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Molecular Characterization of *Low Molecular Weight Glutenin (LMW)* Genes in Triticeae Species with *D* Genome

M. Goldasteh[1], I. Mehregan[1*], M. R. Naghavi[2], and T. Nejadsattari[1]

ABSTRACT

Low Molecular Weight Glutenin Subunits (LMW-GS), encoded by GLU-3 loci located on the short arm of homologous chromosomes of wheat, play an important role in the bread making quality. Some of the most important gens for quality are located on the *D* genome, which are interesting in wheat breeding programs. In addition to the bread wheat (*Triticum aestivum*), some species of *Aegilops* including *Ae. cylindrica*, *Ae. tauschii*, *Ae. crassa*, *Ae. juvenalis*, and *Ae. vavilovi* carry *D* genome. In this study, the phylogenetic relationship among *Aegilops* species with *D* genome and bread wheat has been studied based on the sequence of low molecular weight glutenins loci. The results indicated a great diversity for these loci. Presence of several numbers of common protein bands among species suggested a close relationship and high genetic flow among species. Three primers for the LMW-GS proteins were able to reveal the relationship between the species. The results showed a close relationship among bread wheat (*T. astivum*) and *Ae. tauschii* species. *Ae. crassa* species is more distant from bread wheat. Also, the results indicated a close relationship between the *Ae. cylindrica*, *Ae. juvenalis*, and *Ae. vavilovi*. A great diversity of LMW-GS in wild relatives and close relationship between these species and wheat suggest them as a potential source of genes for wheat breeding programs.

Keywords: *Aegilops* species, Breeding programs, Phylogeny, Triticum.

INTRODUCTION

The storage proteins in wheat seed consist of two main components: The first component is glutenins, a polymer containing High Molecular Weight Glutenin Subunits (HMW-GS), and Low Molecular Weight Glutenin Subunits (LMW-GS), which totally form 20% of the endosperm storage proteins. The second component is gliadins, which is composed of monomer gliadin units (Payne *et al.*, 1980; Payne, 1987). LMW-GS includes about one-third of the storage proteins and 60% of glutenins in cereal seeds (Bietz *et al.*, 1973; Masci *et al.*, 2002). It has been shown that allelic diversity of LMW-GS plays an important role in the properties of dough prepared from different varieties of bread wheat (Gupta *et al.*, 1989, 1994; D'Ovidio and Masci, 2004) and durum wheat (Pogna *et al.*, 1990; Ruiz *et al.*, 1993).

Low molecular weight glutenin subunits are encoded by the Glu-3 loci located on the short arm of the homologous chromosomes group 1 (A1, B1, D1) near the centromere. The Glu3 loci are strongly linked with gliadin encoding sites. The role of some of these subunits is recognized in the food product quality (Payne *et al.*, 1987). These subunits are classified into three types: *B*, *C*, and *D* based on their molecular weight on the SDS-PAGE (Jackson *et al.*, 1983). Most of type *B* and some of type *C* of LMW-GS

[1] Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Islamic Republic of Iran.
[2] Department of Agronomy and Plant Breeding, University of Tehran, Karaj, Islamic Republic of Iran.
*Correspondence: e-mail: maryam.goldasteh@srbiau.ac.ir

proteins are coded by a group of tight linkage genes at Glu-A3, Glu-B3, and Glu-D3 loci located on the short arm of chromosomes 1A, 1B, and 1D, respectively (Jackson *et al.*, 1983; Gupta and Shepherd 1988; Masci *et al.*, 2002). It is estimated that from 10-15 copies (Harberd *et al*., 1985), up to 35-40 copies (Cassidy *et al.*, 1998; Sabelli and Shewry 1991) are present in each set of protein encoding genes. Sissons *et al.* (1998) proved the relationship between type *B* subunits and dough quality. LMW-GS subunits type *D* contain ω-gliadins and types *B* and *C* subunits contain α, β, and γ-gliadins (Gianibelli *et al.*, 2001; D'Ovidio and Masci, 2004; Appelbee *et al.*, 2009). Due to the relative similarity between the genomes of different species of wheat relatives, similar glutenins and gliadins alleles are found on similar loci of their genomes (Ghorbani *et al.*, 2013). High diversity of these proteins is found in different wheat cultivars and its wild and domestic relatives (Jaffaraghai *et al.*, 2013; Ghasemzade *et al.*, 2008; Tahernezhad *et al.*, 2012). Due to the significant role of the *D* genome in bread wheat quality, in this study, the genetic diversity and evolutionary relationships of *LMW-GS* genes at loci of *D* genomes in bread wheat (*Triticum aestivum* L.) and five Aegilops species with *D* genome were evaluated by using specific primers of LMW-GS of the *D* genome.

MATERIALS AND METHODS

Plant Materials

The plant materials consisted of 50 accessions from six species, namely, *Ae. tauschii* Cosson., *Ae. crassa* Boiss., *Ae. Juvenalis* (Thell.) Eig., *Ae. cylindrical* Host., *Ae. Vavilovi* (Zhuk.) Chennav., and *T. aestivum* L., all of which carry the *D* genome. These accessions were provided from the gene bank of the University of Tehran or collected from the natural habitats (Table 1).

SDS-PAGE Analysis

In order to study the diversity of LMW-GS alleles in evaluating species, the seed storage glutenin was separated on SDS-PAGE and the *B*-type LMW-GS scored on the gels. Then, for all accessions, the binary matrix of zero and one was used based on the absence or presence of bands, respectively.

