Journal of Plant Molecular Breeding (JPMB)
Vol. 2/No. 2/ December 2014/ 28-42

PCR-based markers for identification of some allelic variation at *Glu-1* and *Glu-3* loci in common wheat

E. Mehrazar[1], A. Izadi-Darbandi[1*], M. Mohammadi[2] & G. Najafian[2]

1. Department of Agronomy and Plant Breeding Sciences, College of Aburaihan, University of Tehran, P.O.Box, Tehran, Iran.
2. Seed and Plant Improvement Institute, Cereals Research Department, Karaj, Iran.
[*]Corresponding author: aizady@ut.ac.ir

Received: June 2014 Accepted: December 2014

Abstract
Marker assisted selection (MAS) is a tool for breeding, screening, and genetic characterization of germplasm. Allelic variation of both high and low molecular weight glutenin subunits (HMW/LMW-GS) is associated with the rheological properties of wheat flour. In this study, we investigated glutenin pattern using SDS-PAGE and their PCR based on DNA markers in 60 advanced wheat lines and cultivars with different origins. Specific DNA markers regarding to *Glu-1* loci, such as 1319 bp, 669 bp and 450 bp fragments were respectively validated for 2*, 17+18, 5+10 alleles. These alleles showed the highest allelic percentage in *Glu-1* loci in studied cultivars. However the Null, 7+8 and 5+10 alleles showed the highest allelic percentage in advanced lines. In this study, 23%, 40% and 37% of cultivars respectively, got good (10), moderate (8-9) and weak (4-7) quality scores. In advanced lines, 18%, 44% and 38% got good, moderate and weak quality scores respectively. Ten specific DNA PCR markers were also detected for genotyping *Glu-B3* alleles. The most frequent *Glu-B3* alleles in wheat cultivars were *i, a, b* and *d* with 24%, 21%, 20% and 12%, respectively.Specific PCR markers regarding to the reported *Glu-B3* alleles were produced as 621bp, 1095bp, 1570 bp and 662bp consequently. The most frequent *Glu-B3* alleles in advanced lines belonged to *a, i* and *d* alleles with 35%, 26% and 21% respectively. The results provided useful information for breeding program to improve breadmaking quality and develop new cultivars.
Key words: *Glu-1*, *Glu-B3*, Wheat.

Introduction

Wheat (*Triticum aestivum* L.) quality is mainly determined by the seed storage proteins in the grain's endosperm (Shewry and Halford 2002; Peymanpour *et al*. 2012; Majzoobi *et al*. 2012). These proteins composed of two major fractions, gliadin and glutenin that play a main role in rheological properties of bread wheat dough. Glutenins principally consist of two types of subunits: high-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS), which are cross-linked to form glutenin polymer by intermolecular disulphide bonds. The HMW-GS depict nearly 10% of the seed storage proteins. LMW-GS depict about one-third of seed storage proteins of the total grain and also 60% of glutenins (Luo *et al*. 2001; Azizi *et al*. 2006). HMW-GS are encoded by the *Glu-A1*,

Glu-B1 and *Glu-D1*locithat are located on the distal of the long arm of wheat chromosomes 1A, 1B and 1D, respectively (Payne *et al*. 1987). While the LMW glutenin subunits (LMW-GS) encoded by the *Glu-A3*, *Glu-B3* and *Glu-D3*loci, are located on the distal of the short arm of these chromosomes (Wang *et al*. 2009). HMW-GS include less number of subunits and extensive studies have been done on them, while LMW-GS include a larger number of polypeptides and their relationship to grain processing quality have not been studied to the same degree as for the HMW-GS yet (D'Ovidio and Masci 2004). Although the role of HMW-GS on bread making quality (Dough strength) was recognized obviously, LMW glutenin subunits also play a significant role on dough viscosity and formation of large polymers. It is found that some allelic forms of LMW-GS have greater effects on the quality than HMW-GS (Gupta *et al*. 1991; Luo *et al*. 2001).Studies have shown that the allelic variation of both HMW-GS and LMW-GS are associated with the rheological properties of wheat flour (Payne *et al*. 1987). Most types of LMW-GS are in group B in which the *Glu-B3* locusis located between *Gli-B1* locus (at 2cM) and the centromere (Wang *et al*. 2009). Afterwards, six protein alleles were found for the *Glu-A3* locus, nine for the *Glu-B3* locus and five for the *Glu-D3* locus. Dough strength or Rmax (maximum dough resistance), is mostly controlled by *Glu-3*loci with the following ranking order: *i*>*b* = a >*e* = *f* = *g* = *h*>*c* (Gupta *et al*. 1991). To identify different HMW-GS and LMW-GS subunits, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) or reversed phase high performance liquid chromatography (RP-HPLC) (Singh *et al*. 1991) were used in the past, while nowadays to overcome some conflicts on allelic detection of glutenin subunits, the development of specific PCR-based markers are used (Xu *et al*. 2008 ; Wang *et al*. 2009). Different studies showed the usefulness of marker assisted selection for identification of variant ω-gliadin genes (Chen *et al*. 2011), allelic variants encoded at the *Glu-D3* locus (Appelbee *et al*. 2009), allelic variation at the *Glu-A3* and *Glu-B3* loci (Zhang *et al*. 2004; Si *et al*. 2012), allelic variation at the *Glu-1* and *Glu-3* loci and the presence of 1B-1R translocation lines (Liu *et al*. 2005). Therefore an important goal in wheat quality improvement is the identification of specific HMW-GS and LMW-GS alleles (Gupta *et al*. 1991; Gale 2005). It has been recognized that three alleles comprise 5+10, 2* and 17+18 which are allelic variants, are connected with good quality characteristics in bread wheat cultivars and lines (Payne *et al*. 1987; Mohammadi *et al*. 2013). Studies by Uthayakumaran *et al* (2006) and Kuchel *et al* (2007) showed that the marker assisted selection (MAS) for HMW-GS and LMW-GSusing sequence tagged sites (STS) DNA markers can speed up breeding programs. In this study we used STS-PCR method (Abdel-Mawgood 2008; Izadi-Darbandi and Yazdi-Samadi 2012; Goutam *et al*. 2013; Mohammadi et al.

2013) for screening of *Glu-1* and *Glu-B3* loci in wheat genotypes.

Materialsand Methods

Materials: Thirty-four advanced wheat lines and 26 hexaploid wheat cultivars originated from CIMMYT, Iran, Canada, Australia, USA, Turkey, France and Russia, which is kindly provided by the GenBank of Seed and Plant Improvement Institute of Iran were investigated in this study (Table 1).

SDS-PAGE analyses: For the glutenin extraction, the sequential extraction procedure described by Singh *et al*. (1991) was employed.A gel system consisting of two layers i.e., stacking and gradient acrylamide separating layers was used. A linear gradient acrylamide gel from 8.1% to 12.5% with 1% crosslinker concentration (bisacrylamide/acrylamide ratio) allowed better visualization of HMW and LMW glutenin subunits (Izadi-Darbandi *et al*. 2010).). Payne nomenclature system (Payne et al.1988) was applied to detect HMW-GS.

