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Identification of the Parents for Bread-making Quality Improvement in Bread Wheat Based on RAPD and Seed Storage Protein (HMW-GS) Markers

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Abstract: Identification of the parent combinations with long genetic distance and desirable HMW-GS compositions is one of the most important steps for improving bread-making quality in bread wheat. We used RAPD and seed storage protein markers for identifying the best parent combinations of 28 Iranian wheat cultivars and advanced breeding lines for improving bread-making quality in wheat. We considered genetic distance less than 0.1 (<0.1) and quality score equal to or more than eight as criteria for selecting the best parent combinations. Six combinations out of studied cultivars including Khazar1/Darab1, Azadi/Darab1, Chenab/Darab1, Navid/Darab1, Star/Darab1 and Deihim/Darab1, were considered as suitable parents for future crosses in wheat breeding programs for breed-making quality in Iran. We suggest it should also be considered other important factors such as parents performance and complementarily for important agronomic traits in parent selection. However, integrated data of RAPD and HMW-GS compositions can make useful parent selection for breeding programs related to bread-making quality.

Key words: RAPD, HMW-GS composition, bread-making quality, bread wheat

INTRODUCTION

Wheat (*Triticum aestivum*) is the most agronomically crop that the main part of its consumption is for bread production. Of all the cereal grains, wheat is unique, because wheat flour alone has the ability to form a dough that exhibits the rheological properties required for the production of leavened bread. The unique properties of the wheat grain reside primarily in the gluten-forming storage proteins of its endosperm including glutenin and gliadin. Glutenins are divided to two main categories containing HMW-GS and LMW-GS. Although HMW-GS constitute only 10% of storage proteins of wheat endosperm, but they are key factors in the processes of bread-making. Significant correlation between bread-making quality and HMW-GS have been observed in wheats from several countries (Lukow *et al*., 1989; Uhlen, 1990).Therefore in wheat breeding programs for bread making quality, It should be considered HMW-GS as a criteria for parental selection.

Screening for desirable combinations of HMW-GS in the development of new wheat varieties in order to maintain and improve bread-making quality has been successful in the UK in part (Lookhart *et al*., 1993). Also some breeding programs such as those at the former Plant Breeding Institute (PBI) and CIMMYT have already used HMW glutenin subunits composition as a criterion for selecting parents for improving bread-making quality. In the other hand, another important factor for parent selection in breeding programs is genetic distance. Determination of the parents with long genetic distance and different desired allelic compositions can potentially guarantee producing desired progenies with acceptable genetic diversity. Also, genetic diversity between parents is necessary to derive transgressive segregation from a cross. Different methods including morphological data, isozymes, proteins and DNA markers have been used for estimating genetic diversity in crop populations and each method has own special advantages and limitations. More recently, DNA molecular markers have became the favorite tools for estimating genetic diversity and predicting heterosis in crop plants such as wheat (Martin *et al*., 1995, Liu *et al*., 1999, Corbellini *et al*., 2002) maize (Shieh and Thseng 2002; Betran *et al*., 2003) and oilseed rape (Diers *et al*., 1996; Yu *et al*., 2005). In particular, RAPD markers have become one of the most widely used marker system in studies related to plant genetic resources. Liu *et al*. (1999) suggested that based

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on RAPD markers, it is possible to differentiate wheat lines with different performance and that the classification of parents from these markers is of predictive value for developing superior hybrid, but genetic distance based on RAPD markers was not significantly correlated with hybrid performance.

Therefore it seems that integrated data of the RAPD and HMW-GS proteins can become more informative for parent selection in breeding programs of bread-making quality, because both genetic distance and HMW-GS composition are key factors in producing varieties with high quality breads. Wheat breeding programs in Iran have mainly focused on biotic and abiotic stresses so that improvement of the bread-making quality has been ignored which this has caused increasing bread losses, even recently it has reached at 30%.

This study was designed to identify parents with long genetic distance and desired composition of HMW-GS in some agronomically important Iranian wheat cultivars and lines based on RAPD and seed storage protein (HMW-GS). To our knowledge, there aren't any papers that has been directly used both mentioned-markers for identifying suitable parents for breeding programs of the bread-making quality. For first time, we integrated data of the RAPD and HMW-GS markers for predicting parents of the crosses in a bread making quality improvement program in wheat.