DNA Extraction and PCR Amplification

Genomic DNA was extracted with CTAB (Cetyltrimethylammonium bromide) from young leaves (Saghai-Maroof *et al*., 1984). PCR amplifications were conducted in 20 µL reaction volume, containing a 10 µL master mix (prepared by Sina Clone Company), 0.5 µL of each primer, 0.5 µL genomic DNA and 8.5 µL double distilled water. Four specific primers for LMW-GS loci were selected according to Table 2. PCR conditions included primary denaturation at 95°C for 1 minute, followed by 37 cycles denaturation at 95°C for 1 minute, annealing at 45 to 60°C depending on the pair primer sets for 45 seconds, and extension at 72°C for 45 seconds, and a final extension at 72°C for 5 minutes. PCR products were separated on a 1% agarose gel. PCR products were separated on a 1% agarose gel.

PCR Product Sequencing

For each species, one accession was selected and PCR amplifications were conducted for each primer pair in 50 µL reaction volume. PCR products were used for sequencing after observing target bands on 1% agarose gel. DNA sequencing was performed by Fazza Pagoh Company. Comparison of the similarities of DNA

Table 1. Number of specimens and genome characteristics of the species.

No	Name/Accession no	Species	Genome	Origin
1	12160	*T. aestivum*	*AABBDD*	
2	12143	*T. aestivum*	*AABBDD*	
3	Morvarid	*T. aestivum*	*AABBDD*	
4	Niknegad	*T. aestivum*	*AABBDD*	
5	Voroby	*T. aestivum*	*AABBDD*	
6	Sirvan	*T. aestivum*	*AABBDD*	
7	Weebill-1	*T. aestivum*	*AABBDD*	
8	Estar	*T. aestivum*	*AABBDD*	
9	Tagan	*T. aestivum*	*AABBDD*	
10	Rooshan	*T. aestivum*	*AABBDD*	
11	1445	*Ae. cylindrica*	*CCDD*	West-Azarbayejan
12	1487	*Ae. cylindrica*	*CCDD*	West- Azarbayejan
13	1531	*Ae. cylindrica*	*CCDD*	Kermanshah
14	1692	*Ae. cylindrica*	*CCDD*	Alborz
15	1953	*Ae. cylindrica*	*CCDD*	Semnan
16	1966	*Ae. cylindrica*	*CCDD*	Ghazvin
17	1999	*Ae. cylindrica*	*CCDD*	Golestan
18	2080	*Ae. cylindrica*	*CCDD*	East-Azarbayejan
19	369	*Ae. cylindrica*	*CCDD*	Ardebil
20	318	*Ae. cylindrica*	*CCDD*	West-Azarbayejan
21	50136	*Ae. tauschii*	*DD*	Khorasan Razavi
22	1225	*Ae. tauschii*	*DD*	Kermanshah
23	2011	*Ae. tauschii*	*DD*	Mazenderan
24	844	*Ae. tauschii*	*DD*	Mazenderan
25	1985	*Ae. tauschii*	*DD*	Mazenderan
26	1366	*Ae. tauschii*	*DD*	Khorasan Razavi
27	1349	*Ae. tauschii*	*DD*	Khorasan Razavi
28	1773	*Ae. tauschii*	*DD*	Mazenderan
29	1769	*Ae. tauschii*	*DD*	Mazenderan
30	50067	*Ae. crassa*	*DDMM*	West-Azarbayejan
31	973	*Ae. crassa*	*DDMM*	Kermanshah
32	948	*Ae. crassa*	*DDMM*	Kermanshah
33	2060	*Ae. crassa*	*DDMM*	West-Azarbayejan
34	1490	*Ae. crassa*	*DDMM*	West-Azarbayejan
35	50021	*Ae. crassa*	*DDMM*	Markazi
36	1384	*Ae. crassa*	*DDMM*	Zanjan
39	1145	*Ae. crassa*	*DDMM*	Kermanshah
40	794	*Ae. juvenalis*	*DDMMUU*	Ilam
41	50040	*Ae. juvenalis*	*DDMMUU*	Kermanshah
42	911	*Ae. juvenalis*	*DDMMUU*	Kermanshah
43	473	*Ae. juvenalis*	*DDMMUU*	Ilam
44	1101	*Ae. juvenalis*	*DDMMUU*	Kermanshah
45	50122	*Ae. juvenalis*	*DDMMUU*	
46	908	*Ae. juvenalis*	*DDMMUU*	West-Azarbayejan
47	845	*Ae. tauschii*	*DD*	Mazenderan
48	1355	*Ae. vavilovi*	*DDMMSS*	Khorasan Razavi
49	753	*Ae. vavilovi*	*DDMMSS*	Fars
50	50131	*Ae. vavilovi*	*DDMMSS*	Khorasan Razavi
51	1347	*Ae. vavilovi*	*DDMMSS*	Khorasan Razavi

sequences were performed by using BLASTN in NCBI.

Statistical Analysis

Genetic diversity parameters for seed storage proteins were calculated by using GenAlex and PopGene softwares. In addition, the phylogenetic tree of 6 species was formed by using the MEGA 7 software and the Maximum Parsimony method, based on the target DNA sequences.

