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Genetic diversity for high- and low-molecular weight glutenin subunits in local and commercial bread wheat cultivars released since 1951 in Iran: II- Rainfed

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ABSTRACT

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Allelic variations at the *Glu-1* and *Glu-3* loci play an important role in determining dough visco-elastic properties and bread making quality. Thirteen bread wheat cultivars released in Iran since 1930 from three different agro-climate zones adapted for dryland conditions, were examined for their high (HMW-GS) and low (LMW-GS) - molecular-weight glutenin subunits composition, controlled at the *Glu-1* and *Glu-3* loci, respectively. In addition, the presence of the 1B.1R translocation was investigated. Three, six, and two allelic variations were present at *Glu-A1*, *Glu-B1*, and *Glu-D1* loci, respectively. Subunits 2*, 7+8, and 2+12 are the dominant HMW-GS, at *Glu-A1*, *Glu-B1*, and *Glu-D1*, with frequencies of 77, 46.2, and 54%, respectively. Four, five and five allelic variations were present at the *Glu-A3, Glu-B3* and *Glu-D3* loci, with *Glu-A3e*, *Glu-B3i and Glu-D3b* LMW-GS dominating, with frequencies of 46.2, 54 and 69.2%, respectively. The frequencies of allelic variation at *Glu-1* and *Glu-3* differ greatly in different regions. Among the 13 cultivars/varieties examined in this study, three genotypes were local varieties (23%), four (31%) were bred by Iran's national breeding program, and six (46%) originated from international nurseries. The average quality scores based on HMW-GS for local varieties, genotypes from the national breeding program and international nurseries was the same with a score of 8. Research led to the conclusion that integrating desirable subunits at *Glu-1* such as 1, 7+8, 5+10, should be the main focus of the country's rainfed wheat breeding program, to achieve the goal of improving of gluten quality in Iranian bread wheat cultivars adapted to dryland conditions.

Key words: Glutenin, allelic variation, SDS-PAGE, *Triticum aestivum*

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is the most widely grown field crop in the world. The total wheat-growing area is currently estimated at about 220 million hectares with a total annual production of about 749 million tons (FAOSTAT). Wheat is the major crop in 43 countries and supplies food for at least 35% of the world population (Trethowan and Pfeiffer 1999).

World population approached has reached 7.6 billion people and is projected to increase to around 9.0 billion by 2050 (UNFPA, 2017). Wheat (*Triticum aestivum L.*) has a great economic and nutritional importance. It is the most widely produced and consumed cereal grain in the world. The technological quality of a wheat plant can be defined as the result of the interaction between different factors, such as the genetic potential of the cultivar, the effects of soil conditions and climate, susceptibility to pests and diseases, and the ease of crop management, harvesting, drying, and storage (Shewry *et al.*, 2003). In wheat, seed storage proteins are categorized into gluten and non-gluten families. Gluten, formed via cross-links between monomeric gliadins and multimeric glutenins, is a protein complex that confers dough viscosity and extensibility (Shewry *et al.*, 2002). Glutenins are divided according to their molecular weights into high-molecular-weight (MW = 67,000–88,000) and low-molecular-weight (MW = 32,000–35,000) glutenin subunits (HMW-GS and LMW-GS, respectively) (Wieser, 2007). The subunits are linked together through disulphide bonds to form some of the largest polymeric proteins found in

nature with MWs up to tens of millions. These polymeric proteins affect the rheological properties of wheat dough (Wrigley, 1996). The HMW glutenin subunits are encoded by two types of genes (x:y) located at *Glu-Al, Glu-Bl* and *Glu-Dl*, loci close to the centromere on the long arm of the homologous chromosomes lA, 1B and lD, respective1y (Payne et al., 1982). The x-gene encodes for high molecular weight x-type subunit, whereas the y-gene encodes for LMW y-type subunit (Shewry *et al.*, 1992; Gu *et al.*, 2006). Gliadins are classified into α, β, γ, and ω types, based on their mobility in A-PAGE, and are mainly related to dough extensibility and ductility (Metakovsky *et al.*, 1984; Payne, 1987).