DNA extraction: Genomic DNA was isolated from fresh seedling leaves by modified CTAB procedure as reported by Murray and Thompson (1980).

PCR amplification: Polymerase chain reaction (PCR) was done on some wheat genotypes based on their protein banding patterns to validate their correspondence specific DNA markers. Then nine genotypes containing our interesting alleles at protein level was selected. Ten primer sets (Table 2) were used to amplify different *Glu-B3* alleles based on the detected SNPs (Wang *et al* 2009). The complete information for primers and their correspondence allele-specific PCR product are shown in Table 2. Each PCR reaction was performed in 25µl final volume, consisting of 1U Taq DNA polymerase, 2.5µl PCR buffer, 1.5 mM $MgCl_2$ and 2.5mM of each dNTP, 0.4pmol of each primer and 50ng genomic DNA. PCR amplifications of *1Ax2**, *1Bx17*, *1Dx5* and*Glu-B3*loci within germplasm were tested by using primers and protocols reported in previous researches (Ma *et al*. 2003, Vjell, 1998 and Wang *et al*. 2009). PCR reactions for *Glu-1* alleles were performed using an initial denaturing step at 94°C for 2min, followed by 35 cycles of denaturation at 94°C for 45s, annealing at [51-58]°C for 45s, an extension at 72°C for 90s. The PCR conditions for *Glu-B3* allele-specific markers consisted of an initial denaturing step at 94°C for 5min, followed by 35 cycles of denaturation at 94°C for 45s, annealing at [55-62]°C for 45s, an extension at 72°C for 90s. All PCR reactions were terminated by a final extension at 72°C for 10min.

The purity of studied advanced lines was checked for 1*AL-1RS* and 1*BL-*1*RS*wheat-rye translocation lines that will present 1*RS* alleles byusing one pair of specific primers as: Forward:5′-TGACAACCCCCTTTCCCTCGT-3′and Reverse:5′- TCATCGACGCTAAGGAGGACCC-3′ (Saal and Wricke 1999).

Table 1. Advance lines evaluated using SDS-PAGE and allele-specific PCR marker with a genome score based on Payne (1988).

No. advance lines	Advance Line Pedigree	Origin	Analysis allele-specific PCR marker				Analysis SDS-PAGE Protein			
				HMW-GS		LMW-GS		HMW-GS		quality
			Glu-A1b	*Glu-B1i*	*Glu-D1d*	*Glu-B3*	*Glu-A1*	*Glu-B1*	*Glu-D1*	score
1	SOOTY-9/RASCON-37	CIMMYT	2*	17+18	*	C	2*	17+18	*	6
2	AUK/GUIL//Green	CIMMYT	Non-2*	17+18	*	d	null	17+18	*	4
3	RASCON_37/BEJAH-7	CIMMYT	2*	Non-17+18	*	d	2*	6+8	*	2
4	ZAGBA_2/BICHENA	CIMMYT	Non-2*	17+18	*	d	null	17+18	*	4
5	CNDO/PRIMADUR//HAI-OU_17/3/SNITAN	CIMMYT	2*	17+18	*	d	2*	17+18	*	6
6	SULA/AAZ_5//CHEN/ALTAR84/3/AJAIA_12/F3LO	CIMMYT	Non-2*	Non-17+18	*	d	null	7+8	*	4
7	Seri82//Shuha"S"/4/Rbs/Anza/3/Kvz/Hys//Ymg/Tob	CIMMYT	Non-2*	17+18	5+10	i	1	17+18	5+10	10
8	Alvd//Aldan"s"/las58/4/kal/Bb//cj"s"/3/Hork"s"	Iran	Non-2*	Non-17+18	Non-5+10	f	null	7+8	2+12	6
9	1-66-22/5/1-66-31/4/Anza/3/Pi/Nar/Hys/6/M-75-7	Iran	Non-2*	Non-17+18	5+10	a	1	7+8	5+10	10
10	1-66-22/5/1-66-31/4/Anza/3/Pi/Nar/Hys/6/M-75-7	Iran	Non-2*	17+18	Non-5+10	e	1	17+18	2+12	8
11	Hereward/Siren/5/Gov/Az//Mus/3/DoDo/4/Bow	Iran	Non-2*	Non-17+18	Non-5+10	e	null	7+8	2+12	6
12	KAUZ//ALTAR84/AOS/3/PASTOR	CIMMYT	Non-2*	Non-17+18	5+10	a	1	7+9	5+10	9
13	PRL/2*PASTOR	CIMMYT	Non-2*	Non-17+18	5+10	d	1	7+9	5+10	9
14	KAUZ/PASTOR	CIMMYT	Non-2*	Non-17+18	5+10	a	1	7+9	5+10	9
15	Alvd//Nanjing8343/Kauz	Iran	Non-2*	Non-17+18	5+10	a	1	7+8	5+10	10
16	Alvad//Aldan/las/3/Rsh	Iran	2*	Non-17+18	5+10	i	2*	7+9	5+10	9
17	Alvd//Nanjing8343/Kauz	Iran	2*	17+18	5+10	d	2*	17+18	5+10	10
18	PBW343*2/CHAPLO	CIMMYT	Non-2*	Non-17+18	5+10	a	1	7	5+10	8
19	PBW343*2/KUKUNA	CIMMYT	Non-2*	17+18	5+10	a	1	17+18	5+10	10
20	CHAPLO	CIMMYT	2*	Non-17+18	Non-5+10	a	2*	20	2+12	6
21	Alvd//Aldan"s"/las58/4/kal/Bb//cj"s"/3/Hork"s"	Iran	Non-2*	17+18	5+10	f	null	17+18	5+10	8
22	GF-gy54/Attila	Iran	Non-2*	Non-17+18	Non-5+10	a	null	7+8	2+12	6
23	Sakha8/Darab#2//1-66-22	Iran	2*	17+18	5+10	i	2*	17+18	5+10	10
24	GV/D630//Ald"s"/3/Azd/4/1-75-104	Iran	Non-2*	Non-17+18	Non-5+10	a	1	7+9	2+12	7
25	Hmd//1-66-22//Inia	Iran	Non-2*	Non-17+18	Non-5+10	i	null	7+8	2+12	6
26	Hmd//1-66-22//Inia	Iran	Non-2*	Non-17+18	Non-5+10	i	null	7+8	2+12	6
27	1-66-22/3/Alvd//Aldan/Los	Iran	Non-2*	Non-17+18	5+10	a	null	7+8	5+10	8
28	Desprez80/Rsh//1-66-22/Inia	Iran	Non-2*	Non-17+18	5+10	i	null	7+8	5+10	8
29	v82.187/1-66-22/5/Kvz/cgn/4/Hys//Drc*z/7c/3/2*Rsh	Iran	2*	Non-17+18	5+10	i	2*	7+9	5+10	9
30	snb"s"//Emu"s"/Tjb84-1543/3/kauz/stm	Iran	2*	Non-17+18	Non-5+10	b	2*	7+8	2+12	8
31	Alborz/5/K62909/4/Cno//k58/Tob/3/wa/5/ehen	Iran	2*	Non-17+18	Non-5+10	i	2*	7+8	2+12	8
32	Kau2*2/Opata//kauz//kauz/3/sakha8/4/kauz/srkhtm	Iran	Non-2*	17+18	Non-5+10	i	null	17+18	2+12	6
33	GV/D630//Ald"s"/3/Azd/4/Flt	Iran	Non-2*	17+18	5+10	a	null	17+18	5+10	8
34	1-66-22/passarinho/3/Vee/Nac//1-66-22	Iran	Non-2*	Non-17+18	Non-5+10	a	null	7+8	2+12	6

Table 1. continued.