RESULTS

SDS-PAGE Analysis

SDS-PAGE analysis revealed overall 31 LMW-GS bands in all accessions of 6 species (Figure 1). In the recognized bands, the minimum number of effective alleles was 1.97 and the genetic diversity indices [Nei (h)] varied from 0.04 to 0.49 in different species. Maximum and minimum genetic diversity for LMW-GS bands were observed in *Ae. crassa* and *Ae. cylindrica*,

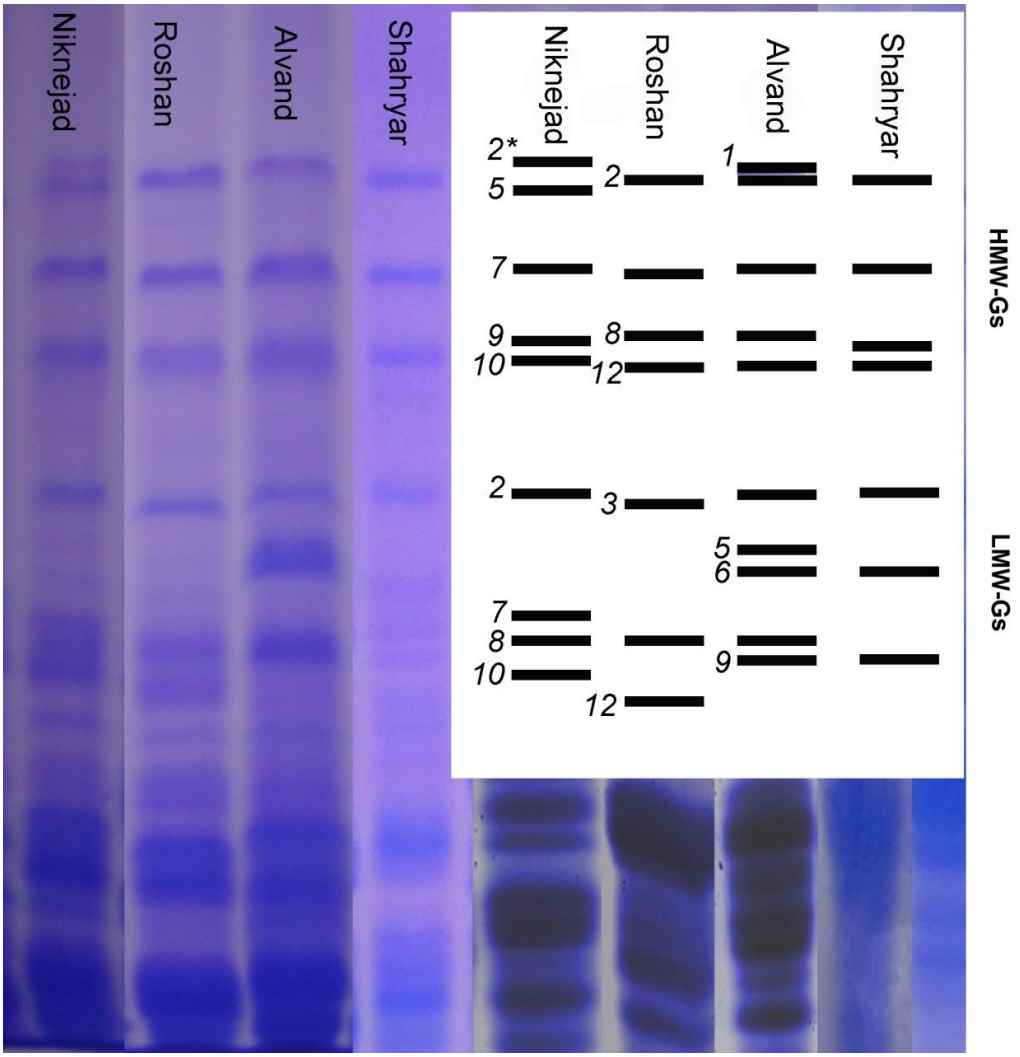

Figure 1. Bands identifying and naming order for LMW-GS subunits type *B* in hexaploid wheat accessions. Similar order is used for identifying and naming bands in other species.

respectively (Table 2).

In *Ae. tauschii* species, 14 LMW-GS bands were polymorphic from 31 observed bands, and 17 bands were not found, so, polymorphic ratio was 41.18% in this species. Usually 3 to 4 bands were observed in each accession. The minimum number of effective alleles was 1 and the maximum was 2, and the Shannon diversity index was at most 69% in *Ae. tauschii* accessions.

In a similar evaluation for HMW-GS diversity in 13 *Ae. tauschii* accessions, the number of effective alleles was 1.11 and the Shannon diversity index was 10% (Ghorbani *et al.*, 2013).

In *Ae. crassa* accessions, 19 polymorphic alleles were found, and the polymorphic ratio was 55.8%. In each accession, 4-6 bands were observed. The number of effective alleles varied from at least 1 to at most 2, and the average of the Shannon diversity index for LMW-GS bands was 66% in accessions of this species. In a similar study of HMW-GS diversity in 13 *Ae. crassa* accessions, the number of effective alleles was 1.07 and the Shannon diversity index was 4% (Ghorbani *et al.*, 2013).

In *Ae. cylindrica* accessions, the number of polymorphic alleles and polymorphism ratio for LMW-GS bands were 5 and 14.71%, respectively. In this species, 1 to 2 bands were observed in each accession and the number of effective alleles was 1 to 1.92. The Shannon Diversity Index for the observed bands was 67% in this species. In a similar study to evaluate HMW-GS diversity in 13 *Ae. cylindrica* accessions, the number of effective alleles and Shannon Diversity Index were reported as 1.06 and 3%, respectively (Ghorbani *et al.*, 2013).

