Assessment of Genetic Relation for Different Ploidy Levels of Aegilops and Triticum Possessing Different Genome-Bearing Species using Start Codon Target Marker

Nariman Salih Ahmad (nariman.ahmad@univsul.edu.iq)
University of Sulaimani https://orcid.org/0000-0003-4712-9074

Research Article

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Posted Date: July 21st, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3171739/v1

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Assessment of Genetic Relation for Different Ploidy Levels of *Aegilops* and *Triticum* Possessing Different Genome-Bearing Species using Start Codon Target Marker

N S Ahmad

College of Agricultural Engineering Sciences, University of Sulaimani, Sulaimani, Kurdistan of Iraq

Abstract:
The Fertile Crescent of Southwest Asia holds high genetic diversity of crop species of the grass tribe Triticeae, which provide staple food for the majority human population. Genetic diversity and their relationships were studied among 51 accessions from *Aegilops* and *Triticum* species from Iraqi Kurdistan. Eighteen SCoT markers were used to generate a total of 139 alleles. The discriminating power of the markers was assessed with polymorphism information content (PIC) and marker index (MI), that valued at 0.56 and 3.7, respectively. These results indicate the informativeness of the markers used for studying genetic diversity among the *Aegilops* and *Triticum* species. A high level of diversity was identified among the accession, to separate three distinct groups based on the SCoT data. All the accessions from different species with an ‘A’ genome were clustered together in a group, including *Aegilops* tauschii (possessing D genome). While the other *Aegilops* (U genome) were clustered in the other two groups. Analysis of molecular variance (AMOVA) partitioned 62% of variability among populations, while 38% of variation was considered between accessions within populations. Principal coordinate analysis (PCoA) for SCoT data showed that the first two components clarified 54.73% of the total variation, indicating consistent patterns of genetic relationships between the accessions. Genetic relationships inferred from UPGMA dendrogram analysis were matched with the PCoA, indicating that the grouping patterns of the accessions were in agreement with their botanical classifications. These results obtained could allow for future insight into wheat breeding programs.

Keywords: Genetic diversity, Start Codon Target Marker, *Aegilops*, *Triticum*, PCoA, Polymorphic information technique

Introduction:
*Aegilops-Triticum* group involves diploid, tetraploid and hexaploid species and all cultivated forms of this group belong to the genus *Triticum* and *Aegilops* are closely related to wheat, and they include forms with different ploidy levels (Spetsov et al. 2006). Genetic drift of germplasm in cultivated wheat is one of the motivations for studying genetic diversity in its wild relatives (Aalami et al. 2014). Studying the genetic relationships among *Aegilops* species (including the wild relatives of cultivated wheat) is important for broadening the cultivated wheat gene pool. They are potential sources of genetic variation and numerous unique alleles for breeding purposes of *T. aestivum* and can serve as a secondary gene pool of this species (Ghobadi et al. 2021; Ivanizs et al. 2019). The introduction of genes from these genera can contribute to improving quality (Ahmadi and Pour-Aboughadareh 2015), yield performance (Mahjoub et al. 2016; Schneider et al. 2008) and resistance to biotic (Alsaleh et al. 2022) and abiotic stresses (Suneja et al. 2019; Trono and Pecchioni 2022).

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Wild relatives of common wheat have been widely distributed in the majority of Iraqi Kurdistan, being one of the primary centers of distribution of wild wheat in the Fertile Crescent (Özkan et al. 2011). Analysis of the genetic diversity of the wheat gene pool provides the information basis for the conservation and improvement of this crop (Pour-Aboughadareh et al. 2017). Genomic DNA markers could be used properly to explore the genetic variation and the degree of relatedness between the Aegilops and Triticum species posing different ploidy levels. Different marker techniques have been applied, including random DNA markers to functional and gene-targeted markers, to study the genetic relationship between Triticum and Aegilops (Asaf et al. 2022; Poczai et al. 2013; Sansaloni et al. 2020). Start codon-targeted (SCoT) polymorphism as a novel, simple, and reliable gene-targeted marker system are among the markers used (Nouri et al. 2021; Xia et al. 2022). The system is based on the short-conserved region and can use a single primer designed to anneal flanking regions of the translation initiation codon (ATG) on both DNA strands (Collard and Mackill 2009). SCoT markers with high polymorphism and high efficiency have been successfully applied in different crop species such as peanut (Xiong et al. 2011), Maize (Vivodík et al. 2016), durum wheat (Etminan et al. 2016) and wheat-Aegilops (Pour-Aboughadareh et al. 2018).