Most common wheat varieties express only three to five subunits because some genes, particularly those encoding the y subunits at the Glu-A1 locus, are silent (Payne and Lawrence, 1983). Thus, HMW-GS are divided into five types of proteins; 1Ax encoded at Glu-A1, 1Bx and 1By encoded at Glu-B1, and 1Dx and 1Dy encoded at Glu-D1. The allelic compositions and expression levels of HMW-GS are related to bread-making properties (Vawser, 2004; Barro *et al.*, 1997). Genes encoding for LMW glutenin subunits are located on the short arms of homologous group 1 chromosomes at *Glu-A3*, *Glu-B3* and *Glu-D3* loci, and are tightly linked to *Glu-1* loci (Liu *et al.*, 2010). The *Glu* loci make unique contributions to dough strength. Gupta *et al.* (1994) reported that the contributions by loci could be ranked as *Glu-D1 >Glu-B1 >Glu-B3 >Glu-A3>Glu-D3 =Glu-A1*, with respect to maximum dough resistance. Zhang *et al.* (2009) reported that *Glu-D1*and *Glu-B3* play the most important roles in determining dough properties. Moreover, the presence or absence of specific HMW glutenin subunits largely determines the bread making quality of wheat. It is well known that the HMW-GS pair 5+10 encoded by *Glu-D1d* contributes to strong dough and good bread-making quality (Campbell *et al.*, 1987; Lagudah *et al.*, 1987). HMW-GS 1 and 2* encoded by *Glu-A1a* and *Glu-A1b*, respectively, and 7+8, 7+9, and 17+18 encoded by *Glu-B1b*, *Glu-B1c*, and *Glu-B1i*, respectively, also contribute to strong dough and good bread making quality (Eagles *et al.*, 2002; He *et al.*, 2005). In general, subunits null, 6+8, and 2+12 at *Glu-A1*, *Glu-B1* and *Glu-D1*, respectively, are negatively related to break making quality (Payne *et al.*, 1987; Weegels *et al.*, 1996). The present study was conducted to determine high molecular weight glutenin subunits of Iranian commercial bread wheat varieties suited to dryland conditions released over the past 80 years.

MATERIALS AND METHODS

Plant material

Thirteen bread wheat cultivars released for dryland areas since 1930 in Iran were evaluated for high and low molecular weight (HMW & LMW) glutenins subunit composition. Grains from single heads were collected from demonstration plots at the Cereal Research Department of the Seed and Plant Improvement Institute (SPII) in the 2012-13 cropping seasons. The samples were sent to the International Maize and Wheat Improvement Center (CIMMYT) quality laboratory in Mexico for SDS-PAGE electrophoresis analysis. They scored high and low molecular weight glutenin subunits.

SDS-PAGE analysis

HMW-Glutenins and LMW-Glutenins were separated by sodium-dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) based on the extraction method described by Singh *et al.* (1991), with modifications reported by Liu *et al.* (2005) and He *et al.* (2005). The presence of the 1B.1R translocation was determined by SDS-PAGE of alcohol-soluble and alcohol-insoluble protein extracts, detecting the presence of *Sec-1* secalins in the first test and the presence of the *Glu-B3j* allele in the second one.

RESULTS AND DISCUSSION

Allelic variation at *Glu-1*

Identification of the HMW-GS composition of the varieties included in the historical series revealed differences between breeding periods (Table 1). Three alleles were observed at *Glu-A1*, subunit 2* allele predominated, with frequencies of 77%, whereas subunit 1 was the least frequent (8%). The 2* allele predominated both in local and improved varieties, but subunit 1 was seen only in one cultivar released in 2011 and selected from international nurseries. The highest frequency of the 2* subunit was in varieties that were adapted and released for cold and warm zones (Table 1 & 2). Six *Glu-B1* alleles were present, subunits 7+8 were found in high proportion (46.2), followed by subunits 7+9 (15.4%) and subunits 6+8 (15.4%). Subunits 17+18, 13+16 and 7 were found in one genotype, respectively. The low-frequency subunit, 13+16, was seen in the Karim cultivar. Karim is adapted to warm zones, selected from the International Center for Agricultural Research in the Dry Areas (ICARDA) international nurseries and released for use in Iran's southern warm and dry zone. It is still commercially planted in many farmers' fields (Tables 1 & 2).

Two allelic variations were observed at the *Glu-D1* locus. The frequencies of 2+12 and 5+10 were

Table 1. Description of the dryland bread wheat varieties used in the study and their allelic composition at the high molecular weight glutenin subunits (HMW-GS) loci

Variety	Pedigree	Year of Release	High Molecular Weight Glutenin Subunits		
			Glu-*A1*	Glu-*B1*	Glu-*D1*
Sardari	Local variety	1930	2*	7+8	2+12
Gahar	Nd/Vg9144//Kal/Bb/3/Yaco"s"/4/Vee#5"s"	1996	2*	7	5+10
Nikenejad	F134-71/Crow"s"	1997	2*	7+9	5+10
Zagross	Tan"s"/Vee"s"//Opata	1997	2*	17+18	5+10
Azar#2	Kaz/Ym71/3/Maya/Bb//Inia/4/Sefid	1999	2*	7+8	2+12
Koohdashat	Bb/Ron//Cno67/Tota/3/Jar	2000	2*	7+8	2+12
Rasad	Fengkang 15/Sefid	2008	null	7+9	2+12
Homa	Sardari-39	2009	2*	7+8	2+12
Ohadi	14 Gene Bank	2010	2*	7+8	2+12
Karim	Hamam-4	2011	1	13+16	5+10
Rijaw	Pato/Cal/3/7C//Bb/Cno/5/Cal//Cno/Sn64/4/ Cno//Bad/Dar/3/Kl/6/Sabalan	2011	2*	6+8	5+10
Baran	Ptz Niska/Ut1556-170//Unknown	2013	2*	7+8	2+12
Prave	Sabalan/4/Vrz/3/orf1148/Tdi//Blo	2017	null	6+8	5+10