In*Ae. juvenalis* accessions, 6 polymorphic alleles were observed, so, the polymorphic ratio was 17.65% and between 4 to 5 bands were observed in each accession. Six polymorphic alleles were observed in *Ae. vavilovi* accessions and 25 remaining bands were not observed, so, the polymorphic ratio was 17.65% in this population. In this species, 2 to 3 bands were observed in each accession. In the accessions of *T. aestivum*, 17 alleles were polymorphic, so, the polymorphism ratio was 50%, and 3 to 6 LMW-GS bands were observed in each accession.

A harmonic average of exchanging alleles in all populations suggested that 15 bands from among 31 observed bands showed high genetic flow between species. These included b1 - b3.3 - b5.1 - b5.2 - b6.1 - b6.2 - b7 - b7.2 - b8.1 - b9 - b10 -b10.1 -b12 -b13 –b14 and were common among most species. However, for some bands such as b2-b3.4-b4-b14.2, the genetic flow was very low and these bands were often limited to a specific species, which were discriminant between species. Bozorgmehr *et al.* (2014) identified 13 LMW-GS patterns of bands in Iranian wheat landraces by using some primers of *D* genome. Khoshro *et al.* (2010) evaluated low molecular weight protein diversity in *Ae. tauschii* species by using two primers of *D* genome, and identified 18 different alleles among accessions. They concluded that there was a significant diversity for low molecular weight glutenins

Table 2. Sample size, number of observed alleles (na), effective number of alleles (ne), heterozygosity index (h) and diversity index (I) of LMW-GS bands in different accessions of evaluated species.

Species	Sample Size	*na*	*ne*	*h*	*I*
T. aestivum	10	1/50±0/51	1/27±0/33	0/16±0/18	0/25±0/27
Ae. tauschii	10	1/41±0/50	1/24±0/34	0/14±0/19	0/21±0/28
Ae. cylindrica	10	1/15±0/36	1/08±0/22	0/05±0/13	0/08±0/19
Ae. crassa	8	1/56±0/50	1/34±0/38	0/20±0/20	0/30±0/29
Ae. juvenalis	7	1/18±0/39	1/15±0/35	0/08±0/18	0/11±0/25
Ae. vavilovi	4	1/18±0/39	1/11±0/23	0/07±0/15	0/10±0/22
All	49	1/91±0/29	1/25±0/24	0/18±0/13	0/30±0/18

among Aegilops species.

PCR Analysis and Sequencing

All specific primers for LMW-GS loci produced monomorphic fragments, except primer P3, which was polymorphic (Table 3).

The lengths of the obtained fragments were almost similar to the reported fragments in the previous research (Naghavi *et al.*, 2013, Vafadar *et al.*, 2016), and a fragment with similar length in previous reports was selected for the primer P3. In comparing the obtained sequences with the reported sequences at NCBI, 14 sequences showed over 95% coverage with the first sequence of a *LMW-GS* gene in NCBI (Table 4).

For the first Primer pairs (P1), a band with 600 nucleotides was observed on agarose gel, of which 542 to 576 nucleotides were sequenced among different species. The sequencing results showed that the obtained sequences in the *T. aestivum*, *Ae. cylindrica*, *Ae. crassa*, *Ae. juvenalis*, and *Ae. vavilovi* species were, respectively, 98, 91, 98%, 98, and 91% similar to the sequence of *T. aestivum* accession in NCBI database. The sequence from the *Ae. tauschii* accessions was 84% similar to the recorded sequence for an *Ae. tauschii* accession (Table 4). In a similar study, by using the same primer, in *T. aestivum*, *Ae. cylindrica*, *Ae. crassa*, and *Ae. tauschii* species a fragment with 606 nucleotides was sequenced, which was 99% similar to a LMW-GS locus registered in NCBI database (Naghavi *et al.*, 2013).

For the second pairs of Primers (P2), a band about 700 bps was observed, of which 631 to 676 nucleotides were sequenced among the different species. The sequences from the *T. aestivum*, *Ae. tauschii*, *Ae. juvenalis*, *Ae. vavilovi* species were, respectively, 98, 98, 91, and 98% similar to a sequence registered in the NCBI database for a *T. aestivum* accession. The sequence from the *Ae. cylindrica* species was 96% similar to the sequence of a *Ae. tauschii*, and the sequence form *Ae. crassa* species was 96% similar to the sequence of a *Triticum zhukovskyi* Menabde & Ericzjan accession, both registerd in the NCBI database (Table 4). In a similar study on LMW-GS loci, by using the same primers, a fragment with 606 bps length from *T. aestivum*, *Ae. cylindrica*, *Ae. crassa* and *Ae. tauschii* was sequenced, which was 99% similar to a gene sequence of LMW-GS, registered in NCBI database (Naghavi *et al.*, 2013).

Table 3. Sequence, annealing temperature, location site and length of the amplified fragments of primers.