Ac-Barrie	Neepawa/Columbus//BW90	Canada	2*	Non-17+18	5+10	h	2*	7+8	5+10	10
Inia	SUMAI-2[1747]; FUNO/TAIWANMAI[2959];	CIMMYT	Non-2*	Non-17+18	5+10	d	1	13+16	5+10	10
Ac-Crystal	HY377/L8474-D1	Canada	Non-2*	Non-17+18	5+10	i	1	7+8	5+10	10
argentine	LP-585-67/KLEIBER[667][851][1258][2846];	USA	Non-2*	Non-17+18	Non-5+10	e	null	7	2+12	4
aqoa	(S)CRIMEAN[37][39][1111];	Turkey	Non-2*	Non-17+18	Non-5+10	b	1	7+8	2+12	8
bezostaya	Import cultivar32338	Russia	2*	Non-17+18	5+10	d	2*	7+9	5+10	9
Cadet	(S)BEZOSTAYA-4[37][80][104][10][11];	Canada	2*	Non-17+18	5+10	b	2*	7+8	5+10	10
Aroona	MERIT/THATCHER[37][616][39][1111];	Australia	Non-2*	Non-17+18	Non-5+10	b	1	7+9	2+12	7
Tobari-66	WW-15/RAVEN[113][626][851];	CIMMYT	Non-2*	Non-17+18	Non-5+10	i	null	7+8	2+12	6
panjamoo	Tzpp/Sn64A	CIMMYT	2*	Non-17+18	Non-5+10	f	2*	7+8	2+12	8
soisson	FKN/N10B	France	2*	Non-17+18	5+10	b	2*	7+8	5+10	10
Glenlea	IENA(JENA)/(HYBRIDE-NATUREL)HN-35[1346][1413][1665][1764][1790][2845];	Australia	2*	Non-17+18	Non-5+10	g	2*	7	2+12	6
Katepwa	UM-530/(MEX)CB-100[39][1323][2331];	Canada	2*	Non-17+18	5+10	h	2*	7+9	5+10	9
Norstar	NEEPAWA*6/RL-2938/3/NEEPAWA*6//CI-8154/2*FROCOR;	Canada	Non-2*	Non-17+18	5+10	b	1	7+8	5+10	10
Ac-Vista	HY44/ 7915QX76B2)LOSPROUT//HY358*3/BW553;	Canada	Non-2*	Non-17+18	Non-5+10	i	1	7+8	2+12	8
Celtic	ANGUS/LEN[1318];	Canada	2*	Non-17+18	5+10	b	2*	7+9	5+10	9
Ac-Reed	ANGUS/LEN[1318];	Canada	2*	Non-17+18	Non-5+10	b	2*	20	2+12	6
Ac-Foremost	HY-320*5/BW-553//HY-320*6/7424BW-5-B-4[1323];	Canada	Non-2*	Non-17+18	Non-5+10	i	1	7+8	2+12	8
Ac-Taber	HY-320*3/BW-553[1125][1315][1323][113];	Canada	2*	Non-17+18	5+10	i	2*	7+9	5+10	9
Hope	YAROSLAV-EMMER/MARQUIS[47][1102][1111]; VERNAL-EMMER(TR.DN)/MARQUIS[39];	USA	2*	Non-17+18	Non-5+10	b	2*	6+8	2+12	6
Marquis	HARD-RED-CALCUTTA/RED-FIFE[10][201][1446];	Canada	Non-2*	Non-17+18	5+10	b	1	7+9	5+10	9
Anza	LERMA-ROJO-64//NORIN-10/BREVOR/3/3*ANDES-ENANO;	USA	Non-2*	Non-17+18	Non-5+10	b	1	7+8	2+12	7
Selkirk	MCMURACHY/EXCHANGE//3*REDMAN,CAN[39]	Canada	Non-2*	Non-17+18	5+10	g	1	6+8	5+10	8
Laura	BW-15/BW-517[229][592][1318][1323][113];	Canada	Non-2*	Non-17+18	5+10	h	1	7+8	5+10	10
Pasqua	BW-63*2/COLUMBUS[1323][1429];	Canada	2*	Non-17+18	5+10	g	2*	7+9	5+10	9
Ac-Eatonia	LEADER/LANCER[1323][1411];	Canada	2*	Non-17+18	5+10	h	2*	7+8	5+10	10

*: Don't have D genome and advance lines are durum.

A touch down PCR condition with an initial denaturation at 94°C for 3min, followed by 40 cycles, 15 cycles of which were performed for 45s at 94°C, 40s at 65°C, 40s at 72°C and were reduced by 1°C per cycle in annealing temperature, at the end, the PCR was continued by 25 cycles of 94°C for 45s, 40s at 50°C, 40s at 72°C and then final extension of 72°C for 5min. PCR products (10μl) were separa-ted by electrophoresis on 1.5% Agarose gels using TAE buffer and then stained with Ethidium bromide (0.5 $\mu g/ml$ final concentration) before being visualized under UV light.

Table 2. Allele-specific molecular markers and PCR conditions used in this study.

