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Classification of high and low molecular weight glutenin subunits and related genes in tetra-hexaploid wheat landraces

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Abstract

Wheat is the first and the most important grain of the world and its bakery property depends on gluten quality. Gluten is a part of endosperm hard proteins that causes increased stickiness quality. Wheat glutenin is divided into two groups according to their molecular weight, glutenin subunits with high molecular weight (HMW-GS) and glutenin subunits with low molecular weight (LMW-GS). In the present study, 97 Iranian wheat landraces were analyzed for diversity in high and low molecular weight glutenin subunits. In hexaploids, 11 different high molecular weight subunits were identified, three of which were related to *Glu-A1*, five for *Glu-B1* and three were related to *Glu-D1*. Interestingly, the subunit 2.1+10* was observed in three landraces. In tetraploids, nine subunits were identified, of which two were related to *Glu-A1* and seven were related to *Glu-B1*. Low molecular weight glutenin subunit encoding genes were investigated using seven DNA primer pairs for *Glu-A3*, *Glu-B3* and *Glu-D3* loci. Five alleles were identified for *Glu3-A.2* with frequencies ranging from 3.092 to 96.907. Two alleles were identified by *Glu3-A3* with relative frequencies of 0.412 and 0.608%. For *Glu3-B.1*, 10 alleles were identified, with frequencies ranging from 1.030 to 92.783. However, three alleles a, b and c were identified by *Glu3-B.2* with frequencies of 0.01, 0.78 and 0.20, respectively. Three specific primers were used for *Glu-D3* locus and because durum wheat lacks DD genome, it was not estimated in this genetic block. Using PCR amplification, nine alleles were identified for *Glu3-D1* with frequencies ranging from 5.34 to 86.67. Also two and three alleles were identified using *Glu3-D.3* and *Glu3-D.4*, respectively.

***Key words*:** Classification, Glutenin, HMW-GS, LMW-GS.

INTRODUCTION

Bakery property in wheat depends on gluten quality. Gluten is a part of endosperm hard proteins that causes increased stickiness quality. The first study on the wheat seed proteins was performed by Osborn in 1907. Wheat seed proteins were divided into four groups according to their solubility. Two groups are albumin and globulin soluble in water and salt solutions which possess metabolic activities. Two other groups are inactive and non-dissolvable and contain glutelins or glutenins and prolamins or gliadins (Weegels *et al*., 1996; Dangi *et al*., 2019). Wheat glutenins and gliadins are located in glutelin and prolamin groups, respectively (Weegels *et al*., 1996). Accurate studies in the field of genetic correlation demonstrated that nine loci with independent separation control gluten proteins (Payne *et al*., 1984). Wheat glutenin is divided into two groups according to molecular weight, glutenin subunits with high molecular weight (HMW-

GS) and glutenin subunits with low molecular weight (LMW-GS). HMW-GS are coded by Glu1 loci located on the long arm of chromosomes group 1, and each locus includes two coding genes for subunits type X and Y (Shewry *et al*., 1992; Guo *et al*., 2019). Genes X and Y control subunits with slower and faster movement, respectively (Chen *et al*., 2019). LMW-GS are coded by *Glu-3* genes including *Glu-A3*, *Glu-B3* and *Glu-D3* that are like a genetic block and located on the short arm of 1A, 1B and 1D chromosome (Masci *et al*., 1998; Wang *et al*., 2016). Six patterns were identified for loci *Glu-A3*, five for *Glu-D3* and nine patterns for *Glu-B3*. For the first time, glutenin subunits were divided into three groups A, B and C according to their movement on the SDS-PAGE gel (Payne *et al*., 1981; liang *et al*., 2015). It was shown that group A belonged to HMW-GS and groups B and C belonged to LMW-GS. Gliadins with high molecular weight show similar movements with B and C and LMW subunits. In addition to groups B and C that have previously been identified for LMW by Payne *et al*. (1981), a new group called D has been identified according to their pattern on the SDS-PAGE gel (Jackson *et al*., 1983). Frequently, special LMW-GS belong to the group B, although a small ratio of similar parts of gliadin sequences are present in this group (Masci *et al*., 2002; Wang *et al*., 2016). According to their N terminal sequences, LMWs are divided into three groups; LMW-m, LMW-s and LMW-i, whose amino acid sequence starts with methionine, serine and isoleucine, respectively. Moreover, type LMW-s subunits are the most abundant types in all studied genotypes and their molecular mass (35000-45000) is higher than LMW-m subunits (30000-40000) (Tao *et al*., 1989; Cloutier *et al*., 2001). LMW-s subunits amino acid sequence is SHIPGL, while the LMW-m has more variable sequences of METSHIGPL, METSSCIPGL, or METSRIPGL (Masci *et al*., 1995; Masci *et al*., 1998). Also, LMW-i subunit sequences usually begin with repeated ISQQQ (Masci *et al*., 1995; Cho *et al*., 2019).