Primer pairs	Sequence	Annealing temperature (°C)	Location	Length (bps)	Author
P1	*5'-ATGGAGACTAGATGCATCCCT-3'* *5'-AGATTTGGATGGAACCCTGAAC-3'*	60	1DS	600	H. Long *et al.*, 2005
P2	*5'-ATGGAGACTAGCTGCATCT-3'* *5'-CTGCAAAAAGGTACCCTGTA-3'*	57	1DS	700	H. Long *et al.*, 2005
P3	*5'-CCACATCCCTAGCTTGGAGAA -3'* *5'-ATGGTATTTGTTGTTGCGGA-3'*	57	1DS	479	S. VanCampen hout *et al.*, 1995
P4	*5'-CGTCTTGCTAGGTCGCAAATG-3'* *5'-CAGATTGACATCCACACAATGCC-3'*	60	1DS	626	T. M. Ikeda *et al.*, 2002

Table 4. The similarity between obtained sequences from six evaluated species which carry *D* genome and similar sequences registered in the NCBI database.

No[a]	Species	Seq_size	E-Value	BLAST	Similar seq in NCBI database
110	*T. aestivum*	550	0	100%	*Triticum aestivum* cultivar Keumkang haplotype *GluD3-21K2 Low-Molecular-Weight Glutenin Subunit* (LMW-GS) gene, complete cds
114	*Ae. cylindrica*	542	0	91%	*Triticum aestivum* clone TaE15038F08 low molecular weight glutenin mRNA, complete cds
125	*Ae. tauschii*	561	*E= 2e-149*	84%	*Aegilops tauschii* clone SC-10 *low molecular weight Glutenin subunit (glu-3)* gene, complete cds
130	*Ae. crassa*	560	0	98%	*Triticum aestivum Low Molecular Weight glutenin (AuLMW-m1)* gene, complete cds
138	*Ae. juvenalis*	576	0	98%	*Triticum aestivum Low Molecular Weight glutenin (AuLMW-m1)* gene, complete cds
149	*Ae. vavilovi*	310	*E= 3e-118*	91%	*Triticum aestivum* isolate PH82-2-2 Low Molecular Weight Glutenin Subunit (LMW-GS) pseudogene, partial sequenc
28	*T. aestivum*	664	0	98%	*Triticum aestivum* cultivar Keumkang haplotype *GluD3-42K1 Low-Molecular-Weight Glutenin Subunit (LMW-GS)* gene, complete cds
214	*Ae. cylindrica*	676	0	96%	*Aegilops tauschii* chromosome 1Ds *prolamin* gene locus, complete sequence
224	*Ae. tauschii*	644	0	98%	*Triticum aestivum* cultivar Keumkang haplotype *GluD3-42K1 Low-Molecular-Weight Glutenin Subunit (LMW-GS)* gene, complete cds
232	*Ae. crassa*	649	0	96%	*Triticum zhukovskyi* strain PI 355706 *LMW-m1 glutenin subunit (LMW-m1)* gene, complete cds
240	*Ae. juvenalis*	658	*E= 5e-56*	LOW 70%	*Triticum aestivum* cultivar Daqingmang *low-molecular-weight glutenin subunit Glu-A3 gene*, partial cds
249	*Ae. vavilovi*	511	*E= 6e-126*	83%	*Triticum aestivum* clone Y34AB-1 Low-Molecular-Weight Glutenin Subunit (LMW-GS) pseudogene, complete sequenc
34	*T. aestivum*	429	*E= 2e-154*	90%	*Aegilops cylindrica* isolate TN0775 *Low Molecular Weight glutenin subunit t128 (LMW)* gene, partial cds
320	*Ae. cylindrica*	432	0	95%	*Aegilops cylindrica* isolate TN0775 *Low Molecular Weight glutenin subunit t128 (LMW)* gene, partial cds
326	*Ae. tauschii*	437	0	98%	*Triticum aestivum* cultivar Jinghong 5 *Low-Molecular-Weight Glutenin Subunit (LMW-GS)* gene, LMW-GS-D3-575 allele, complete cds
334	*Ae. crassa*	426	*E= 1e-176*	94%	*Aegilops crassa* isolate TN0744 *Low Molecular Weight glutenin subunit t128 (LMW)* gene, partial cds
341	*Ae. juvenalis*	406	*E= 4e-150*	91%	*Aegilops cylindrica* isolate TN0775 *Low Molecular Weight glutenin subunit t128 (LMW)* gene, partial cds
349	*Ae. vavilovi*	437	0	98%	*Aegilops tauschii* isolate TN0698 *Low Molecular Weight glutenin subunit t128 (LMW)* gene, partial cds
44	*T. aestivum*	580	0	98%	*Triticum aestivum LMW-GS P-32 (GluD3-3)* gene, GluD3-32 allele, complete cds
419	*Ae. cylindrica*	553	0	99%	*Triticum aestivum LMW-GS P-21 (GluD3-2)* gene, GluD3-22 allele, complete cds
427	*Ae. tauschii*	579	0	97%	*Triticum aestivum* cultivar Jiangdongmen *low-molecular-weight glutenin subunit Glu-D3* gene, complete cds
432	*Ae. crassa*	547	0	98%	*Aegilops tauschii Pt-37 protein (GluDt3-3)* gene, GluDt3-37 allele, complete cds
443	*Ae. juvenalis*	427	*E= 1e-86*	84%	*Triticum aestivum* Low Molecular Weight glutenin subunit LMW-Wan49 pseudogene, complete sequence
446	*Ae. vavilovi*	532	0	98%	*Aegilops tauschii Pt-37 protein (GluDt3-3)* gene, GluDt3-37 allele, complete cds

[a] The first digit is primer number and two other digits show the number of accessions.

