendorfs;

2. Add 200 µl CTAB, preheated to 65 °C;

3. Grind the contents with a pestle;

4. Add 250 μl 2 \times CTAB and 450 μl H₂O;

5. Mix by turning 30 times;

6. Put in a water bath at 65 $^{\circ}$ C with a rocking chair for 80 for 1.5-2 hours with periodic manual tube turning;

7. Cool to room temperature;

8. Add 600 µl of chloroform-isoamyl (24:1) (to the cap);

9. Stir by turning for 30 minutes until the aqueous fraction becomes milky white;

10. Unscrew 10 minutes at 3,000 rpm;

- 11. Select the supernatant in a clean;
- 12. Unscrew 10 minutes at 3,000 rpm;
- 13. Transfer the aqueous fraction to a clean tube, leaving debris at the bottom;
- 14. Add 700 µl of isopropanol (2/3 volume);
- 15. Mix by turning 30 times;
- 16. Unscrew 15 minutes at 13,400 rpm;
- 17. Drain the isopropanol;
- 18. Add 70 % ethanol 100 µl;
- 19. Unscrew 10 minutes at 13,400 rpm;
- 20. Ethanol drain;
- 21. Repeat p/n. 18-20;
- 22. Dry;
- 23. Add 150 µl of water;
- 24. Leave overnight in the refrigerator + 4 °C.

Carrying out a polymerase chain reaction (PCR)

The presence of high molecular weight glutenin locus with a combination of 5 + 10 or 2 + 12 subunits was determined using primers: Dx5F, DxF, DxR.

The composition of the reaction mixture during PCR (final concentration of reagents is given) is 25 μ l:

Buffer	1x
dNTP	0.2 mM
MgCl ₂	1.5 mM
Dx_F primer	0.3 µM
Dx5_F primer	0.1 µM
Dx_R primer	0.4 µM
DNA	50—100 ng
Tag polymerase	1 unit

Program for amplification:

Initial denaturation: 95 °C, 5 min. 32 cycles: Denaturation: 95 °C, 30 sec Primer annealing: 65 °C, 30 sec Elongation: 72 °C, 2 min Final elongation: 72 °C, 7 min Storage: 4 °C The results were detected on 2 % agarose gel in 1xTBE buffer.

The main criteria for the quantity and quality of gluten

The determination of crude gluten content in soft wheat flour was carried out mechanized according to GOST R 52189—2003 (Russian State Standard), used in the analysis of grain (Wheat flour. General specifications) — Quality indicators of wheat baking flour.

Gluten gradation (mass fraction of raw gluten from flour, %).

Variety of baking flour:

- Extra — not less than 28.0 %;

- Higher — not less than 28.0 %;

- Grit — not less than 30 %;

- The first — not less than 30 %;

- The second — not less than 25.0 %;

- Large flour (Wallpaper) — not less than 20 %.

At the same time, quality of SDS gluten should not be lower than the second group (Classification standards used by the Central Laboratory of the State Commission for Variety Testing of Agricultural Crops to characterize wheat varieties by baking quality).

Strong wheat:

– excellent improver — not less than 34.0 %;

good improver — not less than 32.0 %;

– satisfactory improver — not less than 30.0 %.

Moreover, quality of gluten (SDS) 45-75 units;

Valuable wheat — not less than 27.0 %, SDS 45—85 units.

Wheat Fillers:

- Good — not less than 25.0 %, SDS 35—90 units;

- Satisfactory — not less than 23.0 %, SDS 20—100 units.

Weak wheat — not less than 18.0 %, SDS 0—120 units.

Evaluation of the "strength" of flour on the basis of the swelling index (0.5 g flour sample) was carried out by evaluating the sedimentation (ml) at a certain grinding size (silk sieve no. 43) [12].

- Very strong — more than 60 ml;

- Strong 60—40 ml;

– Average 40—20 ml;

– Weak — less than 20 ml.

The indicator of sedimentation of flour was determined according to the methodology of the laboratory of grain technology in Agricultural Research Institute of the Central Regions of Non-Chernozem Zone.

Results and discussion

The percentage of high-molecular glutenins is known to exert the biggest impact on the grain's baking characteristics. We used a score of these characteristics in our research determined by the Glu alleles [13]. Researchers have previously discovered a correlation between the existence of certain subunits of elevated molecular weight glutenins and strength, measured by the sedimentation of sample quantity by SDS [14]. Based on this, a score was developed for each allelic state of high molecular weight glutenins [15].

The higher the Mark was assigned to one or another allele, the more significant influence it had on baking qualities (Table 1). Therefore, the best quality of baking corresponds led to the greatest value (4 Marks — in the presence of subunits HMW 5+10).

Thus, it is feasible to assess baking characteristics of wheat variety with the assistance of this classification by adding three alleles expressed in its genotype. However, this evaluation shows only the prospective characteristics of the variety, as bakery characteristics are mainly dependent on setting, agrotechnology, and a number of other variables.