Name Marker	Sequence (5′-3′)	Allele	Product Size (bp)	Annealing Temperature (°C)
gluB3a	F: CACAAGCATCAAAACCAAGA R: TGGCACACTAGTGGTGGTC	a	1095	55
gluB3b	F: ATCAGGTGTAAAAGTGATAG R: TGCTACATCGACATATCCA	b	1570	56
gluB3c	F: CAAATGTTGCAGCAGAGA R: CATATCCATCGACTAAACAAA	c	472	56
gluB3d	F: CACCATGAAGACCTTCCTCA R: GTTGTTGCAGTAGAACTGGA	d	662	58
gluB3fg	F: TATAGCTAGTGCAACCTACCAT R: CAACTACTCTGCCACAACG	fg	812	62
gluB3g	F: CCAAGAAATACTAGTTAACACTAGTC R: GTTGGGGTTGGGAAACA	g	853	60
gluB3h	F: CCACCACAACAAACATTAA R: GTGGTGGTTCTATACAACGA	h	1022	60
gluB3i	F: TATAGCTAGTGCAACCTACCAT R: TGGTTGTTGCGGTATAATTT	i	621	58
gluB3bef	F: GCATCAACAACAAATAGTACTAGAA R: GGCGGGTCACACATGACA	bef	750	60
gluB3e	F: GACCTTCCTCATCTTCGCA R: GCAAGACTTTGTGGCATT	e	669	58
Ax2*	F: ATGACTAAGCGGTTGGTTCTT R: ACCTTGCTCCCCTTGTCTTT	2*	1319	53
Bx17	F: CGCAACAGCCAGGACAATT R: AGAGTTCTATCACTGCCTGGT	17+18	669	58
Dx5	F: GCCTAGCAACCTTCACAATC R: GAAACCTGCTGCGGACAAG	5+10	450	51

Results

HMW-GS identification by SDS-PAGE: In order to confirm the STS-PCR markers, protein allelic patterns of advanced lines, containing all of our target alleles were detected through SDS-PAGE system for 1AX2*, 1BX17 and 1DX5 alleles. The presence of each specific PCR band was confirmed by the expression of desired alleles at protein level (Figure 1).

PCR analysis: Specific primers (Table 2) for 2*, 17+18, and 5+10 alleles in

wheat advanced lines produced 1319 bp, 669 bp and 450 bp fragments, respectively (Figure 2a, Figure 2b, Figure 2c).

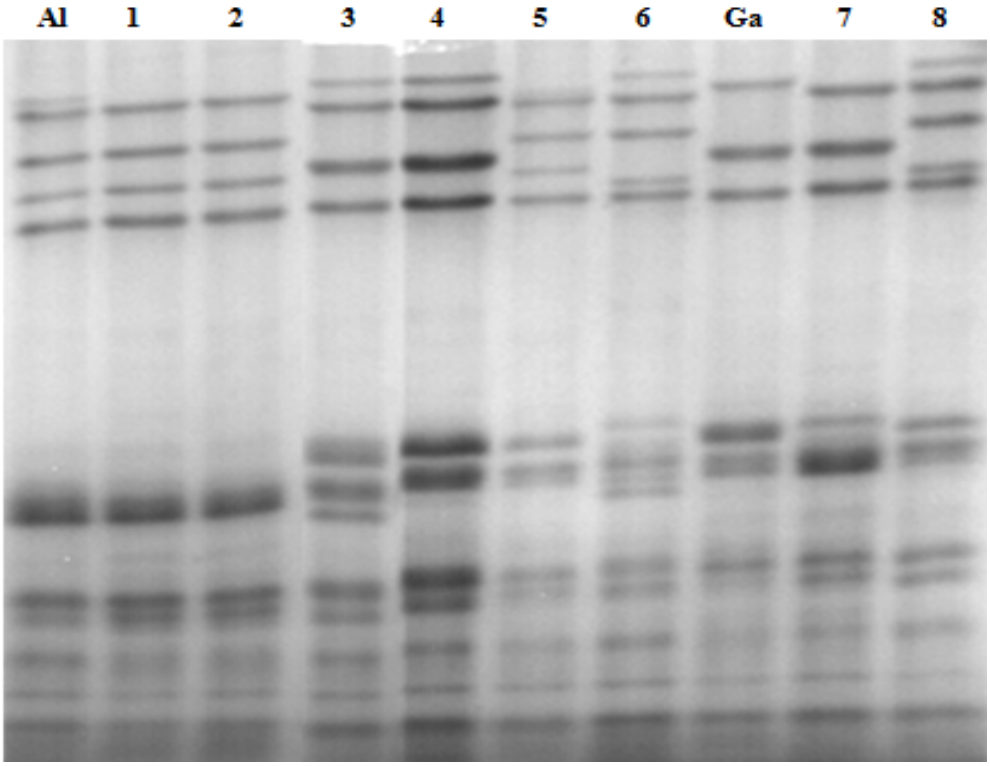

Figure 1. SDS-PAGE patterns of glutenin subunits of wheat advanced lines.1 no. 34, 2 no. 25, 3 no. 19, 4 no. 7, 5 no. 28, 6 no. 13, 7 no. 33, 8 no. 14 in Table 1. Gabo (Gb) and Alvand (Al) were used for the identification of their banding patterns.

Among 34 wheat advanced lines, only a 1,095-bp PCR fragment was amplified in three advanced lines (No.9, 19, and 33) using specific primer set for *Glu-B3a* allele (Figure 3a). One PCR fragment as 1,549-bp was detected for the *Glu-B3b* allele in advanced lines (Figure 3b). For the *Glu-B3c* allele in one line (No 1), a unique 472 bp PCR product was generated (Figure 3c). Primer set *Glu-B3d* was used to identify the d allele in seven lines, producing a 662-bp band (Figure 3d). For advanced lines containing the *e* allele (No.10 and 11 of 34), a specific 669-bp PCR product was generated using the primer set *Glu-B3e* (Figure 3e). Primer set *Glu-B3i* Specifically amplified a 621 bp PCR fragment in 9 lines (Figure 3h). Since it was difficult to design a specific primer set for *Glu-B3f*, primer set *Glu-B3fg* was used to amplify *Glu-B3f* and *Glu-B3g* in two advanced lines (Figure 3f). In combination with *Glu-B3f*, this primer set can be used to identify *f* allele. Additionally, primer set *Glu-B3bef* was used to amplify *Glu-B3b*, *e* and *f* in 5 advanced lines producing a 750-bp band

(Figure 3g). This set can be used to verify the former primer sets. The *Glu-B3g*primer set was used to detect the *g* allele in lines with a 853-bp PCR fragment. Primer set *Glu-B3h*, which generated a 1022bp band in lines, was used to discriminate the *h* allele from others. The results of Table 1 indicates that none of these 34 advanced lines showed the *Glu-B3g* and *h* alleles that were present in commercial cultivars. The absent of specific PCR products attributed to the 1RS of Rye representing their purity of advanced lines without any translocation.