In the present study, genetic diversity and relationships were studied at different levels of genome-bearing species of Aegilops and Triticum. SCoT marker was used to understand the extent and pattern of diversity among diploid and polyploid wild populations from Aegilops and Triticum sampled from Kurdistan of Iraq. Detection of their relationship with wheat cultivars was investigated to facilitate the incorporation of novel genes from wild relatives into domestic wheat cultivars.

Materials and Methods:

**Plant materials:** A total of 51 genotypes were included in this investigation. Forty-nine accessions representing different ploidy levels of nine Aegilops and three Triticum species were collected from different areas of Kurdistan, Iraq (Fig 1 and Table 1).
Two bread wheat cultivars of Aras and Sabir-beg (*Triticum aestivum* L.) were included as a common wheat crop in the Kurdistan of Iraq. The accessions represent different geographical distributions and
morphological characters of *Aegilops* and *Triticum* species in the region. Sorting and classification of the collected accessions were performed at HKS Herbarium, Forests and Rangelands Research Department, Kurdistan Agricultural and Natural Resources, Research and Education Center, AREEO, Sanandaj, Iran.

**DNA extraction:** Total genomic DNA was extracted from the young fresh leaves of a two-week-old seedling. Up to 12 individuals per accession were pooled for DNA purification, using BETA Bayern extraction Kit (Germany), following manufacturers’ instructions. The quality and quantity of the DNA were assessed on agarose gel (1% w/v), in 1×TBE buffer, using known concentrations of DNA lambda.

**PCR amplification:** Out of 22 SCoT primers previously described (Gholamian et al. 2019; Khodaee et al. 2021; Pour-Aboughadareh et al. 2020), eighteen were amplified properly and applied to screen the current accessions. PCR amplification were carried out in a standard PCR machine (MultiGene OptiMax Thermal Cycler, Labnet Company), at the laboratories of the College of Agricultural Engineering Sciences, University of Sulaimani. PCR amplifications for all the primers was carried out in a 20μl reaction volume, containing a mixture of 10μl of amaR 2X PCR mix, 2μl of DNA (15ng/μl), 2μl of each forward reverse primer (20ng/μl), completed to the final reaction volume with double distilled water. The running program was set up for one initial denaturation cycle at 94°C for 5 minutes, followed by 40 cycles of denaturation at 94 °C for 45 seconds, primer annealing at 54–61°C (varied based on primer used) for 45 seconds and primer elongation at 72 °C for 2 minutes. The final extension was 10 min at 72 °C. The amplification reaction products were separated on 1.5% denaturing agarose gels, stained with ethidium bromide (0.5 μg/ml) at a rate of 3μl for 150ml agarose gel and the electrophoresis was run at 80 V for one hour. The separated fragments were visualized under UV Transilluminators, using electrophoresis Gel Doc (ENDURO™ GDS Touch, Labnet, model no. GDST1302).

**Data analysis:** Visible and clear polymorphic bands in the SCoT profile were scored manually as ”1” and ”0” for the presence and absence of alleles, respectively. Polymorphism percentage was calculated for all polymorphic markers according to the method of Blair et al. (1999). Gene diversity and polymorphic information content were estimated on the basis of frequencies of identified alleles. The informativeness of the markers was quantified through the estimation of polymorphic information content (PIC), which also depends on the scored binary data Botstein et al., 1980). The marker index (MI) was estimated to indicate the overall utility of the maker system according to the method of Chesnokov and Artemyeva (2015). Binary matrix data was applied for the calculation of Jaccard’s similarity coefficient using the XLSTAT 2017 software. Jaccard’s coefficient was converted to a dissimilarity matrix to create a dendrogram using the unweighted pair-group method with arithmetic averages (UPGMA). To determine the relationship between different genotypes, the principal coordinate analysis (PCoA) was conducted based on the dissimilarity matrix of the accessions screened with the SCoT markers. GenAlEx V6.5 was implemented to conduct Analysis of Molecular Variance (AMOVA). Nei Genetic Distance and heterozygosity were estimated for the accessions’ populations relying on a pairwise population matrix.
Results:

The collected accessions represented different environmental conditions in Iraqi Kurdistan within a range of 800m elevation from 807m to 1619m abs. The collected accessions represented a range of Triticeae tribe growth in the region that belongs to the Fertile Crescent of Southwest Asia.