For the third pairs of Primers (P3), a band about 475 bps was observed, of which 398 to 443 nucleotides were sequenced among the various species. The sequences from the *T. aestivum*, *Ae. cylindrica* and *Ae. juvenalis* species were, respectively, 90, 95, and 91% similar to a sequence registered in the NCBI database for an *Ae. cilindrica* accession. The sequence from the *Ae. tauschii* was 98% similar to the same sequence of a *T. aestivum* accession registered in the NCBI. Besides, the sequences from the *Ae.crassa* were 94% similar to the same sequence in a *Ae. crassa* accession registered in the NCBI, and the sequence from the *Ae.vavilovi* was 98% similar to the same sequence in a *Ae. tauschii* accession registered in the NCBI database (Table 4). In a similar study by using the same primer, a fragment with 480 bps length from *T. aestivum*, *Ae. cylindrica*, *Ae. crassa* and *Ae. tauschii* was sequenced, which was 98% similar to a gene sequence of LMW-GS, registered in NCBI database (Naghavi *et al.*, 2013). In another study, by using this primer in some accessions of *Ae. tauschii* species, 4 alleles with 550 to 650 nucleotides long were found (Khoshro *et al.*, 2010).

For the fourth pairs of Primers (P4), a band about 625 bps was observed, of which 398 to 580 nucleotides were sequenced among different species. The sequences from *T. aestivum*, *Ae. cylindrica*, *Ae. tauschii* and *Ae. juevanalis* species were, respectively, 98, 99, 97, and 84% similar to a sequence registered in the NCBI database form a *T. aestivum* accession, and the sequences from *Ae. crassa* and *Ae. vavilovi* species were, respectively, 97 and 98% similar to a sequence registered in the NCBI database form a *Ae. tauschii* accessions (Table 4). In a similar study by using the same primer, a sequence with 660 nucleotides length from *T. aestivum*, *Ae. cylindrica*, *Ae. crassa* and *Ae. tauschii* was 98% similar to a gene sequence of LMW-GS, registered in NCBI database (Naghavi *et al.*, 2013). In another study, by using the same primer on some accessions of *Ae. tauschii*, 3 alleles with 650 to 750 nucleotides lengths were found (Khoshro *et al.*, 2010).

The phylogenetic tree based on the diversity of LMW-GS bands clustered two species; *Ae. vavilovi* and *Ae. cylindrica* in one group, and the species *Ae. juvenalis* and *T. aestivum* in a separate group. *Ae. tauschii* was also in a further branch, and finally the *Ae. crassa* species was in a branch apart from the other five species (Figure 2-a).

In a study, by using diversity of HMW-GS proteins, among several Aegilops species, *Ae. tauschii*, *Ae. cylindrica*, and *Ae. crassa* species were located in separated clusters (Ghorbani *et al.*, 2013). In another study, in order to investigate the genetic relationships among four species of *T. aestivum*, *Ae. tauschii*, *Ae. cylindrica*, and *Ae. crassa* by using SSR markers of *D* genome, *Ae. tauschii* and *Ae. cylindrica* were located in the same cluster and *T. aestivum* species was located in a different cluster close to them. However, *Ae. crassa* was located in a separate cluster far away from them (Naghavi *et al.*, 2009).

Based on the DNA sequence of primer pairs P1 on the second phylogenetic tree, *Ae. crassa* and *Ae. juvenalis* were closed in the same group, and *T. aestivum* was at less distance and followed by *Ae. cylindrica*, *Ae. tauschii* and *Ae. vavilovi*, respectively, at farther distance (Figure 2-b). Based on the DNA sequence of primer pairs P2 on the third phylogenetic tree, species clustered in 3 groups. *T. aestivum* and *Ae. tauschii* and *Ae. juvenalis* were in the same group and *Ae. cylindrica* and *Ae. vavilovi* were in a separate group close to the first one, and *Ae. crassa* was also in a group, apart from others (Figure 2-c). Based on the DNA sequence of primer pairs P3 on the fourth phylogenetic tree, the *T. aestivum*, *Ae. tauschii* and *Ae. juvenalis* were clustered in the same group, two other species, *Ae. cylindrica* and *Ae. vavilovi*, were located in a separate group and the *Ae. crassa* was in a dedicated group, also apart from others