MATERIALS AND METHODS

Plant materials: Twenty-eight cultivars and breeding lines, included Azadi, Khazar1, Karaj1, Karaj2, Arvand1, karaj3, Moghan1, Moghan2, Navid, Chenab, Rashid, Star, Ghafgaz, Deihim, Alamoot1, Alamoot2, Darab1, Darab2, New adl, Old adl, Azar and advanced breeding lines: 273, 583, 518, 524-6, 7107, 5806-3, 7007/2-6, were supplied from Field Crop Research and Genetic Resources Unit of agriculture faculty of Tehran University.

Protein extraction and electrophoresis analysis: Total proteins were extracted from single seed of each cultivar and line. The seeds were crushed finely after removal of the embryo. The flour was mixed in extraction buffer of 62.5 mM Tris-HCl (pH 6.8) buffer containing 12% (w/v) glycerol, 2% (w/v) sodium dodecyl sulfate (SDS), 0.003% (w/v) boromophenol blue and 5% 2-mercaptoethanol. The samples were boiled for 5 minutes and then centrifuge for 5 min at 1000 rpm; 15 mL of each sample extract were loaded on the gel. Proteins were fractionated by SDS-PAGE according to Laemmli (1970) using stacking and separating gels containing 4% acrylamide, 0.3% bis-acrilamid, 0.1% SDS and 0.125 M Tris-HCl (pH 6.8) and 8.7% acrylamid, 0.3% bis-acrylamid, 0.1% SDS and 0.38 M Tris-HCl (pH 8.8), respectively. Gels were stained overnight with 0.13% Coomassie Brilliant Blue R250 in water and acetic acid (53:40:7 v/v) and destained overnight in water, butanol and acetic acid (65:25:10 v/v).

Scoring: The HMW-GS were identified using the numbering system suggested by Payne and Lawrence (1983). To determine the electrophoretic mobility of HMW glutenin subunit by SDS-PAGE, standards (Bezostaya-1, Champlein, Chinese Spring, Danchi, Dunav, Federation, Gabo, Hobbit, Hope, Lancota, Norin 61, Sappo and Serbian) that included the spectra of the subunits expected were used (Payne and Lawrence, 1983). A quality score was assigned to each HMW-GS of a subunit pair using the method described by Payne (1987).

DNA extraction and RAPD-PCR reactions: Total genomic DNA was isolated from young leaves of 15 old-days seedling by Dellaporta (1983) method. Extracted genomic DNA was stored at 4°C. The quality and concentration of DNA was assessed by a spectrophotometer and 0.1% agarose gel electrophoresis. PCRs were carried out using 50 arbitrary decamer primers produced by Cinagene Company. PCR was performed in a volume of 25 μL containing 100 mM Tris-HCl pH(8.8), 50 mM KCL, 0.01% Triton x-100, 1.14 mM $MgCl_2$, 0.175 mM of each dNTP, 0.5 μM primer, 5 ng of genomic DNA and 1 unit of Taq DNA polymerase. DNA amplifications were performed in a DNA thermal cycler (Pharmacia LKB ATAQ Controller) programmed for an initial denaturation step of 120 sec at 94°C, then 45 cycles at 92°C (60), 35°C (1 min), 72°C (2 min) for denaturation, primer annealing and primer extension, respectively and a final primer extension at 72°C for 5 min. The PCR products with 5ul loading buffer were separated by electrophoresis using 6% polyacrylamid gel in TAE buffer. Gels were stained with ethidium bromide (0.5 μg mL^{-1}). DNA fragments were then visualized under UV light and photographed using a gel documentation system.

RAPD-derived data analysis: The gels were scored for the presence (1) or absence (0) for only those major bands showed reproducible pattern among genotypes. The 0,1

Table 1: The HMW-GS and corresponding bread-making quality scores of 28 bread wheats grown in Iran

	HMW-GS at *Glu-1* loci						
Cultivar and lines	*Glu-A1*	*Glu-B1*	*Glu-D1*		Payne score		Quality score
AZADI	2*	7+8	2+12	3	3	2	8
KHAZAR 1	2*	7+8	2+12	3	3	2	8
KARAJ 1	N	7+8	5+10	1	2	4	7
RASHID	N	7+8	2+12	1	3	2	6
GHAFGAZ	N	7+9	5+10	1	2	4	7
STAR	2*	7+8	2+12	3	3	2	8
MOGHAN	N	7+8	2+12	1	3	2	6
MOGHAN 2	N	13+16	2+12	1	3	2	6
NAVID	2*	7	5+10	3	1	4	8
KARAJ 2	N	7+8	5+10	1	3	4	8
ARVAND1	1	7+8	2+12	3	3	2	8
KARAJ 3	2*	7+8	2+12	3	3	2	8
CHENAB	1	17+18	2+12	3	3	2	8
DEIHIM	1	7+8	2+12	3	3	2	8
L.273	N	7+8	2+12	1	3	2	6
L. 524-6	N	7+8	2+12	1	3	2	6
DARAB 2	2*	17+18	2+12	3	3	2	8
AZAR	2*	7+8	2+12	3	3	2	8
ALAMOOT 1	N	7+9	2+12	1	2	2	5
DARAB 1	2*	17+18	5+10	3	3	4	10
L.583	N	7+8	2+12	1	3	2	6
L.7107	N	7+8	2+12	1	3	2	6
ALAMOOT 2	N	7+9	2+12	1	2	2	5
L 518	N	7+8	2***+12'	1	3	2	4+nd
L 5806-3	N	7+8	2+12	1	3	2	6
L 7007/2-6	N	7+8	2+12	1	3	2	6
OLD ADL	N	7+8	2+12	1	3	2	6
NEW ADL	N	13+19	2+12	1	2	2	3+nd