Genetic diversity using SCoT markers: Eighteen primers were capable to amplify the accessions under study. They generated in total 139 alleles, out of which 129 (92.80%) were able to detect polymorphism among the studied accessions (Table 2). The number of polymorphic alleles per locus varied, ranging from 5 for primer SCoT-13 to 9 for SCoT-18. All the SCoT markers showed high levels of genetic diversity. The values ranged from 0.89 for SCoT-6 to 0.31 for SCoT-12, with an average of 0.56. The marker index (MI) ranged from 6.2 to 1.63 for the primers SCoT-6 and SCoT-13, respectively, with an average of 3.7.

Table 2. Start Codon Targeted (SCoT) primers and their amplification results generated in 51 Aegilops and Triticum accessions

<table>
<thead>
<tr>
<th>No.</th>
<th>Marker name</th>
<th>sequence</th>
<th>Annealing temperature (°C)</th>
<th>Total allele number</th>
<th>Polymorphic alleles</th>
<th>PIC</th>
<th>MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SCoT-2</td>
<td>CAACAATGGCTACCACCC</td>
<td>55</td>
<td>8</td>
<td>7</td>
<td>0.49</td>
<td>3.03</td>
</tr>
<tr>
<td>2</td>
<td>SCoT-3</td>
<td>CAACAATGGCTACCACCG</td>
<td>56</td>
<td>9</td>
<td>8</td>
<td>0.53</td>
<td>3.74</td>
</tr>
<tr>
<td>3</td>
<td>SCoT-4</td>
<td>CAACAATGGCTACCACCT</td>
<td>54</td>
<td>8</td>
<td>7</td>
<td>0.60</td>
<td>3.67</td>
</tr>
<tr>
<td>4</td>
<td>SCoT-6</td>
<td>CAACAATGGCTACCACGC</td>
<td>55</td>
<td>7</td>
<td>7</td>
<td>0.89</td>
<td>6.20</td>
</tr>
<tr>
<td>5</td>
<td>SCoT-7</td>
<td>CAACAATGGCTACCACGT</td>
<td>54</td>
<td>9</td>
<td>8</td>
<td>0.43</td>
<td>3.07</td>
</tr>
<tr>
<td>6</td>
<td>SCoT-9</td>
<td>CAACAATGGCTACCAGCC</td>
<td>56</td>
<td>7</td>
<td>7</td>
<td>0.48</td>
<td>3.37</td>
</tr>
<tr>
<td>7</td>
<td>SCoT-10</td>
<td>AAGCAATGGCTACCACCA</td>
<td>54</td>
<td>7</td>
<td>7</td>
<td>0.65</td>
<td>4.52</td>
</tr>
<tr>
<td>8</td>
<td>SCoT-12</td>
<td>ACGACATGGCGACCACAG</td>
<td>58</td>
<td>7</td>
<td>7</td>
<td>0.31</td>
<td>2.15</td>
</tr>
<tr>
<td>9</td>
<td>SCoT-13</td>
<td>ACGACATGGCGACCACAT</td>
<td>58</td>
<td>6</td>
<td>5</td>
<td>0.39</td>
<td>1.63</td>
</tr>
<tr>
<td>10</td>
<td>SCoT-14</td>
<td>ACGACATGGCGACCACCC</td>
<td>60</td>
<td>9</td>
<td>8</td>
<td>0.32</td>
<td>2.31</td>
</tr>
<tr>
<td>11</td>
<td>SCoT-15</td>
<td>ACGACATGGCGACCACCA</td>
<td>61</td>
<td>9</td>
<td>8</td>
<td>0.58</td>
<td>4.15</td>
</tr>
<tr>
<td>12</td>
<td>SCoT-17</td>
<td>CATGGCTACCACCCGACC</td>
<td>57</td>
<td>7</td>
<td>7</td>
<td>0.60</td>
<td>4.22</td>
</tr>
<tr>
<td>13</td>
<td>SCoT-18</td>
<td>ACCATGGCTACCACCGCC</td>
<td>60</td>
<td>9</td>
<td>9</td>
<td>0.40</td>
<td>3.61</td>
</tr>
<tr>
<td>14</td>
<td>SCoT-19</td>
<td>GCACAATGGCTACCACAG</td>
<td>54</td>
<td>6</td>
<td>6</td>
<td>0.80</td>
<td>4.79</td>
</tr>
<tr>
<td>15</td>
<td>SCoT-20</td>
<td>ACCATGGCTACCACCGAC</td>
<td>55</td>
<td>8</td>
<td>7</td>
<td>0.59</td>
<td>3.61</td>
</tr>
<tr>
<td>16</td>
<td>SCoT-21</td>
<td>CACCATGGCTACCACCAT</td>
<td>55</td>
<td>7</td>
<td>6</td>
<td>0.79</td>
<td>4.07</td>
</tr>
<tr>
<td>17</td>
<td>SCoT-24</td>
<td>CCATGGCTACCACCGCCA</td>
<td>55</td>
<td>8</td>
<td>8</td>
<td>0.54</td>
<td>4.35</td>
</tr>
<tr>
<td>18</td>
<td>SCoT-26</td>
<td>ACAATGGCTACCACCATC</td>
<td>55</td>
<td>8</td>
<td>7</td>
<td>0.66</td>
<td>4.02</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td>7.72</td>
<td>7.16</td>
<td>0.56</td>
<td>3.70</td>
</tr>
</tbody>
</table>