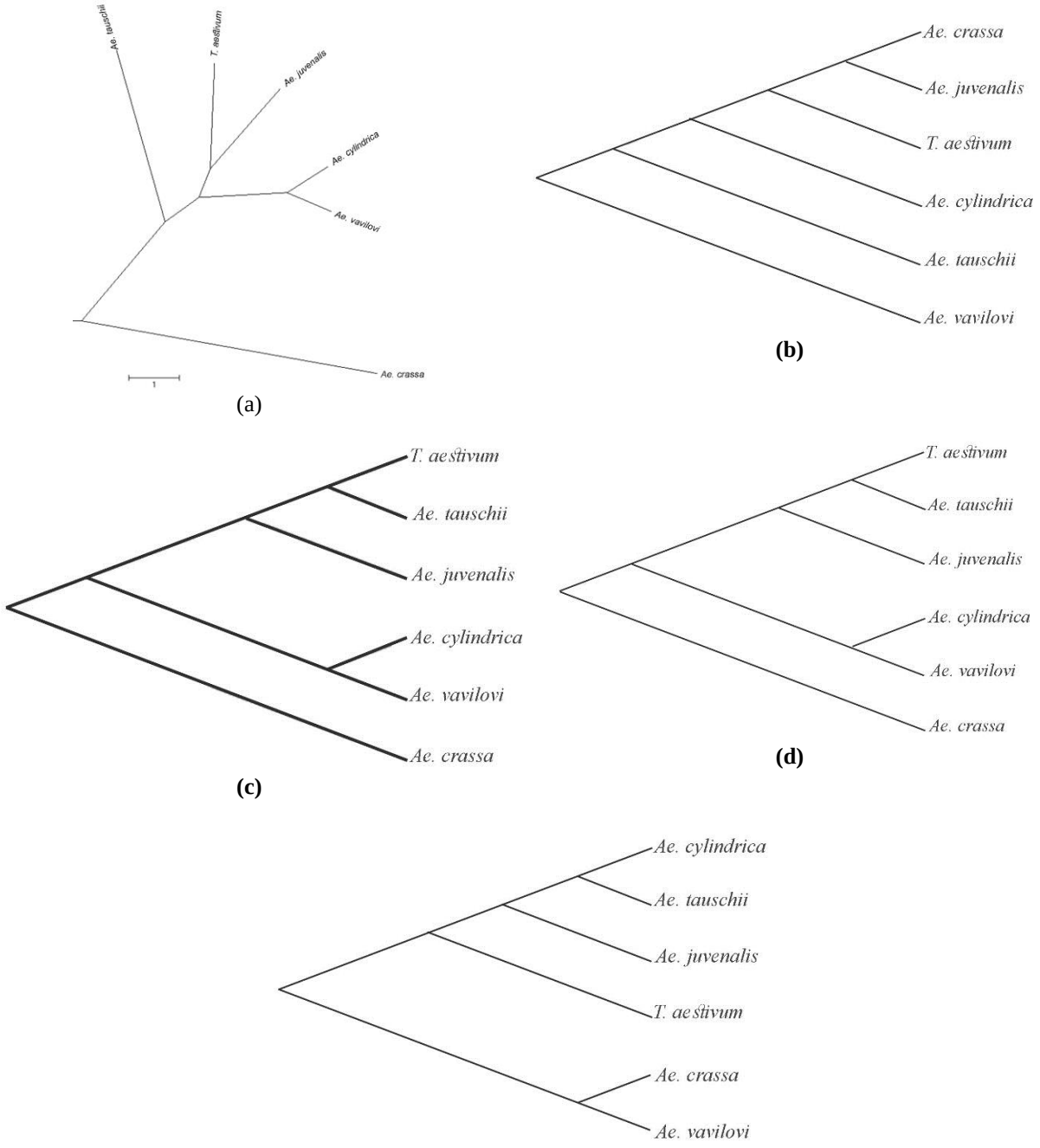

Figure 2. The phylogenetic relationship tree using the Maximum Parsimony method, (a) among the species studied based on the diversity of LMW-GS bands, identified by SDS-PAGE method, (b) of the evaluated species based on the DNA sequence of *LMW-GS* gene amplified by Primer No. 1, (c) of the evaluated species based on the DNA sequence of *LMW-GS* gene amplified by Primer No. 2, (d) of the evaluated species based on the DNA sequence of *LMW-GS* gene amplified by Primer No. 3, (e) of the evaluated species based on the DNA sequence of *LMW-GS* gene amplified by Primer No. 4

(Figure 2-d). Based on the DNA sequence of primer pairs P4 on the fifth phylogenetic tree, the *Ae. cylindrica* and *Ae. tauschii* were clustered in a group and two species, *Ae. juvenalis* and *T. aestivum* were located close to them. Two other species, i.e. *Ae. crassa* and *Ae. vavilovi*, were clustered in another group (Figure 2-e). In a similar study in a drawn phylogenetic tree based on the DNA sequence of LMW-GS loci, *Ae. cylindrica*, *Ae. tauschii* and *Ae. crassa* were the closest species to *T. aestivum* (Naghavi *et al*., 2013).

DISCCUSION

The results of this study indicate a great diversity for LMW-GS proteins among species with *D* genome. It has been reported that low molecular weight glutenins make about one-third of seed storage proteins and 60% of seed glutenin (Bietz and Wall, 1973). It has also been shown in several studies that allelic diversity for LMW-GS loci is associated with good dough quality of bread wheat cultivars (Gupta *et al.*, 1989, 1994) and durum wheat (Pogna *et al.*, 1990; Ruiz and Carrilo, 1993). Therefore, the high diversity detected in species with *D*-genome, potentially can be used as a valuable source in breeding programs of bread making quality in bread wheat cultivars.

Overall, based on the phylogeny results of the diversity of seed storage proteins and LMW-GS sequences, bread wheat (*T. aestivum*) was more similar to *Ae. tauschii* species and usually clustered in the same groups, while the *Ae. crassa* species is more distant than these two species and clustered in a separate group.