Nd: not identified, *: Nomenculture proposed by Payne and Lawerence (1983)

data were used to calculate a genetic similarity matrix with Jacard's coefficient. Cluster analysis was performed using unweighted pair-group method (UPGMA) and dendrogram drawed by SPSS software (version 10).

RESULTS

Seed storage protein data: In total, 13 *Glu-1* alleles were identified, three at the *Glu-A1* , six at the *Glu-B1* and four at the *Glu-D1*. At the *Glu-A1*, the frequencies of occurrence *Null*, 2* and 1 were 60.7, 28.5 and 10.7%, respectively. At the *Glu-B1* subunits, 7, 7+8, 7+9, 13+19, 13+16 and 17+18 were found in 3.57, 67.85, 10.71, 3.57, 3.57 and 10.71% of the cultivars and lines, respectively. At the *Glu-D1* subunits 2+12, 5+10 and 2***+12' were detected with frequency 78.57, 17.85 and 3.51%, respectively. At the *Glu-A1* the prevalent allele was *Null* with frequency of 60.71%. The most frequent HMW-GS at the *Glu-B1* locus was 7+8 as shown in 67.85% of the cultivars and lines. The quality score for individual varieties ranged from 5 to 10. The HMW-GS average quality score for the bread wheat cultivars and lines included in this study was 7 with 46% of the varieties higher than average and 46% lower than average. As no quality data are available for subunits 2***+12' and 13+19, no score was calculated for cultivars possessing these subunits. (Table 1).

Table 2: Various subunit composition for 28 cultivars and lines

Glu-A1	*Glu-B1*	*Glu-D1*	Cultivars and Lines
1	7+8	2+12	Arvand1, Deihim
1	17+18	2+12	Chenab
2*	7+8	2+12	Azadi, Khazar1, Star , Karaj3 and Azar
2*	7	5+10	Navid
2*	17+18	2+12	Darab2
2*	17+18	5+10	Darab1
N	7+8	2+12	Rashid, Moghan, Old adl, L273, L524-6, L583, L7107, L5806-3 and L7007/2-6
N	7+8	5+10	Karaj1 and Karaj 2
N	7+9	5+10	Ghafgaz
N	7+9	2+12	Alamoot1 and Alamoot2
N	13+16	2+12	Moghan2
N	13+19	2+12	New adl
N	7+8	2***+12'	L 518

A subunit pair at the *Glu-D1* locus was detected in L518 with mobility slower than subunits 2+12 at this locus using SDS-PAGE on 10% gel. The x-type subunit of this allelic variant showed mobility faster than '1' at the *Glu-A1* locus. This allelic variant seems similar to subunit 2***+12' which has previously been reported as a novel allele with a very low frequency in Pakistani bread wheat landraces (Tahir and Lafiandra, 1994).

These cultivars could be divided into 13 groups based on allelic composition. Eight groups have only one genotype each and the most frequent HMW-GS composition were *Null*, 7+8, 2+12 which was observed in 9 cultivars (Table 2).