LMW-GS have a more allelic diversity in comparison with HMW-GS, but because of their overlap with gliadins on the SDS-PAGE gel, it is difficult to identify more alleles (Wang *et al*., 2005; Dangi *et al*., 2019). Up to now, several specific primers have been designed for a group of genes present in *GLU-3* genetic block on the protected carboxylic and amine ends. By using specialized indicators it has been made possible to identify alleles that are unidentified due to the overlap of gliadin sequences with LMW-GS subunits (Wang *et al*., 2008; Zhen *et al*., 2014). In this study, the length variations of HMW–GS and LMW-GS genes in Iranian landraces of bread and durum wheat were analyzed.

MATERIALS AND METHODS

Seed protein extraction and analysis

High and Low molecular weight glutenin subunits were assessed in 97 Iranian wheat landraces (Table 1) collected from different provinces in IRAN. Total grain protein was extracted and electrophoresis of HMW glutenin subunits was carried out using SDS-PAGE on 10% poly acrylamide gels following Payne *et al*. method (1981). Gels were stained over night by Commassie Brilliant Blue R-250 in the presence of Tri-chloroacetic acid (TCAA) and ethanol and destained by a 3% NaCl solution. Since sub-units 2* and 2 normally overlap on 10% SDS-PAGE gels, in landraces carrying 2+12 sub-units, 7.5% poly acrylamide gels were employed for their separation. The detected bands were scored following Payne and Lawernce (1983).

Primers designing

Specific primers for Low molecular weight glutenin subunits (*Glu-A3*, *Glu-B3* and *Glu-D3*) were designed according to GS wheat genome by Long *et al*. (2005) (Table 2).

DNA isolation and PCR amplification

Genomic DNA was isolated from fresh wheat leaf tissues using a CTAB modified method as reported by Doyle and Doyle (1990). Polymerase chain reactions (PCR) were run in a volume of 20 µl containing 1X PCR buffer, 30 ng sample DNA, 2.5 µM primer, 200 µM of each dNTP, 2 mM $MgCl_2$ and 1 unit of Taq DNA polymerase. PCR program was started with denaturing at 95 °C for 5 min followed by 35 cycles including: denaturing at 95 °C for 1 min, annealing at 50-70 °C (depending on the annealing temperatures) for 1 min, extension at 72 °C for 1 min and the final extension for 4 min. The PCR products were separated on a 2% agarose gel.

Data analysis

For estimating genetic diversity in Glu3 locus, Nei's genetic variation index (Nei, 1973) was used: In this formula ($H=1- \Sigma Pi^2$) , if Pi is the relative abundance of Mi allele at a genetic site in the population being studied, genetic variation at the genetic site can be calculated.

RESULTS

Surveying allelic diversity of HMW glutenin subunits

The result of SDS PAGE showed that out of 97 studied wheat landraces, 75 were hexaploid bread wheat and 22 were tetraploid durum wheat (Table 1). In hexaploid

Table 1. Allelic combination and genotypic scores based on glutenin high molecular weight subunits for 97 Iranian wheat landraces.