54% and 46%, respectively. The high frequency of subunit 5+12 was observed in commercial varieties released for warm areas. Unfortunately, this subunit was not seen in any commercial cultivars that adapted to cold and moderate cold zones. For these cultivars, subunits 2+12 were detected with high frequency (86%). One of the reasons that wheat products in farmers' fields in these regions is of poor quality, could be related to the lack of 5+10 subunits in commercial bread wheat cultivars released for this zone (Tables 1 & 2).

Table 2. Frequencies (%) of HMW (glutenin subunits) in 13 dryland wheat genotypes from three agro-ecological zones in Iran

Locus	Allele/Subunit	Number	Mean frequency	Zone I	Zone II	Zone III
Glu-*A1*	2*	10	77	50	10	40
	1	1	8	0	0	100
	N	2	15	50	50	0
Glu-*B1*	7+8	6	46.2	83	0	17
	7+9	2	15.4	50	0	50
	6+8	2	15.4	50	50	0
	others	3	23.0	0	0	100
Glu-*D1*	5+10	6	46	0	33	67
	2+12	7	54	86	0	14

Quality Scores Based on HM W-GS composition

HMW-GS quality scores of all genotypes ranged from 5-10 with an average of 8, shown in Table 3. The quality scores for varieties released for the cold zone ranged from 5-8 with high frequency for a score of 5 (100%). In the second temperate zone, the range for quality scores was between 6-8, with a high frequency of 6 (100%) (Table 3).

Table 3. Frequencies (%) and distribution of scores based on HMW-GS in 13 dryland bread wheat genotypes from three agro-ecological zones in Iran

Quality scores Based on Glu-1	Number	Mean frequency	Zone I	Zone II	Zone III
10	2	15.4	0	0	100
9	1	7.7	0	0	100
8	8	61.5	62.5	12.5	25
6	1	7.7	0	100	0
5	1	7.7	100	0	0

In the warm zoe, the range of quality scores was between 8-10 with an average of 8.5. Eighteen percent of studied cultivars related to this zone had a score of 10. Zagross and Karim, CIMMYT and ICARDA lines, respectively, received a score of 10 for this zone. The second cultivar, Karim, is planted in the southern part of Iran and exhibits high adaptability. This variety has high yield potential in dryland conditions and matures early (Table 3).

Of the 13 varieties used in this study, three genotypes were local varieties (23%), four (31%) came from the national breeding program and six (46%) originated from international nurseries. The average of quality scores based on HMW-GS for local varieties, genotypes from national breeding program and international nurseries was 8, 8 and 8, respectively (Table 4).

Table 4. Mean frequency of score based on HMW-GS composition and origin of studied genotypes

Origin	No.	Mean frequency	Quality scores Based on Glu-1
Local varieties	3	23	8
National breeding program	4	31	8
International nurseries	6	46	8

Allelic variation at *Glu-3*

The frequencies of LMW glutenin subunits observed in 13 genotypes are presented in Table 5. Four allelic variations were observed at the *Glu-A3*

locus, predominating allele *Glu-A3e* (46.2%), followed by *Glu-A3c*, *Glu-A3d*, and *Glu-A3b*, respectively. *Glu-A3b* was present only in one genotype (karim cultivar) from the warm zone of Iran. This cultivar is from the ICARDA wheat breeding program and is planted widely in farmers' fields. Five allelic variations were presented at the *Glu-B3* locus, with *Glu-B3i* representing almost 54% of the tested germplasm. *Glu-B3b*, *Glu-B3j*, *Glu-B3h* and *Glu-B3d* represented 7.6, 7.6, 23 and 7.6% of the population, respectively.

For *Glu-D3,* five allelic variants were also observed in the 13 studied genotypes, with *Glu-D3b* representing 69.2%, and each of *Glu-D3a*, *Glu-D3c, Glu-D3d* and *Glu-D3e* subunits represented in one genotype (7.7%). The diversity exhibited in *Glu-3* alleles at the three loci (at least four alleles at each loci) and the low frequency of the Glu-B3j allele in most of the zones (except zone I) is very encouraging with regard to the potential for further improvement of the quality of Iranian wheat for diverse food uses. The high frequency of *Glu-B3*j in zone I warrants further efforts to decrease the presence of the 1B. IR translocation in lines for this zone.