Mark	Chromosome, allele		Mark	Chromosome, allele		Mark	Chromosome, allele				
	1A	1B	1D	Wark	1A	1B	1D	iviar k	1A	1B	1D
4			5+10	3		7+8	—	1	zero		—
3	1		-	3	_	13+16	—	1	_	7	_
3	2*			2	_	7+9	—	1	_	6+8	—
3	—	17+18	—	2	—	—	2+12	1	_	20	—

Mark of baking qualities determining Glu-1 alleles [16]

As can be seen, the highest mark 4 corresponds to an allele that expresses the subunits of 5+10. Therefore, the main protein of the marker for the baking qualities of wheat is a pair of high-molecular glutenins -Dx-5+Dy-10 in the Glu-D1 locus, while the alternative combination Dx2+Dy12 is usually associated with low gluten quality [17].

The enhancement in gluten quality associated with the existence of a mixture of elevated molecular weight glutenin 5 + 10 subunits is primarily due to the existence in the Dx-5 subunit of an extra cysteine residue compared to the Dx-2 subunit like Cysteine, and in comparison with other amino acids, which is contributed in the formation of a greater number of disulfide bonds as well as formatted of polymers with many branches and number of relationships. Eight of the 13 wheat variety samples had an allelic state of GluD1 5+10. Varieties Al-Murug, Sham-6, Tahadi and Sabirbeg were characterized by genotype 2+12.

The 5 + 10 group has a major impact on the sample kneading moment, strength and SDS sedimentation value relative to the 2 + 12 subunit [18, 19]. Different genotypes of wheat differentiate between 3 and 5 subunits of elevated molecular gluten. Allelic variants GluA1a and GluA1b encoding subunits 1 and 2* have a beneficial impact on cooking quality (3 points), the null allele has a score of 1 point [13, 20].

The variety of samples from Iraq were divided into three groups depending on the allele state of genes influencing baking characteristic (Tables 2, 3 and 4). In particular, the first group included varieties (Table 2), whose genotype contained an allelic variant of elevated molecular weight glutenins 5+10-Dx-5+Dy-10 at the Glu-D1 locus, as well as the subunit 2* at the Glu-A1 locus.

Table 2

Chromosome, allele Indicators of quantity and gluten quality, harvest, 2016 A1 D1 B1 2* 5 + 10Fateh Tamuz-3 2* 5+10 Varieties Abighreb-3 2* 5+10 2* 5+10 Irad Maxibak 2* 5 + 10

Content of glutenin in grain of wheat samples from Iraq with allelic state Glu-D1 5+10 and subunit, 2016 (First group)

Varieties in Figure 1 (first group) are characterized by high gluten content (from 31.5 to 35.3 %) characteristic of strong wheat varieties and gluten quality in all five varieties (according to SDS consequences) is only the second group.



Fig. 1. Content of glutenin in grain of wheat from Iraq, 2016 (First group)

Two types of excellent fillers-two varieties Fateh and Maxibak and one variety Abighreb-3 are categorized as precious varieties Tamuz-3 and Iraq. The second group (Table 3) includes varieties combining the variant Glu-D1 5+10 and subunit 1 at the Glu-A1 locus.

Table 3

Content of gluten in grain of wheat samples from Iraq with allelic state Glu-D1 5 + 10 and subunit Glu-A1-1 (Second group)

Indicators of quantity and gluten quality, harvest, 2016		Chromosome, allele				
		A1	B1	D1		
Varieties	Alrasheed	1	_	5+10		
	Ibaa-99	1		5+10		
	Ibaa-95	1	_	5+10		

The qualitative feature of gluten (in accordance with SDS) in the second group of samples is as follows: two varieties Alrasheed and Ibaa-99 are allocated to the second group of quality varieties, while the third group is Ibaa-95 (Fig. 2). The general assessment of the varieties of this group by the quantity and quality of gluten: Alrasheed variety is a valuable variety; Ibaa-95 is a good filler, and the third group involves varieties with an alternative mixture of Dx2 + Dy-12 alleles, generally associated with low quality gluten.



Fig. 2. Content of glutenin in grain of wheat from Iraq, 2016 (Second group)

According to the characteristic of the allelic state of Glu-D1 2+12, two varieties are assigned to the third group. Two varieties of this group — Tahadi and Sabirbeg — are an example of the contrast ratio of gluten content and its quality. The content of gluten in the second grade (Tahadi) is half that (25.5 %), but the qualitative characteristics of gluten are classified as strong varieties, based on the screening of allelic composition of genes related to gluten quality, considering the results of the analysis of content and quality of gluten in the grain of varietal samples from Iraq (Table 4, Figure 3).