Line No.	*Glu-A1*	*Glu-B1*	*Glu-D1*	Genotypic score	Line No.	*Glu-A1*	*Glu-B1*	*Glu-D1*	Genotypic score
1	null	7+8	-	nd	27	null	7+8	2+12	6
2	null	7+8	-	nd	28	null	7+8	2+12	6
3	null	20	2+12	4	29	null	7+8	2+12	6
4	null	20	-	nd	30	2*	7+8	2+12	8
5	null	6+8	-	nd	31	null	7+8	2+12	6
6	null	7+8	2+12	6	32	null	7+8	2+12	6
9	null	7+8	2+12	6	33	null	7+8	2+12	6
10	null	7+8	2+12	6	34	null	7+8	2+12	6
11	null	7+8	2+12	6	35	null	7+8	2+12	6
12	null	7+8	2.1+10*	nd	36	null	7+8	2+12	6
13	null	6+8	2+12	4	37	null	7+8	2+12	6
14	null	7+8	2+12	6	38	null	7+8	2+12	6
15	null	7+8	2+12	6	39	null	7+8	2+12	6
16	null	7+8	2+12	6	40	null	7+8	2+12	6
17	null	7+8	2+12	6	41	null	7+8	5+10	8
18	null	7+8	2+12	6	42	null	7+8	2+12	6
19	null	7+8	2+12	6	43	null	7+8	2+12	6
20	null	7+8	2.1+10*	nd	44	null	7+8	2+12	6
21	null	7+8	2+12	6	45	null	7+8	2+12	6
22	null	7+8	2+12	6	46	null	7+8	2+12	6
23	null	7+8	2+12	6	47	null	7+8	2+12	6
24	null	7+8	2+12	6	48	null	7+8	2+12	6
25	null	7+8	2+12	6	49	null	7+8	2+12	6
26	null	7+8	2+12	6	50	null	7+8	2+12	6
51	null	7+8	2+12	6	77	2*	20	-	nd
52	null	7+8	2+12	6	78	1	7+8	2+12	8
53	null	6+8	2+12	6	79	null	7+8	2+12	6
54	null	7+8	2+12	6	80	null	7+8	2+12	6
55	2*	7	-	nd	81	null	20	-	nd
56	null	7+8	2+12	6	82	null	7+8	2+12	6
57	null	7+8	2+12	6	83	null	20	-	nd
58	null	7+8	2+12	6	84	null	20	-	nd
59	2*	20	-	nd	85	null	7+8	2+12	6
60	2*	7	-	nd	86	null	20	-	nd
61	null	7	-	nd	87	null	7+8	2+12	6
62	2*	20	-	nd	88	null	7+8	2.1+10**	nd
63	2*	7	-	nd	89	null	7+8	2+12	6
64	2*	7	-	nd	90	null	7+8	2+12	6
65	2*	7	-	nd	91	null	13+16	-	nd
66	null	7+8	2+12	6	92	null	7+8	2+12	6
67	2*	20	-	nd	93	null	7+8	2+12	6
68	2*	6+8	-	nd	94	2*	20	5+10	7
69	null	ZZ´	-	nd	95	null	7+8	2+12	6
70	null	7+8	2+12	6	96	null	7+8	2+12	6
71	null	/13+19 7+8	5+10	8/nd	97	null	7	5+10	6
72	null	20	2+12	4	98	null	7+8	2+12	6
73	null	20	2+12	4	99	null	7+8	2+12	6
74	null	7+8	2+12	6	100	null	20	5+10	6
76	null	20	-	nd	94	2*	20	5+10	7

Table 2. Specific primers for low molecular weight glutenin subunits designed according to GS wheat genome by Lang *et al.* (2005).