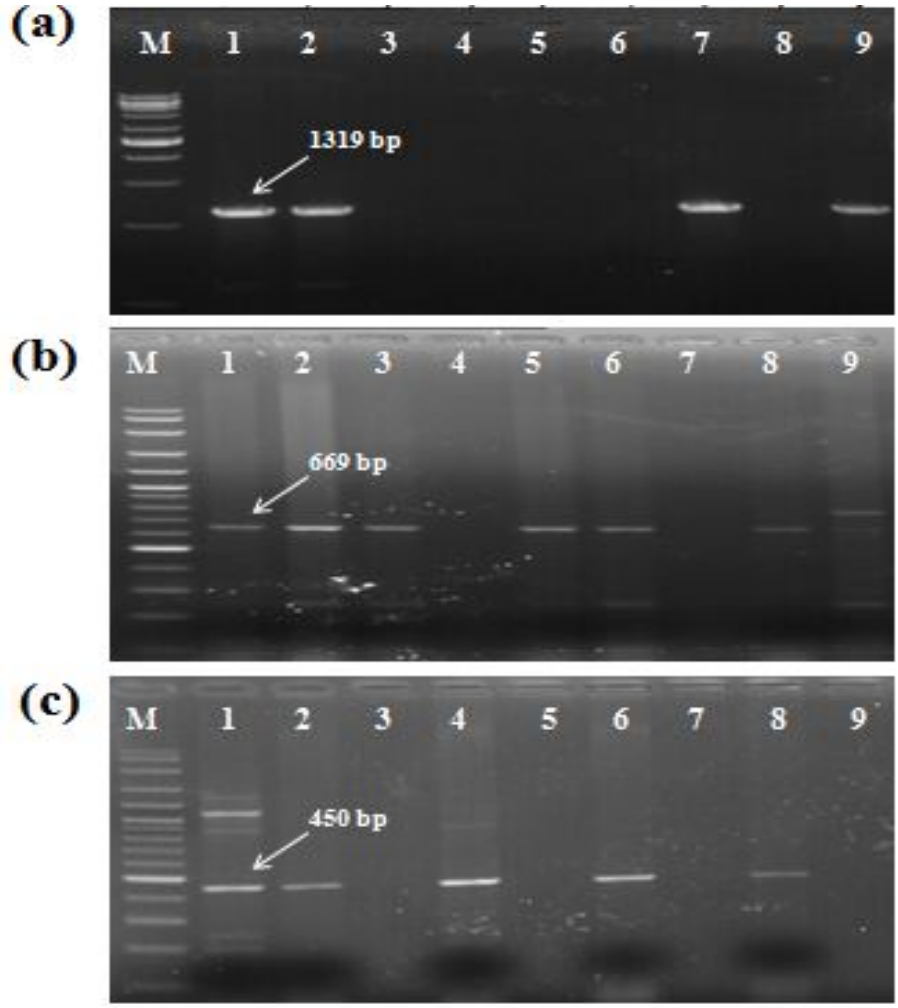

Figure 2. Electrophoresis of PCR products of three gene-specific primer sets on agarose gels. Wheat are used as PCR templates are as listed in Table 1 as lanes 1-9: numbes;.23, 17, 10, 9, 21, 19, 30, 33 and 1. (a) Primer Ax2* for the 2*allele. M, 1kb DNA ladder (b) primer Bx17 for the 17+18 allele M, 100 bp DNA ladder (c) primer Dx5 for the 5+10 allele M, 100 bp DNA ladder.

Discussion

We dissected the allelic variation of *Glu-1*and *Glu-B3* glutenin loci in 34 wheat advanced lines and 26 cultivars. In these study allelic variations at loci were identified using SDS-PAGE and were nominated by using Gabo and Alvand standard genotypes based on their known banding patterns (Izadi-Darbandi *et al*. 2010). The advantages of PCR-based assay compared with SDS-PAGE for selecting HMW-GS and LMW-GS alleles have been reported previously by Abdel-Mawgood (2008) and Wang *et al*. (2009) respectively. In this study the exact and fine looking of HMW-GS composition at *Glu-A1b* (2*), *Glu-B1i* (17+18) and *Glu-D1d* (5+10) loci and 9 alleles of *Glu-B3* locus from LMW-GS were validated by using a set of specific applied primers. DNA markers that had been used in this study were able to identify common HMW-GS alleles with a high quality ranking and *Glu-B3* alleles in wheat breeding programs Wheat dough propertiesis related to its rheology as well as its maximum resistance. Research has shown that HMW-GS components increase the dough strength (Gupta *et al*. 1991). While LMW-GS components, in comparison with HMW-GS components, have more important role in maximizing the dough elasticity (Gupta *et al*. 1991). It has been proven that by increasing the amount of protein, those LMW-GS with more subunits are screened as a result of their higher effects on dough and this has been accepted as a general strategy (Payne *et al*. 1987). Among the LMW-GS, *Glu-B3* locus has the highest allelic diversity and major amount related to the *Glu-D3* and *Glu-A3* loci. Thus, allelic variation in loci associated with this group of chromosomes can be helpful in identifying varieties and their phylogenetic relationship (Long *et al*. 2005). Although studies of LMW-GS proteins have several difficulties, such as gliadin contamination and co-migration on gel, however, Singh *et al*. (1991) proposed the sequential extraction methods and the use of gradient gels for this purpose. The overall implementation of this method is, however, time consuming and hard working. Moreover, due to the large number of bands, the analysis is very difficult and sometimes wrong, though STS-PCR markers have solved such problems and are very informative. The results showed that only 38% of the studied genotypes had 2* alleles and the remaining showed; 1 or null alleles (Figure 4a). 20% of wheat genotypes had the 17+18 allele and 80% of the remaining showed other related alleles at *Glu-B1* locus. In the case of the 5+10 allele, only 52% of the studied advanced lines showed this allele (Figure 4a). The results of PCR with the specific primers for *Glu-B3* locus, confirmed the existence of these nine alleles, $i > a > b > d > h > g = f = e >$ consequently with 24%, 21%, 20%, 12%, 6%, 5%, 5%, 5% and 2% of allelic composition. Allelic frequency at *Glu-B3* loci, showed that there are a bimodal or trimodal distribution in our studied cultivars. Results showed that the alleles of*i* and *a* are more frequent in Iranian genotypes and *b, g* and *h*alleles in this loci were got higher frequency in Canadian genotypes,

howeverthe *d* allele was the most frequent in CIMMYT genotypes.

