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

M. Goldasteh¹, I. Mehregan¹*, M. R. Naghavi², and T. Nejadsattari¹

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 genes 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. aestivum) 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. cylindrica* 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-Marooof 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.
Table 1. Number of specimens and genome characteristics of the species.

<table>
<thead>
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<th>No</th>
<th>Name/Accession no</th>
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<td>DDMMSS</td>
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</table>
sequences were performed by using BLASTN in NCBI.

**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 \([\text{Nei (h)}]\) 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*,

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**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.
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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sample Size</th>
<th>na</th>
<th>ne</th>
<th>h</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. aestivum</em></td>
<td>10</td>
<td>1/50±0/51</td>
<td>1/27±0/33</td>
<td>0/16±0/18</td>
<td>0/25±0/27</td>
</tr>
<tr>
<td><em>Ae. tauschii</em></td>
<td>10</td>
<td>1/41±0/50</td>
<td>1/24±0/34</td>
<td>0/14±0/19</td>
<td>0/21±0/28</td>
</tr>
<tr>
<td><em>Ae. cylindrica</em></td>
<td>10</td>
<td>1/15±0/36</td>
<td>1/08±0/22</td>
<td>0/05±0/13</td>
<td>0/08±0/19</td>
</tr>
<tr>
<td><em>Ae. crassa</em></td>
<td>8</td>
<td>1/56±0/50</td>
<td>1/34±0/38</td>
<td>0/20±0/20</td>
<td>0/30±0/29</td>
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<tr>
<td><em>Ae. juvenalis</em></td>
<td>7</td>
<td>1/18±0/39</td>
<td>1/15±0/35</td>
<td>0/08±0/18</td>
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<td><em>Ae. vavilovi</em></td>
<td>4</td>
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<td>1/11±0/23</td>
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<td>1/25±0/24</td>
<td>0/18±0/13</td>
<td>0/30±0/18</td>
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</table>
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. juvénalis*, 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. juvénalis*, *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 registered 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>
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<th>Location</th>
<th>Length (bps)</th>
<th>Author</th>
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<td>H. Long et al., 2005</td>
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<td>700</td>
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<td>P3</td>
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<td>S. VanCampen hout et al., 1995</td>
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<td>60</td>
<td>1DS</td>
<td>626</td>
<td>T. M. Ikeda et al., 2002</td>
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Table 3. Sequence, annealing temperature, location site and length of the amplified fragments of primers.
The similarity between obtained sequences from six evaluated species which carry D genome and similar sequences registered in the NCBI database.