Table 4

Content of gluten in grain of varietal wheat samples from Iraq with allelic state Glu-D1 2 + 12



Fig. 3. Content of glutenin in grain of Iraqi wheat 2016 (Third group)

In our studies, using the dominant PCR marker for the allelic state of the PinaD1 gene, amplification is observed only for the wild-type allele PinaD1a identified in Sham 6 and Sabirbeg varieties. Amplification was not observed in the Nine cultivars having over the null allele (PinaD1b) associated with hardness. The Ibaa-99 sample was heterogeneous on the basis of soft grain/hardness (Figure 4).



Fig. 4. Electrophoregrams of puroindolins A and in variety samples (1 — Farah, 2 — Al-Murug, 3 — Fateh, 4 — Alrasheed, 5 — Sham 6, 6 — Ibaa-99, 7—Tamuz 3, 8 — Abighreb-3, 9 — Iraq, 10 — Ibaa-99, 11 — Tahadi, 12 — Maxibak, 13 — Sabirbeg)

In accordance with the Classification Standards used by the State Commission for Variety Testing of Agricultural Crops' Central Laboratory for characterizing wheat varieties by baking traits, the "strong" wheat category involves varieties whose gluten quality in grain and flour in normal SDS units ranges from 45 to 75 units: Tahadi, Alrasheed, Ibaa-99. The Saberbeg sample belongs to the third group. The remaining Iraqi genotypes are allocated to the second quality group for this indicator. The sedimentation index is an indirect technique of evaluating the baking characteristics of flour.

It is the consequence of determining the level of flour swelling and rainfall on a unique device in a soft acetic acid solution (2 %) and flour swelling is determined by the milliliter size of the precipitate. The use of indirect techniques to determine the baking characteristics of wheat is prompted by the need for quality assessment of the source material in the early generation of selection, when the breeder has several grams of grain obtained from one plant. For analysis by this method, 2—5 g of grain is sufficient, which is ground in a micro mill.

This technique is commonly used for a preliminary evaluation of grain quality not only in Russia, but also in other countries. The appropriate classification of soft wheat according to sedimentation indicators has been developed: strong — 40 ml and higher; valuable — 20—40 ml; weak — less than 30 ml. An analysis of our collection showed that 8 variety samples can be classified as valuable wheat by sedimentation level. Studies of wheat grain quality and flour also revealed variations in raw and dry gluten content. Fateh and Abighreb-3 had the highest raw gluten content — 35.3 % and 34.4 %, respectively. Farah variation had the smallest proportion (12 %). The same grade had the lowest proportion (2 %) of dry gluten. GOST R 52189-2003 (Russian State Standard) was not met by the amount and quality of gluten varieties Farah and Sabirbeg.

The remaining 11 samples studied accepted the standard's particular criteria.

Conclusions

Thus, soft wheat collection surveys showed the heterogeneity of grain and flour quality variety samples and revealed interesting samples as sources of economically precious selection characteristics, as well as introduction of Middle Eastern wheat in Russia. The Varietal samples Abighreb-3, Tamuz-3, Fateh, Iraq showed elevated outcomes in terms of a set of gluten quality indices and other economically important features connected with the wheat gene allegiance. Short-range forms separated from the collection samples can be used directly for short-range reproduction of wheat and, indirectly, for lodging resistance. Marked samples may be included in the selection method for the quality of soft wheat grain and flour.

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Научная статья

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Определение генов, контролирующих качественные характеристики глютена

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Аннотация. Исследование посвящено анализу аллельных вариантов белка, подходящих для использования в хлебобулочных изделиях, изготавливаемых из сортов иракской пшеницы, а также оценке этих сортов с помощью генетического источника с использованием методики качественного отбора зерна. Испытания сортов проводились на полевой опытной станции Российского аграрного университета им. Тимирязева. Анализ качества зерна пшеницы проведен после сбора урожая в середине августа, с помощью метода полимеразной цепной реакции определено аллельное состояние генов, контролирующих качество клейковины зерна пшеницы. Объектом исследования являлись 12 иракских сортов мягкой пшеницы и 1 сорт твердой пшеницы, характеризующиеся значительными колебаниями содержания глютена и его качества. Пять сортов пшеницы содержат в своем генотипе аллельный вариант высокомолекулярных глютенинов Glu-D1 5 + 10 и субъединицы Glu-A1-2* (Fateh, Tamuz-3, Abighreb-3, Iraq и Maxibak). Наибольшее содержание глютена в зернах этих сортов составляет от 31,5 (Iraq) до 35,3 % (Fateh), при этом качество глютена не опускается ниже второй группы. У сортов Farah, Al-Murug, Sham-6, Tahadi и Sabirbeg встречается интересная комбинация аллельного состояния гена Glu-D1 2 + 12, обычно ассоциирующегося с низким качеством глютена, и субъединицей 2^* для локуса Glu-A1, которая позволяет повысить качественные показатели глютена до уровня изучаемых сортов пшеницы.

Ключевые слова: мягкая пшеница, качество зерна, содержание белка, содержание глютена, аллель, глютенины, хлебопекарные качества

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