Jafaraghaee *et al.* (2007) studied the homology of *D* genomes, based on chiasma frequencies in metaphase of meiosis of interspecific hybrid plants, in four species containing *D* genome including *T. aestivum*, *A. tauschii*, and *Ae. cylindrica*. They found that the genome of *Ae. tauschii* species was similar to the *D* genome of bread wheat. Therefore, the chromosomes of the two species were able to pair with each other and form an average of 11.9 chiasma per cell. However, the *Ae. cylindrica* species chromosomes were less similar to bread wheat chromosomes and an average of 7.37 chiasma per cell were observed in between them. The similarity of *Ae. crassa* chromosomes with bread wheat was less than others and the chromosomes of this species made only 3.43 chiasma with bread wheat chromosomes. Cassidy *et al*. (1988) studied the diversity of the *D* genome in *T. aestivum* and *Ae. tauschii* by Polymorphism in the lengths of restriction fragments at 53 single-copy loci, the rRNA locus Nor3, and the high-molecular-weight glutenin locus Glu1. They suggested *Ae. tauschii* subsp *strangulata* as donor of the *D* genome of *T. aestivum*.

Considering the results of this study, based on the diversity of seed storage proteins and sequences revealed by primers 2 and 3, *Ae. cylindrca* and *Ae. Vavilovi* clustered in the same group. Also, based on the diversity of seed storage proteins and sequences revealed by primers 2, 3, and 4, the *Ae. juvenils* and *Ae. tauschii* species were grouped together in the same cluster. Badaeva *et al*. (2001) studied six polyploid Aegilops species containing the *D* genome by *C*-banding and Fluorescence *In Situ* Hybridization (FISH). They found that the *Ae. cylindrica* chromosomes were identical to those of the parental species. Also, the *D* genome of *Ae. crassa* was more similar to the *D* genome of *Ae. ventricosa* Tausch than to the *D* genome of *Ae. tauschii*. Both genomes of *Ae. crassa* were significantly modified as the result of chromosomal rearrangements and redistribution of highly repetitive DNA sequences. Hexaploid *Ae. crassa* and *Ae. vavilovii* arose from the hybridization of chromosomal type *N* of tetraploid *Ae. crassa* with *Ae. tauschii* and *Ae. searsii* (Feldman and Kislev), respectively. The highest level of genome modification in *Ae. juvenalis* indicate that it is the oldest hexaploid species in this group. No chromosome

changes relative to the parental species were detected in *Ae. vavilovii*.

Bordbar *et al*. (2011) analyzed genetic diversity and phylogenetic relationships among *D* genome in bread wheat and some relatives of the genus *Aegilops* SSR, nuclear rDNA ITS, and chloroplast trnL-F markers. They revealed two different *Ae. tauschii* gene pools, and a close relationship among *Ae. crassa*, *Ae. juvenalis*, and *Ae. vavilovii*. Also, they found close relationships among the *D* genome of *Aegilops* species and *T. aestivum*.

Finally, only primer No. 1 was unable to distinguish appropriately among species. But, other primers, as well as seed storage proteins, were suitable discriminant between species to show the relationship between species carrying the *D* genome. Therefore, due to the similarity among LMW-GS sequences in *Ae. juvenalis*, *Ae. cylindrical*, and *Ae. tauschii* to the bread wheat and the importance of these proteins in the bread making quality of wheat cultivars, these species can be used as a potential source in breeding programs of bread wheat cultivars.

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32.

بررسی مولکولی ژن های کد کننده گلوتنین های با وزن مولکولی پائین در گونه های گندمیان حامل ژنوم D

م. گلدسته، ا. مهرگان، م. ر. نقوی، و ت. نژادستاری

چکیده

زیرواحدهای گلوتنین با وزن ملکولی پائین LMW-Gs که توسط مکان ژنی Glu-3 بر روی بازوی کوتاه کروموزوم‌های هومیولوگ گندم قرار دارند، دارای نقش مهمی در کیفیت نانوایی محصول گندم هستند. یکی از سه ژنوم اصلی گندم نان ژنوم D است که اهمیت ژن‌های واقع بر این ژنوم در کیفیت محصول گندم مورد توجه به نژادگران قرار دارد. علاوه بر گندم نان (*Triticum aestivum*) چندین گونه دیگر در جنس آژیلوپس از جمله *Ae.cylindrica*، *Ae.tauschii*، *Ae.crassa*، *Ae.juvenalis*، *Ae.vavilovi* نیز حامل این ژنوم هستند. در این مطالعه رابطه فیلوژنتیکی میان گونه های آژیلوپس حامل ژنوم D و گندم نان براساس توالی ژن‌های کد کننده پروتئین‌های ذخیره‌ای با وزن مولکولی پائین مورد بررسی قرار گرفته است. نتایج بیانگر آن بود که تنوع فوق‌العاده‌ای برای این نوع پروتئین‌ها در گونه‌های خویشاوند گندم نان در دسترس قراردارد.وجود تعداد زیادی باندهای پروتئینی مشابه در میان گونه ها بیانگر آن بود که این گونه ها رابطه نزدیکی با گندم نان دارند. سه تا از پرایمرهای مربوط به گلوتنین های با وزن مولکولی پائین قادر بودند روابط میان گونه ها را نشان دهند. نتایج بیانگر روابط نزدیک میان گونه *Ae. tauschii* و گندم نان بود. گونه *Ae. crassa* در فاصله دورتری از گندم نان قرار داشت. همچنین نتایج بیانگر رابطه نزدیک میان *Ae. cylindrica, Ae. Juvenalis* و *Ae. Vavilovi* بود. روابط نزدیک میان این گونه‌ها با گندم نان و تنوع بالای پروتئین‌ها، این خویشاوندان وحشی را بعنوان یک منبع تنوع بالقوه برای استفاده در برنامه‌های به‌نژادی گندم مطرح می نماید.