<table>
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<tr>
<td>1</td>
<td><em>T. aestivum</em></td>
<td>550</td>
<td>0</td>
<td>100%</td>
<td><em>Triticum aestivum</em> cultivar Keumkang haplotype GluD3-21K2 Low-Molecular-Weight Glutelin Subunit (LMW-GS) gene, complete cds</td>
</tr>
<tr>
<td>11</td>
<td><em>Ae. cylindrica</em></td>
<td>542</td>
<td>0</td>
<td>91%</td>
<td><em>Triticum aestivum</em> cultivar TaE15038F08 low molecular weight glutelin mRNA, complete cds</td>
</tr>
<tr>
<td>2</td>
<td><em>Ae. tauschii</em></td>
<td>561-560</td>
<td>E= 2e-149</td>
<td>84%</td>
<td><em>Aegilops tauschii</em> clone SC-10 low molecular weight Glutelin subunit (glu-3) gene, complete cds</td>
</tr>
<tr>
<td>3</td>
<td><em>Ae. crassa</em></td>
<td>576</td>
<td>0</td>
<td>98%</td>
<td><em>Triticum aestivum</em> Low Molecular Weight glutulin (AeLMW-m1) gene, complete cds</td>
</tr>
<tr>
<td>4</td>
<td><em>Ae. juvenalis</em></td>
<td>310</td>
<td>0</td>
<td>98%</td>
<td><em>Triticum aestivum</em> Low Molecular Weight glutulin (AeLMW-m1) gene, complete cds</td>
</tr>
<tr>
<td>5</td>
<td><em>Ae. vavilovi</em></td>
<td>564</td>
<td>E= 3e-118</td>
<td>91%</td>
<td><em>Triticum aestivum</em> isolate PH82-2-2 Low Molecular Weight Glutelin Subunit (LMW-GS) pseudogene, partial cds</td>
</tr>
<tr>
<td>6</td>
<td><em>T. aestivum</em></td>
<td>664</td>
<td>0</td>
<td>98%</td>
<td><em>Triticum aestivum</em> cultivar Keumkang haplotype GluD3-42K1 Low-Molecular-Weight Glutelin Subunit (LMW-GS) gene, complete cds</td>
</tr>
<tr>
<td>7</td>
<td><em>Ae. cylindrica</em></td>
<td>696</td>
<td>0</td>
<td>96%</td>
<td><em>Aegilops tauschii</em> chromosome 1Ds prolamin gene locus, complete sequence</td>
</tr>
<tr>
<td>8</td>
<td><em>Ae. tauschii</em></td>
<td>694</td>
<td>0</td>
<td>98%</td>
<td><em>Triticum aestivum</em> cultivar Keumkang haplotype GluD3-42K1 Low-Molecular-Weight Glutelin Subunit (LMW-GS) gene, complete cds</td>
</tr>
<tr>
<td>9</td>
<td><em>Ae. crassa</em></td>
<td>699</td>
<td>0</td>
<td>96%</td>
<td><em>Triticum zhukovskyi</em> strain PI 355706 LMW-m1 glutelin subunit (LMW-m1) gene, complete cds</td>
</tr>
<tr>
<td>10</td>
<td><em>Ae. juvenalis</em></td>
<td>98</td>
<td>E= 5e-56</td>
<td>LOW 70%</td>
<td><em>Triticum aestivum</em> cultivar Daqingmang low-molecular-weight glutelin subunit Glu-A3 gene, partial cds</td>
</tr>
<tr>
<td>11</td>
<td><em>Ae. vavilovi</em></td>
<td>511</td>
<td>E= 6e-126</td>
<td>83%</td>
<td><em>Triticum aestivum</em> clone Y34AB-1 Low-Molecular-Weight Subunit (LMW-GS) pseudogene, complete sequence</td>
</tr>
<tr>
<td>12</td>
<td><em>T. aestivum</em></td>
<td>422</td>
<td>E= 2e-154</td>
<td>90%</td>
<td><em>Aegilops cylindrica</em> isolate TN0775 Low Molecular Weight Glutelin subunit 1128 (LMW) gene, partial cds</td>
</tr>
<tr>
<td>13</td>
<td><em>Ae. cylindrica</em></td>
<td>417</td>
<td>0</td>
<td>95%</td>
<td><em>Aegilops cylindrica</em> isolate TN0775 Low Molecular Weight Glutelin subunit 1128 (LMW) gene, partial cds</td>
</tr>
<tr>
<td>14</td>
<td><em>Ae. tauschii</em></td>
<td>437</td>
<td>0</td>
<td>98%</td>
<td><em>Aegilops crassa</em> isolate TN0744 Low Molecular Weight Glutelin subunit 1128 (LMW) gene, partial cds</td>
</tr>
<tr>
<td>15</td>
<td><em>Ae. crassa</em></td>
<td>438</td>
<td>E= 1e-176</td>
<td>94%</td>
<td><em>Aegilops crassa</em> isolate TN0744 Low Molecular Weight Glutelin subunit 1128 (LMW) gene, partial cds</td>
</tr>
<tr>
<td>16</td>
<td><em>Ae. juvenalis</em></td>
<td>490</td>
<td>E= 4e-150</td>
<td>91%</td>
<td><em>Aegilops crassa</em> isolate TN0775 Low Molecular Weight Glutelin subunit 1128 (LMW) gene, partial cds</td>
</tr>
<tr>
<td>17</td>
<td><em>Ae. vavilovi</em></td>
<td>437</td>
<td>0</td>
<td>98%</td>
<td><em>Aegilops tauschii</em> isolate TN0698 Low Molecular Weight Glutelin subunit 1128 (LMW) gene, partial cds</td>
</tr>
<tr>
<td>18</td>
<td><em>T. aestivum</em></td>
<td>560</td>
<td>0</td>
<td>99%</td>
<td><em>Triticum aestivum</em> LMW-GS P-21 (GluD3-2) gene, GluD3-32 allele, complete cds</td>
</tr>
<tr>
<td>19</td>
<td><em>Ae. cylindrica</em></td>
<td>553</td>
<td>0</td>
<td>99%</td>
<td><em>Triticum aestivum</em> LMW-GS P-21 (GluD3-2) gene, GluD3-32 allele, complete cds</td>
</tr>
<tr>
<td>20</td>
<td><em>Ae. tauschii</em></td>
<td>575</td>
<td>0</td>
<td>97%</td>
<td><em>Triticum aestivum</em> cultivar Jiangledongmen low-molecular-weight glutulin subunit Glu-D3 gene, complete cds</td>
</tr>
<tr>
<td>21</td>
<td><em>Ae. crassa</em></td>
<td>567</td>
<td>0</td>
<td>98%</td>
<td><em>Aegilops tauschii</em> Pr-37 protein (GluD3-3) gene, GluD3-37 allele, complete cds</td>
</tr>
<tr>
<td>22</td>
<td><em>Ae. juvenalis</em></td>
<td>577</td>
<td>E= 1e-86</td>
<td>84%</td>
<td><em>Triticum aestivum</em> Low Molecular Weight glutelin subunit LMW-Wan49 pseudogene, complete sequence</td>
</tr>
<tr>
<td>23</td>
<td><em>Ae. vavilovi</em></td>
<td>555</td>
<td>0</td>
<td>98%</td>
<td><em>Aegilops tauschii</em> Pr-37 protein (GluD3-3) gene, GluD3-37 allele, complete cds</td>
</tr>
</tbody>
</table>