Gene locus	Primer sequence (5'-3')	Annealing temperature (°C)
Glu3.A.2	AGTGCCATTGCGCAGATGAAT AACGGATGGTTGAACAATAGA	60
Glu3.A.3	ATGGAGACTAGCTGCATCC CTGCAAAAAGGTACCCTTTT	60
Glu3.B.1	GCACAAATGGAGAATAGCCAC GGAGTAGACGATAGCACGGAT	59
Glu3.B.2	CCTAGCTTGGAGAAACCATT GGCAACATTGTTGCTGCATCAC	50
Glu3.D.1	CCTGGCTTGGAGAAACCATC ACCCAGTTGTTGTTGTTGAG	50
Glu3.D.3	ATGGAGACTAGATGCATCCCT AGATTGGATGGAACCCTGAAC	60
Glu3.D.4	ATGGAGACTAGCTGCATCT CTGCAAAAAGGTACCCTGTA	52

wheat landraces, 11 different subunits were identified (Figure 1), three of which were related to *Glu-A1*, five to *Glu-B1* and three were related to *Glu-D1*. Moreover, each pattern included three to five bands of HMW glutenin subunits. Null allele of *Glu-A1* locus was observed in 96.5% of the lines, while among 1380 cultivars collected from all over the world, the frequency of this allele in genetic locus *Glu-A1* has been reported to be 36% (Morgunov *et al.*, 1993). Also the frequency of allele 2* was 2.63% in this genetic locus (Table 3). On the other hand, in *Glu-B1* locus (Figure 1), subunits 7+8 (Glu-B1b), 6+8 (Glu-B1d), 7 (Glu-B1a), 20 (Glu-B1e), and 13+19 (Glu-B1g) were observed. Subunits 7+8 were present in 88.15% of lines, while among 1380 cultivars collected from all over the world, the genetic diversity was reported to be around 25% in *Glu-B1* locus (Morgunov *et al.*, 1993). We found the most abundant alleles for Glu-B1b (subunit 7+8) and Glu-B1e (subunit 20) with 88.15 and 6.58% frequencies, respectively (Table 3). Concurrently, diversities of subunits 5+10 (Glu-D1d) and 2+12 (Glu-D1a), encoded by *Glu-D1* locus, were 7.89 and 88.15%, respectively. While among 1380 cultivars collected from all over the world, these diversities were 41% for Glu-D1d and 35% for Glu-D1a (Morgunov *et al.*, 1993). According to the previous studies, subunit 5+10 is the most valuable subunit for flour quality and bakery. Its desirability in comparison with subunit 2+12 is due to excessive cysteine amino acid in subunit 5 in comparison to subunit 2. As a result, it would create opposite peptide chains reactions and increase dough strength (Shewry *et al.*, 1997). Interestingly, the subunit 2.1+10* encoded by *Glu-D1* locus was reported as a new allele with a very