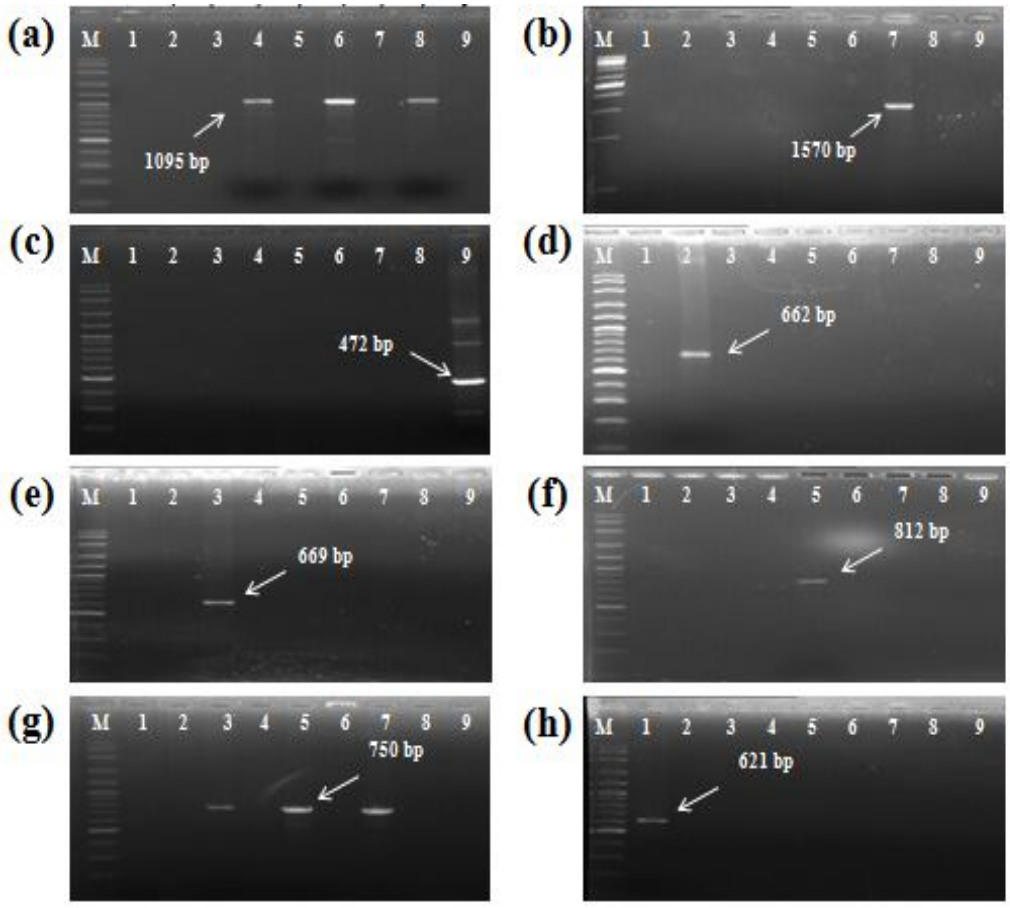

Figure 3. Electrophoresis of ten allele-specific PCR markers for genotyping Glu-B3 alleles in nine wheat advance lines on agarose gels. (a) glu-B3a, (b) glu-B3b, (c) glu-B3c, (d) glu-B3d, (e) glu-B3e, (f) glu-B3fg, (g) glu-B3bef and (h) glu-B3i. Wheat are used as PCR templates were as lanes 1-9 as listed in Table 1: numbers:.23, 17, 10, 9, 21, 19, 30, 33 and1M, 1kb and 100 bp DNA ladder.

The most frequent *Glu-B3* alleles in advanced lines of breeding programs of Iran belonged to *a, i* and *d* alleles with 35%, 26% and 21%, respectively. This type of distribution and a high percentage of alleles *a, i, b* and *d* in the studied advanced lines and cultivars are consistent with other results (Izadi-Darbandi *et* al. 2010) (Figure 4a). Gupta *et al.* (1991) were ranked the quality of *Glu-B3* alleles as following order: $i > b = a > e = f = g = h > c$. and therefore existing of the *a* and *i* as the frequent alleles with high ranking on bread-making quality in studied advanced lines is expected to show their good potential in breeding programs. The existence of*d* allele in our advanced lines is also

expected to show their potential for being used as noodle consumes (Gale *et al*. 2005). Wang *et al*. (2009) reported that alleles Glu-B3d and Glu-A3d are better application for Chinese noodles. Wheat cultivars and advanced lines showed the highest frequency (38%) for 2* or *b* allele at *Glu-A1*locus. At the *Glu-B1*locus, the highest frequency was detected for the 7+8 allele with 43%, 7+9 allele with 22% and 17+18 allele with 20%. The same frequencies were reported at *Glu-A1* locus in Australian wheat and existing of the null allele (c) represents poor quality in studied genotypes (Gupta *et al*. 1991). Allele 1By was Null in the line No. 18 and cultivars of Argentina, Glenlea containing 1Bx (7) allele. Furthermore line No. 20 and Ac-Reed cultivar expressed 1Bx (20) alone without their 1By linked subunits (Figure 4b). Studies showed that in common wheat, 1Bx, 1Dx and 1Dy alleles are always expressed, while 1Ax and 1By are not always expressed. 1Ay subunit mostly is off in hexaploid wheats whereas it is usually expressed in tetraploid and diploid (Jiang *et al*. 2009; Izadi-Darbandi *et al*. 2010).

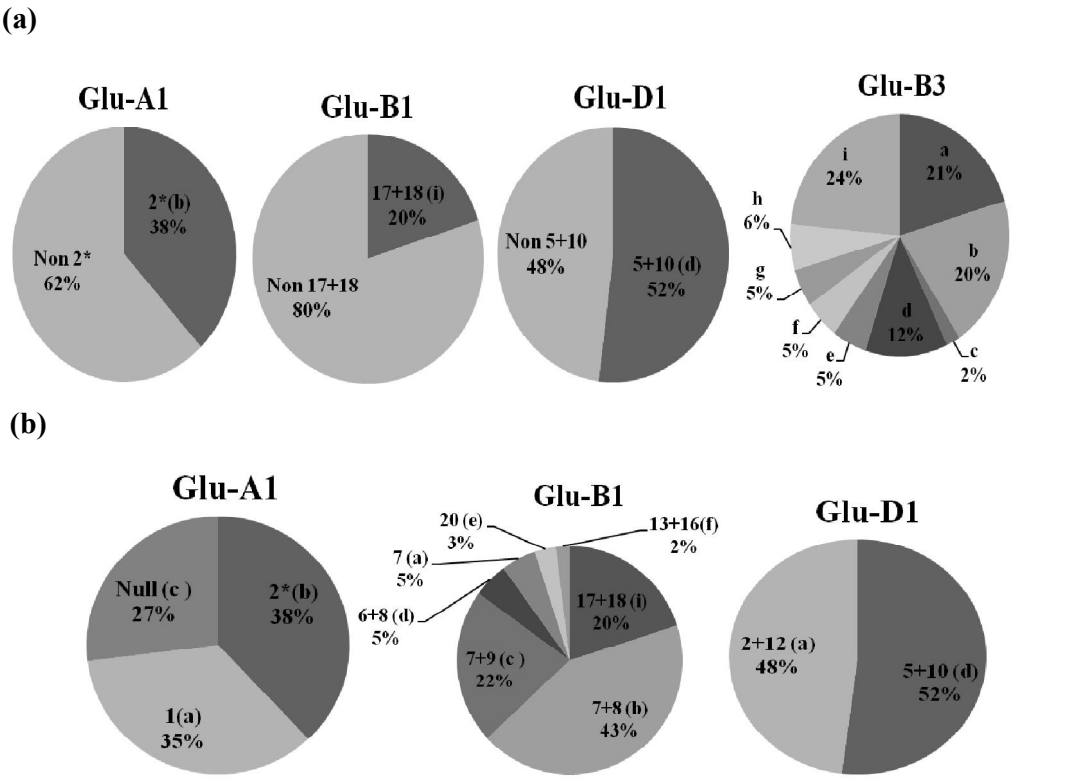

Figure 4. (a) Allelic frequency distributions for *Glu-1* and *Glu-B3* loci are shown in the DNA level (b) Allelic frequency distributions for *Glu-A1, Glu-B1*and *Glu-D1* loci are shown by SDS-PAGE.