Table 5. Frequencies (%) of LMW glutenin subunits in 13 wheat genotypes from three agro-ecological zones in Iran

Locus	Allele/Subunit	Number	Mean frequency	Zone I	Zone II	Zone III
Glu-*A3*	d	2	15.4	50	50	0
	c	4	30.8	25	25	50
	e	6	46.2	83	0	17
	b	1	7.7	0	0	100
Glu-*B3*	b	1	7.6	0	0	100
	i	7	54	72	0	28
	j	1	7.6	100	0	0
	h	3	23	0	33	67
	d	1	7.6	0	100	0
Glu-*D3*	a	1	7.7	100	0	0
	b	9	69.2	44.4	11.1	44.4
	c	1	7.7	0	100	0
	d	1	7.7	100	0	0
	e	1	7.7	0	0	100

Table 6. Allelic composition at the low molecular weight glutenin subunits (LMW-GS) loci and 1B/1R translocation status in studied genotypes.

Variety	Low Molecular Weight Glutenin Subunits			Status of 1B/1R Translocation
	Glu-*A3*	Glu-*B3*	Glu-*D3*	
Sardari	e	i	b	1B/1B
Gahar	c	h	b	1B/1B
Nikenejad	c	b	b	1B/1B
Zagross	e	i	b	1B/1B
Azar#2	e	i	a	1B/1B
Koohdashat	d	i	b	1B/1B
Rasad	c	j	b	1B/1R
Homa	e	i	b	1B/1B
Ohadi	e	i	b	1B/1B
Karim	b	h	e	1B/1B
Rijaw	d	h	c	1B/1B
Baran	e	i	d	1B/1B
Prave	c	d	b	1B/1B

The frequency (15%) of the 1B.1R translocation in the studied genotypes was relatively low. Most of the commercial varieties evaluated in this study did not have this translocation. However, only the Rasad cultivar from Iran's national breeding program at the Dryland Agricultural Research Institute (DARI), showed the 1B/1R translocation (Table 6).

The Rasad cultivar originated from crosses between Sefid (Sardari cultivar), and one Hungarian bread wheat line, which had winter growth habits, was the only variety that had 1B.1R translocation among the studied dryland cultivars. This cultivar was released for cold areas and has narrow adaptability in some locations in the cold and moderate cold zone in Iran.

CONCLUSIONS

This study confirmed wide genetic variability for both in HMW-GS and LMW-GS in different cultivated bread wheat released since 1930. At locus *Glu-1A*, the 2* subunit was found in large frequency. Subunits 7+8 at locus *Glu-1B*, were very frequent in the studied varieties. Surprisingly, *Glu-1D* subunit 2+12 was found in high frequency among the 13 studied varieties. The quality scoring system for HMW-GS developed by Payne *et al.* (1987), in which individual subunits are graded with numbers based on dough visco-elastic parameters,

means that a given cultivar can be assigned a HMW-GS quality score, which is the sum of contributions of each of the three *Glu-1* loci. The HMW-GS quality score has more influence in some sets of wheat than in others (MacRitchie and Wrigley, 1990). Among the cultivars adapted to wheat dryland areas that were planted in three agro-ecological zones, only two cultivars (Karim and Nikenejad) showed the maximum HMW-GS quality score of 10, which would be best suited for bread making. Normally, the lines with 5+10 subunits have strong gluten and thus their HMW-GS quality score is at the higher side (Payne *et al.*, 1987, MacRitchie and Wrigley, 1990).

Among the 13 studied cultivars, some genotypes possessed low HMW-GS quality scores of 5 and 6. The lowest quality score 5 was found in Rasad, a modern variety released in 2008 adapted for cold and moderate cold zones. A HMW-GS score of 6 was found Prave cultivar, a new variety released in 2017 for temperate zones in dryland conditions. These lines with low HMW-GS scores, consisted of 2+12 and 6+8 subunits for *Glu-D1* and *Glu-B1*, respectively. These subunits typically have weak gluten levels and thus their quality score should have been on the lower side. The study also revealed that a few cultivars in use over the past two decades have low HMW-GS quality scores, as well as the presence of the undesirable 1B.IR translocation. This finding warrants putting more emphasis on improving the glutenin composition (*Glu-1* and *Glu-3*) through breeding and stricter quality testing. The electrophoretic analysis of glutenin subunit composition was very useful in cultivar and end use quality product identification.

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