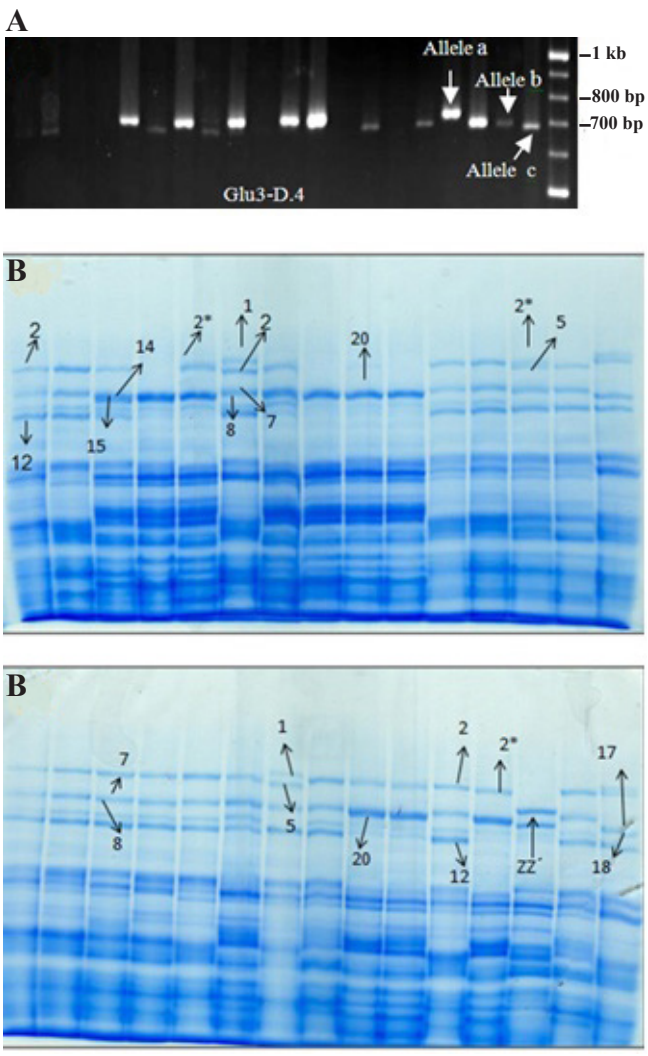

Figure 1. A: PCR amplification for low molecular weight glutenin subunit gene alleles (for genome D by primer Glu3.D.4) and **B:** high molecular weight glutenin subunits (arrow numbered) in Iranian tetra-hexaploid wheat landraces.

Table 3. High molecular weight glutenin subunits (HMW-GS) and their composition patterns in 75 hexaploid wheat landraces.

Locus	Subunit	Number of lines	Frequency (%)	Subunit composition	Number of lines	Frequency (%)
Glu- A1	null	73	96.05	null, 20, 2+12	3	3.94
	2*	2	2.63	null, 7+8, 2+12	60	78.94
	1	1	1.31	null, 7+8, 2.1+10*	3	3.94
	13+19	1	1.31	null, 6+8, 2+12	2	2.63
Glu- B1	7+8	67	88.15	2*, 7+8, 2+12	1	1.31
	20	5	6.57	null, 7+8, 5+10	2	2.63
	6+8	2	2.63	null, 13+19, 5+10	1	1.31
	7	1	1.31	2*, 20, 5+10	1	1.31
Glu- D1	2+12	67	88.15	null, 7, 5+10	1	1.31
	5+10	6	7.89	null, 20, 5+10	1	1.31
	2.1+10*	3	3.94	1, 20, 2+12	1	1.31

Table 4. High molecular weight glutenin subunits (HMW-GS) and their composition patterns in 22 tetraploid wheat landraces.

Locus	Subunit	Number of lines	Frequency (%)	Subunit composition	Number of lines	Frequency (%)
Glu- A1	null	12	54.55	null, 7+8	2	9.09
	2*	10	45.45	null, 20	6	27.27
Glu- B1	7+8	2	9.09	null, 6+8	1	4.54
	20	10	45.45	2*, 7	5	27.27
	7	6	27.27	2*, 20	4	18.18
	6+8	2	9.09	2*, 6+8	1	4.54
	13+16	1	4.54	null, zz´	1	4.54
	ZZ´	1	4.54	null, 13+16	1	4.54
				null, 7	1	4.54

low diversity in Pakistan native varieties (Tahir *et al*., 1994), this allele was also observed in 3 lines (3.94%) in our study. Bahraei *et al*. (2004) also reported a high diversity of this subunit in Iranian landraces grown in Sistan and Balouchestan area. Among 75 landraces, in 11 hexaploid lines a different allele composition was observed (Table 3) and the most common composition of HMW glutenin subunits was null, 7+8 and 2+12 which was observed in 60 lines. Also, compositions 2*, 7+8, 2+12, null, 7+8, 5+10 and 1, 7+8, 2+12 that give grade 8 quality to the lines possessing them, were observed in one, two, and one lines, respectively. More importantly, subunit 7+8 which has a great impact on dough expansion was observed in 67 lines, while subunit 6+8 (Glu-Bd) that is the glutenin low quality indicator (Payne *et al*., 1984) was observed only in two lines.