Payne *et al*. (1988) showed that the presence of subunits 7+8 compared with subunits 7+9 which is coded at *Glu-B1* locus is associated with higher dough strength. At *Glu-D1* locus, the highest frequent allele was 5+10 (d) allele with 52% frequency which is among the most valuable alleles in the bakery. The frequency of this allele was lower than 2+12 in Iranian bread wheat cultivars Figure 4b). In fact, the majority of Iranian wheat cultivars showed null allele at locus *Glu-A1*, thus it can be mentioned that they have a lower rating quality (Payne *et al*. 1987). As it can be seen, only 23% of the studied advanced lines got good quality score about 40% of lines were well worth to the bakery because their genome scores were around 8-9 and 37% of lines are considered weak with genome scores 4-7 (Table 1). In various studies, the positive effect of subunits 7+8 and 5+10 on the baking quality has been reported. The frequencies of these two subunits in the studied lines were higher than other alleles which indicate their high bread making quality. Alleles 2+12 and 5+10 at *Glu-D1* locus, respectively accompany with the weakest and strongest impact on the brad making quality. Screening for some useful alleles such as 5+10 is highly recommended in Iranian wheat breeding programs. However, screening at early generation of breeding program can increase the frequency of suitable alleles lead to improving bread making quality. Nine *Glu-B3* and three *Glu-1* allele-specific markers with high effects on bakery usage were validated in 60 wheat advanced lines and cultivars (Table 1). The results of allelic variation at *Glu-B3* and Glu-1 loci obtained by PCR based markers was quite verified with those of detected by SDS-PAGE. The overall results of this study showed that the protein mobility alleles determined by SDS-PAGE were consistent with the screening results obtained using the allele specific markers in 60 wheat advanced lines and cultivars. SDS-PAGE is one method for identification of allelic components in quality scoring of wheat cultivars, but the mobility of subunits in this system does not exactly correspond with the size and sometimes makes the interpretation of banding pattern difficult. However, marker assisted selection can help avoid misinterpretation of results from SDS-PAGE. Wheat quality identification can be done in early stage of growth development without having to wait for seed set and analysis of their glutenin composition. In this study, STS-PCR markers verified for the sequences of three and nine different alleles is located on the *Glu-1* and *Glu-B3* loci respectively. We can use these markers as an alternative method in wheat quality breeding program for detecting poor or good qualities in early stage of growth. Marker assisted selection by DNA markers which have verified in this study can be used for both quality classification and accelerating breeding program for bread-making quality.

Reference

Abdel-Mawgood, A.L. 2008. Molecular markers for predicting end-products

quality of wheat (*Triticum aestivum* L.). *Afr. J. Biotechnol*., 7: 2324-2327.

Appelbee, M.J., Mekuria, G.T., Nagasandra, V., Bonneau, J.P., Eagles, H.A., Eastwood, R.F. and Mather, D.E. 2009. Novel allelic variants encoded at the *Glu-D3* locus in bread wheat. *J. Cereal Sci*., 49: 254–261.

Azizi, M.H., Sayeddin, S.M. and Payghambardoost, S.H. 2006. Effect of flour extraction rate on flour composition, dough rheological characteristics and quality of flat bread. *J. Agric. Sci. Technol*.,8: 323-330.

Chen, F., Yang, L., Zhao, F., Min, H. and Xia, G. 2011. Molecular cloning and variation of ω-gliadin genes from a somatic hybrid introgression line II-12 and parents (*Triticum aestivum* cv. Jinan 177 and *Agropyron elongatum*). *J. Genet.,* 90: 137-142.

D'Ovidio, R. and Masci, S. 2004. The low-molecular-weight glutenin subunits of wheat gluten. *J Cereal Sci.*, 39:321–339.

Gale, K.R. 2005. Diagnostic DNA markers for quality traits in wheat. *J Cereal Sci*, 41:181–192.

Goutam, U., Kukreja, S., Tiwari, R., Chaudhury, A., Gupta, R.K., Dholakia, B.B. and Yadav, R. 2013. Biotechnological approaches for grain quality improvement in wheat: Present status and future possibilities. AJCS*Aust. J. Crop Sci.*, 7. 469-483.

Gupta, R.B., Bekes, F. and Wrigley, C.W. 1991. Prediction of physical dough properties from glutenin subunit composition in bread wheats. *Cereal Chem*., 68: 328–333.

Izadi-Darbandi, A. and Yazdi-Samadi, B. 2012. Marker assisted selection of highmolecular weight glutenin alleles related to bread-making quality in Iranian common wheat (*Triticum aestivum* L.). *J. Genet.,* 91: 193-198.

Izadi-Darbandi, A., Yazdi-Samadi, B., Shahnejat-Boushehri, A.A. and Mohammadi, M. 2010. Allelic variations in *Glu-1* and *Glu-3*loci of historical and modern Iranian bread wheat (*Triticum aestivum* L.) cultivars. *J. Genet*., 89: 193-199.

Jiang, Q.T., Wei, Y.M., Wang, F., Wang, J.R., Yan, Z.H. and Zheng, Y.L. 2009. Characterization and comparative analysis of HMW glutenin *1Ay*alleles with differential expressions. *BMC Plant Biol*. 6: 9-16.

Kuchel, H., Fox, R., Reinheimer, J., Mosionek, L., Willey, N., Bariana, H. and Jefferies, S. 2007. The successful application of a marker-assisted wheat breeding strategy. *Mol. Breed*. 20: 295-308.

Liu, L., He, Z., Yan, J., Zhang, Y., Xia, X. and Pena, R. 2005. Allelic variation at the Glu-1 and *Glu-3*loci, presence of the 1B. 1R translocation, and their effects on mixographic properties in Chinese bread wheats. *Euphytica*. 142: 197-204.

Long, H., Wei, Y. M., Yan, Z.H., Baum, B., Nevo, E. and Zheng, Y.L. 2005. Classification of wheat low-molecular-weight glutenin subunit genes and its chromosome assignment by developing LMW-GS group-specific primers.*Theor. Appl. Genet*., 111: 1251–1259.

Luo, C., Giyn, W. B., Branlard, G. and McNeil, L. 2001. Comparison of low and high molecular-weight wheat glutenin allele effects on your Quality.*Theor. Appl. Genet*., 102: 1088–1098.

Ma, W., Zhang, W. and Gale, K.R. 2003. Multiplex-PCR typing of high molecular weight glutenin alleles in wheat. *Euphytica*, 134: 51-60.

Majzoobi, M., Farhoodi, S., Farahnaky, A. and Taghipour, M.J. 2012. Properties of

dough and flat bread containing wheat germ.*J. Agric. Sci. Technol*.,14: 1053-1065.