Table 3: The sequence, total bands, percentage of polymorphic bands, fragment size range (bp) for primers showed polymorphism

Primer	Sequence (5'-3')	Total bands	Polymorphic bands (%)	Fragment size range (bp)
UBC1	CCTGGGCTTC	160	14	100-758
UBC3	CCTGGGCTTA	46	37	100-1230
UBC5	CCTGGGTTCC	273	11	100-812
UBC13	CCTGGGTGGA	142	7.7	144-616
UBC64	GAGGGGGGGA	207	14.4	107-977
UBC66	GAGGGCGTGA	285	9.1	100-691
UBC76	GAGCACCAGT	58	17.2	478-1288
UBC77	GAGCACCAGG	104	13.4	109-467
UBC82	GGGCCCGAGG	139	10.8	102-588
UBC84	GGGCGCGAGT	222	10.4	102-524
UBC89	GGGGGGCTTG	62	19.4	331-1258
UBC95	GGGGGGTTGG	110	15.5	363-1288
UBC96	GGCGGCATGG	57	17.5	602-1348
UBC100	ATCGGGTCCG	119	18.5	426-1500
TOTAL		1984		260

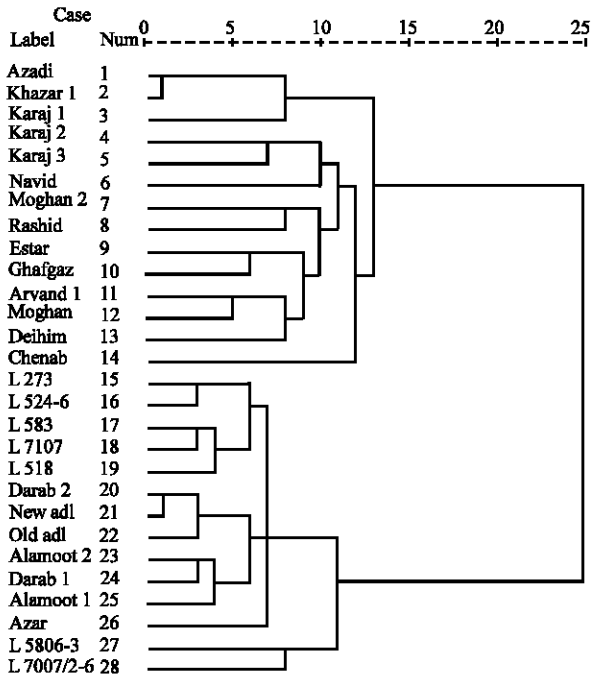

Fig. 1: UPGMA dendrogram of 28 bread wheat genotypes based on RAPD data

RAPD data: Fourteen out of 50 10-mer arbitrary primers were polymorphic. The characteristic fragments generated by 14 decamer oligonucleotides employed as single, arbitrary primers are summarized in Table 3. The percentage of polymorphic fragments ranged from 7.7 for UBC13 to 37 for UBC3 with an average of 15.42. Amplified fragments ranged in size from 100 to 1500 bp for all primers were included in the analysis (Table 3) .

Cluster analysis using jacard's similarity coefficient based on UPGMA revealed two main groups. Each group was included 14 cultivars. The first group was included Azadi, Khazar1, Karaj1, Karaj2, Karaj3, Navid, Moghan2, Rashid, Estar, Ghafgaz, Arvand1, Moghan, Deihim and Chenab. Advanced breeding lines 273, 524-6, 7107, 518, 5806-3, 7007/2-6 and cultivars Darab2, New adl, Old adl, Alamoot2, Darab1, Alamoot 1 and Azar were located in the second group. All breeding lines were in the second group and in the same subgroups. This shows the similarity of studied lines to the cultivars located in the second group and low genetic diversity of them. RAPD-derived dendrogram is shown in Fig. 1.

Table 4: Genetic distance and quality score of the selected combinations

	Quality score		
Cross	Patent 1	Parent 2	Genetic similarity
Khazar1/Darab1	8	10	0.05
Darab1/ Azadi	10	8	0.07
Darab1/Chenab	10	8	0.08
Darab1/ Navid	10	8	0.07
Darab1/Star	10	8	0.08
Darab1/ Deihim	10	8	0.09

The genetic similarity ranged from 0.04 to 0.67 with an average of 0.26. Azadi and Khazar1 had the most similarity, while Navid and 7007/2-6 had the lowest similarity. The average of genetic similarity for cultivars (0.26) was less than that for the studied lines (0.5). This makes necessary attending to genetic base in developing breeding lines in Iran.

Parental selection based on protein and RAPD data : We defined two criteria to integrate RAPD and HMW-GS data based on study aims. Genetic similarly less than 0.1 and quality score equal to or more than eight were determined as indices for parent selection. Six combinations out of studied cultivars and lines were candidated based on defined indices. Indeed, these were included parent pairs from the similarity matric with long genetic distance and desired HMW-GS allelic composition. The genetic distance and quality score of them are shown in Table 4.