* 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. juvenalis* species were, respectively, 98, 99, 97, and 84% similar to a sequence registered in the NCBI database for 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 for an *Ae. tauschii* accession (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).

**DISCUSSION**

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 stranugalata 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. cylindrica* 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. vavilovi* 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.

**REFERENCES**


32.
بررسی مولکولی زن‌های کد کننده گلوتین‌های با وزن مولکولی پایین در گونه

D

های گندم‌های حامل زن‌های D

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

چکیده

زمینه

ِبی گلًتىيه بب يزن ملکًلي پبئيه LMW-Gs کٍتًظط مکبن شوي Glu-

-3 بر روي Gs
gen در D

اميت شن بب زیت کًتبٌ کريمًزیم َبی ًَميًلًگ
gیا بگىدم قرار
dارود، دارای وقش مُمي در کيفيت وبوًايي محصًل گىدم َعتىد.

یکي از ظٍ شوًم اصلي گىدم وبن شوًم

D

اظت کٍ اَميت شن

َبی ياقع بر ايه شوًم در کيفيت محصًل گىدم َعتىد.

Ae.crasa Ae.tauschii Ae.cylindrica

* Ae.vavilovi Ae.juvenalis

نزی حامی این زن‌های D

وگي وبن براظبض تًالي شن

َبی ذخيرٌ

ای بب يزن مًلکًلي پبئيه مًرد بررظي قرا

ر گرفتٍ اظت.

وتطج بيبوگر آن بًد کٍ تىًع فًق

العبدٌ

ای برای ايه وًع

پريتئيه

َب در گًوٍ

َب خًيشبيود گىدم وبن

در دظترض قراردارد.

يجًد تعداد زيبدی بب گىدم وبن دارود. ظٍ تب از پرا

يمرَبی مربًط بٍ گلًتىيه َبی بب يزن مًلکًلي پبئيه قبدر بًدود ريابط ميبن گًوٍ َب را وشبن دَىد. وتبيج بيبوگر

رباط وسديک ميبن

Ae. tauschii

ي گىدم وبن بًد.

رواط وسديک ميبن

Ae. vavilovi Ae. Juvenalis

مربوط به گلوتین‌های با وزن مولکولی پایین قادر بودند وربط میان گونه‌ها را نشان دهد. نتایج بیانگر

Ae. crassa

رواط نزدیک میان گونه‌ها از

Ae. tauschii

و Ae. cylindrica, Ae. Juvenalis

گندم نان قرار داشت. همچنین نتایج بیانگر رابطه نزدیک میان

Ae. Vavilovi

خویشاندند وخشی را بعنوان یک منبع منبع تنواع بالقوه برای استفاده در برنامه‌های به‌نژادی گندم مطرح می

نماید.