Mohammadi, M., Mehrazar, E., Izadi-Darbandi, A. and Najafian, G. 2013. Marker assisted selection for complementation of *Glu-A1b*, *Glu-B1i*, and *Glu-D1d*alleles in early segregating generations of wheat. *Wheat Inf. Serv*. 116:3-8. www.shigen.nig.ac.jp/ewis.

Murray, M. and Thompson, W.F. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res*., 8: 4321–4325.

Payne, P.I., Holt, L.M., Krattiger, A.F. and Carrillo, J.M. 1988. Relationship between seed quality characteristics and HMW glutenin subunit composition determined using wheats grown in Spain. *J. Cereal Sci*. 7: 229–235.

Payne, P.I., Nightingale, M.A., Krattiger, A.F. and Holt, L.M. 1987. The relationship between HMW glutenin subunit composition and the bread-making quality of british-grown wheat varieties.*J. Sci. Food. Agric*.,40: 51–65.

Peymanpour, Gh., Rezaei, K., Sorkhilalehloo, B., Pirayeshfar, B. and Najafian, G. 2012. Changes in rheology and sensory properties of wheat bread with the addition of oat flour.*J. Agr. Sci. Tech*., 14: 339-348.

Saal, B. and Wricke, G. 1999. Development of simple sequence repeat markers in |rye (*Secale Cereale*L). *Genome*, 42: 964-972.

Shewry, P.R. and Halford, N.G. 2002. Cereal seed storage proteins: structures, properties and role in grain utilization. *J. Exp. Bot*., 53: 947-958.

Si, H., Gao, Y., Liu, F., Li, Z. and Ma, C. 2012. Distribution of low-molecular-weight glutenin subunit *Glu-B3* alleles in mini core collections of Chinese wheat germplasms. *Aus. J. Crop. Sci*. 6: 1390-1394.

Singh, N.K., Shepherd, K.W. and Cornish, G.B. 1991. A simplified SDS-PAGE procedure for separating LMW subunits of glutenin.*J. Cereal Sci*., 14: 203-208.

Uthayakumaran, S., Listiohadi, Y., Baratta, M., Batey, I.L. and Wrigley, C.W. 2006. Rapid identification and quantitation of high-molecular-weight glutenin subunits. *J. Cereal Sci*., 44: 34-39.

Vjell, P. 1998. Vyuzitl Genetickych Markeru Pro Tvorbu Dihaploidn Psenice Obecne (*Triticum aestivum* L.). [Disertatacn prace.] CZU, *praha*, 344s.

Wang, L.H., Zhao, X.L., He, Z.H., Ma, W., Appels, R., Pena, R.J. and Xia, X.C. 2009. Characterization of low-molecular-weight glutenin subunit *Glu-B3*genes and development of STS markers in common wheat (*Triticum aestivum* L.). *Theor Appl Genet*, 118: 525–539.

Xu, Q., Xu, J., Liu, C.L., Chang, C., Wang, C.P., You, M.S., Li, B.Y. and Liu, G.T. 2008. PCR-based markers for identification of HMW-GS at *Glu-B1x*loci in common wheat.J. Cereal Sci.,47: 394–398.

Zhang, W., Gianibelli, M.C., Rampling, L.R. and Gale, K.R. 2004. Characterization and marker development for low molecular weight glutenin genes from *Glu-A3* alleles of bread wheat (*Triticum aestivum*. L). *Theor Appl Genet*, 108: 1409–1419.

تعیین نشانگرهای اختصاصی DNA برای برخی ترکیبات آللی مکان‌های ژنی *Glu-1* و *Glu-3* در گندم نان

الهام مهرآذر[۱]، علی ایزدی‌دربندی[۱*]، محسن محمدی[۲] و گودرز نجفیان[۲]

۱. گروه زراعت و اصلاح نباتات، دانشگاه ابوریحان، تهران، ایران

۲. موسسه تحقیقات اصلاح و تهیه نهال و بذر، بخش تحقیقات غلات و حبوبات، کرج، ایران

*نویسنده مسئول: aizady@ut.ac.ir

چکیده

انتخاب به کمک نشانگر روشی مناسب جهت به‌گزینی و ارزیابی ژنتیکی ژرم پلاسم است. خواص رئولوژیکی آرد با تنوع زیر واحدهای گلوتنین با وزن مولکولی بالا و پایین گندم همبستگی دارد. در این تحقیق با استفاده از روش های SDS-PAGE و PCR الگوی زیرواحدهای گلوتنین و نشانگرهای اختصاصی DNA مرتبط به آنها در ۶۰ لاین پیشرفته و واریته گندم با منشأ جغرافیایی متفاوت بررسی شدند. نشانگرهای اختصاصی DNA به اندازه‌های ۱۳۱۹، ۶۶۹ و ۴۵۰ جفت باز به ترتیب برای آلل‌های ۲، ۱۸+۱۷ و ۱۰+۵ مکان ژنی *Glu-1* تکثیر شدند. در واریته‌های گندم مورد مطالعه، آلل‌های ذکر شده بیشترین فراوانی را در جایگاه ژنی *Glu-1* نشان دادند. در لاین‌های پیشرفته بیشترین فراوانی مربوط به آلل‌های Null، ۸+ ۷و ۱۰+۵ بود. واریته‌های مطالعه شده از نظر ارزش نانوایی بصورت خوب (۲۳ درصد با نمره ۱۰)، متوسط (۴۰ درصد با نمره ۹-۸) و ضعیف (۳۷٪ با نمره ۷-۴) طبقه‌بندی شدند. این ارزش در لایه‌های پیشرفته به ترتیب صورت ۱۸ درصد خوب، ۴۴ درصد متوسط و ۴۸ درصد ضعیف ثبت شدند. تعداد ۱۰ نشانگر اختصاصی DNA، با استفاده از PCR برای آلل‌های *Glu-B3* شناسایی شدند. در مکان ژنی *Glu-B3* برای واریته‌های بررسی شده آلل‌های i، a، b و d به ترتیب با ۲۴، ۲۱، ۲۰ و ۱۲ درصد بیشترین فراوانی را نشان دادند. نشانگرهای اختصاصی DNAبا اندازه‌های ۶۲۱، ۱۰۹۶، ۱۵۷۰ و ۶۶۲ جفت باز برای آلل‌های یاد شده تولید شدند. در مکان ژنی *Glu-B3* آلل‌های a، i و d به ترتیب با ۲۵، ۲۶ و ۲۱ درصد بیشترین فراوانی را در لاین‌های پیشرفته گندم داشتند. از نتایج این تحقیق در پیشبرد برنامه اصلاحی گندم جهت بهبود کیفیت ارزش نانوایی و تولید ارقام جدید استفاده خواهد شد.

کلمات کلیدی: *Glu-1*، *Glu-B3* و گندم.