In tetraploid landraces, nine different subunits were identified, of which two were related to *Glu-A1* and seven were related to *Glu-B1*. Consequently, each pattern includes one to three bands of HMW glutenin subunits. Null allele of *Glu-A1* was observed in 54.55% of lines, while among 1380 cultivars collected by Morgunov *et al.* (1993), abundance of this allele was 36%. Diversity of allele 2^* in this genetic locus was 45.45% (Table 4). We found more interesting results for *Glu-B1* locus, in which subunits 7+8 (Glu-B1b), 6+8 (Glu-B1d), 7 (Glu-B1a), 20 (Glu-B1e), 13+16 (Glu-B1f, and zz' (unknown)) were observed (Figure 1). One of the outstanding observations was related to subunit 7+8 whose frequency was 9.09%, while among 1380 cultivars collected by Morgunov *et al*. (1993), its abundance was 25% in *Glu-B1*. Moreover, in *Glu-B1* the most diverse alleles were Glu-B1e (subunit 20) and Glu-B1a (subunit 7) with 45.45% and 27.27% diversities, respectively (Table 4). On the other hand, nine different allelic compositions were observed among 22 tetraploids (Table 4). The most common composition of HMW glutenin subunits were (null, 20), (2^*, 7) and (2^*, 20) observed in six, five, and four lines, respectively.

Glutenin allele diversity with low molecular weight (LMW-GS)

In the present study, 75 bread and 22 durum wheat landraces were investigated for HMW-GS using seven specific primer pairs designed by Long *et al*. (2005) from Gs wheat genome including *Glu-A3*, *Glu-B3*, and *Glu-D3* loci. Two pairs of primers were specific for *Glu-A3* locus, primers Glu3-A.2 and Glu3-A.3, which were designed for the AA genome of Chinese Spring, amplifying a 350 bp fragment. If in a reaction the 350 bp fragment is absent and other fragments are present, it should be concluded that a proposed allelic pattern is detectable, according to Long *et al*. (2008). As a result, four patterns were identified with lengths between 350 bp to 400 bp. Generally, five alleles a, b, c, d, and e were characterized for primer Glu3-A.2, with the abundances of 3.092, 90.72, 3.092, 96.907, and 18.556%, respectively (Table 5). Among them allele d with 350 bp length had the highest frequency and was observed in three patterns out of four. Furthermore, allele b was identified in three patterns and 88 lines, but alleles a and c were observed only in three lines. Although the pattern 2 was the most common pattern in both wheat types, pattern 3 was uniquely observed in bread wheat (Table 6). The estimation of genetic diversity using Nei coefficient showed that durum wheat (*T. durum*) had a higher diversity with the coefficient of 0.563 compared to bread wheat (*T. aestivum*) with a diversity coefficient of 0.432. Primer pairs for Glu3-A.3 amplified a 680 bp fragment. In our research the size range of the amplified fragments for Primer pairs Glu3-A.3 was between 650-750 bp. In general, alleles a and b were identified by Glu3-A3 primers with the frequencies of 0.412 and 0.608, respectively. Allele b with the approximate length of 680 bp had the highest abundance (Table 5). Allele b was the most common and was observed in both patterns and 59 lines and allele a was identified in pattern 1 and in 40 lines (Table 6). Durum wheat landraces with 93% had a higher diversity than bread wheat landraces.

Two pairs of primers were used for *Glu-B3* locus, Glu3-B.1 and Glu3-B.2, in which Glu3-B.1 primer was designed for BB genome of Chinese Spring to amplify a 780 bp fragment. However, the length of the amplified fragments in our landraces differed from 700 to 1000 bp. Generally, by Glu3-B.1 primer pair 10 alleles, a to j, were identified, with the abundances of 1.030, 1.030, 1.030, 15.463, 55.670, 92.783, 5.154, 50.70, 69.072 and 58.762%, respectively (Table 5). Among them, allele f with an approximate length of 850 bp had the highest abundance and was observed in nine patterns out of 12 (Table 6). Alleles i, j, h, and e were observed in 67, 57, 55 and 54 lines, respectively. While alleles a, b and c were observed once. Furthermore, estimating genetic diversity using Nei coefficient showed that bread wheat landraces with a diversity of 0.722 had a higher diversity than durum wheat landraces. Similarly, Glu3-B.2 primer was designed for BB genome of Chinese Spring to amplify a 574 bp fragment. However, in the landraces three alleles a, b and c were identified for Glu3-B.2

Table 5. The frequencies of low molecular weight glutenin subunit (LMW-GS) alleles in 97 Iranian wheat landraces (75 hexaploid bread wheat and 22 tetraploid durum wheat).