DISCUSSION

To our knowledge more studies have used DNA and protein markers for herterosis prediction and relationship

of the hybrid performance with genetic diversity in developing hybrids in crop plants (Cheres *et al*., 2000; Shieh and Thseng 2002; Hua *et al*., 2002; Corbellini *et al*., 2002; Betran *et al*., 2003; Jordan *et al*., 2003; Yu *et al*., 2005). As reported in above–mentioned literatures, the relationship between genetic heterozigosity and hybrid performance has not been consistent among the many different studies using different species, different materials or different environments. The major reason for this contradictions may be the particularizes of different agronomic traits and genotype environment interaction in different crops (Yu *et al*., 2005). There have not been any investigation on parent selection in breeding programs of the bread-making quality by integrating DNA and protein markers. HMW-GS are essential for parent selection in bread-making quality in wheat, but, not enough. If we would like to select parents for a cross in a breeding programs and have useful cross, it should be considered genetic distance. Therefore, in order to have a appropriate cross in breeding programs of the bread-making quality, genetic distance and allelic composition, specially HMW-GS should be taken into account. Regarding to bread losses in Iran and the significance of the HMW-GS in bread quality, predicting the desired parent combinations of crosses in breeding programs of quality would be useful based on two above-mentioned factors. Some wheat breeding programs such as those at the former Plant Breeding Institute (PBI) and international maize and wheat improvement center (CIMMYT) have already used HMW glutenin subunit composition as criterion of selecting parents for improving bread-making quality (He *et al*., 1992). Dong *et al*. (1991) and Payne (1987) have shown the significance correlation between some HMW-GS such as 1, 2*, 7+8, 17+18, 13+16, 5+10 with bread-making quality. In our study, six out of parent pairs are candidated based on high quality score defined by Payne (1987) and long genetic distance.

All pairs had a genotype with quality score equal to 10. This was related to cultivar Darab1 possessing desired allelic compositions 2*, 17+18, 5+10 at the *Glu-A1*, *Glu-B1* and *Glu-D1* (Table 4). Lawrence (1986) reported subunit 17+18 as a common and desired subunit in Australian varieties. This subunit, associated with good gluten quality (Payne, 1987) are very common in the CIMMYT bread wheat gerplasm (Morgunov *et al*., 1993) which is the major source of introduction for the Iranian bread wheat-crossing program. Regarding to genetic distance of Darab1 with other parents, it should be intended as a constant parent in crosses. Also other desired subunits such as 2* and 7+8 had the high frequency in candidated parent pairs for crosses. Undesired subunits including 6+8, 14+15 and 20 didn't found in studied genotypes at all, resulting not to found in selected parent pairs and subunit 13+19 as an undesired subunit had a frequency 3.5% in cultivar New adl. The scarcity and lackness of these subunits are advantages, because they have all been associated with poor bread making quality (Dong *et al*., 1991; Payne, 1987). The HMW-GS average score for the bread wheat cultivars and lines included in this study was seven. The main reason for the medium quality scores in this set of cultivars was the high frequency of subunit 2+12 at the *Glu-D1* locus (78.57%) and *Null* allele at the *Glu-A1* locus (60.71%). Therefore, allele 7+8 at the *Glu-B1* locus associated with good bread making quality and presented in 67.85% of studied varieties and lines was responsible for medium quality score. Subunit 2+12 reported as undesired allele was present in five out of six selected parent pairs. Therefore, the existence of this subunit should be checked out in the progenies and progressive generations derived from a proposed cross and negative selection should be done for deleting this allele. One of the other advantages of selected parent pairs was the lack of undesired *Null* allele at the *Glu-A1*. It should be omitted the existence of the undesired alleles in progressive generations and progenies of the crosses if they would have existed in parents. This can simplify to get a population and individuals with the high frequency of alleles related to good bread-making quality. We suggested to integrate HMW-GS and DNA markers especially RAPD(as a routine and common-use marker system) for identifying the parent with long genetic distance possessing desired HMW-GS in breeding programs of quality.

Also, in order to parents selection in breeding programs of bread-making quality in different regions of Iran, it is necessary to take into account resistance to prevalent biotic and abiotic stresses in those region. Of course we think this is not a new idea, because in all breeding programs for any traits, parents are commonly selected based on desired alleles related to trait and genetic distance. This is only the use of prevalent molecular techniques for this purpose.

We suggest to survey large populations and parents for further investigation and also, use morphological and agronomical data as well as other storage proteins such as LMW-GS along with used factors in this study, because parent performance and complementarily for important agronomic traits will cause to get progenies or populations with high performance for other traits. Moreover, in large population it is recommended at first the population is primarily screened based on allelic compositions of storage proteins, then parent pairs with

long genetic distance are selected from the chosen part of the population using one of the most popular molecular markers.

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