Primer	Allele	Number of lines	Frequency (%)
Glu3.A.2	a	3	3.092
	b	88	90.72
	c	3	3.092
	d	94	96.907
	e	18	18.556
Glu3.A.3	a	40	0.412
	b	59	0.608
Glu3.B.1	a	1	1.030
	b	1	1.030
	c	1	1.030
	d	15	15.463
	e	54	55.670
	f	90	92.783
	g	5	5.154
	h	55	50.70
	i	67	69.072
	j	57	58.762
Glu3.B.2	a	1	0.010
	b	76	0.78
	c	20	0.20
Glu3.D.1	a	10	13.34
	b	65	86.67
	c	4	5.34
	d	60	80
	e	7	9.34
	f	60	80
	g	2	2.67
	h	43	57.34
	i	20	26.67
Glu3.D.3	a	2	0.026
	b	58	0.773
Glu3.D.4	a	2	2.67
	b	60	80
	c	10	13.34

Table 6. Patterns of allelic composition by genome specific primers in 97 Iranian wheat landraces (75 hexaploid bread wheat and 22 tetraploid durum wheat).

primer	Pattern	Allele										Number of lines	Frequency (%)
		a	b	c	d	e	f	g	h	i	j		
Glu3.A.2	1		+		+	+						18	0.185
	2		+		+							67	0.690
	3	+	+	+								3	0.03
	4				+							9	0.092
Glu3.A.3	1	+	+									40	0.412
	2		+									19	0.195
Glu3.B.1	1						+					24	0.247
	2								+			1	0.0103
	3					+	+		+	+	+	42	0.432
	4				+	+	+		+	+	+	2	0.020
	5				+	+	+			+	+	4	0.041
	6				+		+			+		4	0.041
	7				+		+			+	+	4	0.041
	8					+	+	+	+	+	+	5	0.051
	9	+	+				+					1	0.0103
	10						+		+	+		4	0.041
	11				+					+		1	0.0103
	12			+		+				+		1	0.0103
Glu3.B.2	1		+									74	0.76
	2			+								20	0.206
	3		+	+								1	0.010
	4	+	+									1	0.010
Glu3.D.1	1					+		+				2	0.027
	2		+		+		+		+			42	0.56
	3		+		+		+					7	0.094
	4		+			+	+			+		10	0.134
	5	+	+							+		5	0.067
	6	+		+		+				+		3	0.053
	7					+				+		1	0.013
	8	+	+		+		+		+			1	0.013
Glu3.D.3	1		+									56	0.746
	2	+	+									2	0.026
Glu3.D.4	1		+									58	0.773
	2			+								10	0.134
	3	+	+									2	0.027

primer that had relative abundances of 0.010, 0.78 and 0.20, respectively (Table 5). Allele b with an amplified fragment of about 620 bp in length had the highest abundance and was observed in three patterns out of four and was present in 76 lines. Also, alleles a and c were observed in 1 and 20 lines, respectively. While pattern 1 was the most common pattern in bread and durum wheat landraces, pattern 3 and 4 were unique in bread wheat (Table 6). The estimation of genetic diversity using Nei coefficient showed that bread wheat landraces with a coefficient of 0.423 had a higher diversity than durum wheat landraces.

We used three specific primer pairs for *Glu-D3* locus and since durum wheat lacks the DD genome, it was not estimated in this genetic block. Although Glu3-D.1 primer was designed for DD genome of Chinese Spring to amplify an 834 bp fragment, the amplified fragments in bread wheat landraces differed from 800 to1000 bp in size. Using PCR amplification, nine alleles, a to i, were identified for Glu3-D1 with the frequencies of 13.34, 86.67, 5.34, 80, 9.34, 80, 2.67, 57.34 and 26.67%, respectively (Table 5). Meanwhile, allele b with a length of about 900 bp had the highest abundance and was identified

in 5 patterns out of 8, in 65 lines. Moreover, alleles d, f, h, I, a, e, c and g were observed in 60, 60, 43, 20, 10, 7, 4, and 2 lines, respectively. Pattern 2 was the most common in bread wheat lines (Table 6) and estimating genetic diversity using Nei coefficient showed that bread wheat landraces had a diversity coefficient of 0.651. Similar to Glu3-D.1, Glu3-D.3 primer was designed for DD genome of Chinese Spring to amplify a 600 bp fragment. However, the amplified fragments varied in length from 600 to 650 bp in wheat landraces. Two alleles, a and b, were identified using Glu3-D.3 primer and had 0.026 and 0.773 relative abundances, respectively (Table 5). Allele b with about 600 bp in length had the highest abundance and was the most common allele and was present in both patterns. Also, pattern 1 was the most common pattern in bread wheat lines (Table 6) and showed a diversity coefficient of 0.442. Although Glu3-D.4 primer was designed for DD genome of Chinese Spring to amplify a 700 bp fragment, but the fragment sizes in the studied lines varied from 700 to 780 bp. Meanwhile, three alleles a, b and c (Figure 1) were identified with 2.67, 80, and 13.34% frequencies, respectively (Table 5). Allele b with about 720 bp in length had the highest frequency and was identified in 58 lines and was observed in two patterns out of three (Table 6). Pattern 1 was the most common with a genetic diversity coefficient of 0.383 in bread wheat landraces.

DISCUSSION

Since gluten is the most important factor affecting seed quality in wheat, we designed the present study to evaluate the bakery properties of some Iranian wheat landraces. It was reported that presence of subunits 1 or 2* (against subunit null) in *Glu-A1* locus causes higher volume of SDS (Payne *et al*., 1987). It was found that despite the fact that the frequencies of 1 and 2* subunits were 1.31 and 2.63%, respectively, the frequency of null subunit was 96.05% in wheat lines. On the other hand in durum lines, 2* and null subunit showed 45.45 and 54.55% frequencies. Graybosch *et al*. (1994) and Rogers *et al.* (1989) reported that they could not find any relation between 1 and 2* subunits and qualitative value. While the presence of 7+8 subunit against 7+9, encoded by *Glu-B1* locus, are related with higher dough strength (Perron *et al*., 1998). They were found in 88.15% of bread wheat and only in 9.09% durum wheat lines. In studies performed for comparing the effects of 2+12 and 5+10 subunits encoded by *Glu-D1* locus, the positive effect of 5+10 subunit was reported in 7.89% lines (Payne *et al*., 1981; Gupta *et al*., 1993).

Most of LMW-GS coding genes include a short (13 amino acid) and high protected amino end and a carboxyl end that includes two third of peptide length and is less sustainable than amino end. Amongst these two ends, there is a repetitive area whose length is too variable among the genes. Repetitive area is formed by some repetitive units, being the main cause of diversity in genes length. This diversity may be due to deletion or addition phenomenon in the repetitive units created by unequal crossing over or sliding along replication (Long *et al*., 2005). Therefore, the primers designed according to amino and carboxyl ends amplify the repetitive areas (Long *et al*., 2005). Then, it is concluded that the observed differences in fragments length are related to deletion and addition phenomenon in DNA level taking place in the repetitive areas. But the reason that some genotypes e.g. line 54 showed six fragments by PCR using Glu3-B.1 primer is that probably there are several copies of one gene in a genetic block. Similar to line 54, several cases were observed in other lines by other primers. Among five identified alleles by Glu3-A.2 primer, one with about 300 bp in length was different to other alleles. Such new alleles can subsequently be sequenced and by performing BLAST with the present sequences in the data banks, one can obtain more information about their length diversity. Also, it is possible that these new alleles contain more cysteine amino acid and since cysteine amino acid residues take part in disulphide bridges and have a considerable role in stretching qualities of dough, therefore, these can be used in remitting projects for improving wheat flour quality. For instance, Xu *et al*. (2006) cloned a new LMW gene called *XYGLUD3-LMW* (Ay263369) from bread wheat type Xiaoyan6. The protein made by this gene contained 9 cysteine amino acids while most of genes identified so far have 8 cysteine amino acids. Their complimentary tests showed that this extra cysteine meaningfully increased LMW qualitative specifications (Xu *et al*., 2006).

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