PIC: polymorphism information content, MI: marker index.

Cluster Analysis: The UPGMA dendrogram (Fig. 2) derived the species accessions into three main groups. All the accessions of Triticum were grouped together. Two commercial cultivars and their wild relatives are clustered in Group 1, however, one accession from Aegilops triuncialis, and two accessions from Ae. tauschii were also clustered in this group. Other accessions of Ae. triuncialis constructed different clusters, to make a sub-group of the second largest group with accessions from Ae. cylindrica and Ae. caudata. The second subgroup comprised accessions from Ae. ovata, Ae. columnaris, Ae. umbellulata and Ae. Kotschyi. All the species here are sharing ‘U’ genome. Two accessions of Ae. lorentii stood apart from other accessions to create the third group on the dendrogram.
Fig 2: Phylogenetic relationship between the 51 Aegilops and Triticum accessions based on 139 allele data of SCoT markers. The genotypes were divided into three main groups. Clustering could be demonstrated by Jaccard’s similarity coefficient using the XLSTAT software.

The dissimilarity matrix of the molecular data was examined to figure out the principal coordinate analysis (PCoA) for the studied accessions. Data obtained from employing SCoT markers were used and the square root correction has been applied. The scatter plot allotted the accessions into three groups (Fig. 3) similar to the dendrogram. The results showed that the first two principal coordinates explained 54.73% of the total variation among the accessions. A two-dimensional plot of PCoA separated all the accessions into three distinct groups. Neighboring groups were with the least genetic distance, while the highest distances were for distant groups. All the Triticum accessions were grouped together with Ae. tauschii and an accession of Ae. triuncialis. Other accessions of Ae. triuncialis constructed different clusters, to make part of the second largest group with some other Aegilops. Two accessions of 46 and 47 from Ae. lorentii dotted in a clear distance from the accession of the belonged taxon.
Fig 3: Scatter plot of PCoA based on the Jaccard distance between individuals of 51 accessions, based on the SCoT data.

**Analysis of Molecular Variance (AMOVA):** The analysis was conducted based on SCoT alleles variability to find out differences among the population (Table 3). The highest percentage of variation was attributable more among populations (62%), while the variation within populations was considered less (38%).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>Est. Var</th>
<th>%</th>
</tr>
</thead>
</table>
| Among Pops      | 12 | 671.026| 55.919| 12.334   | 62%
| Within Pops     | 42 | 314.138| 7.479 | 7.479    | 38% |
| Total           | 54 | 985.164| 63.398| 19.814   |     |

Genetic variation for the species populations was estimated. According to the results, number of band frequency had a wide range from 76 for Ae. tauschii to the highest rate of 122 band frequency for Ae. triuncialis (Table 4). This population also recorded the highest rate of polymorphic loci (62.59%), while the lowest refers to T. aestivum. The same pattern of heterozygosity values was conducted for polymorphic loci to recoded the highest and lowest heterozygosity for Ae. triuncialis and T. aestivum respectively. The percentage of polymorphic loci and heterozygosity had no record for the species T. dicoccon Ae. umbellulata and Ae. columnaris, as they have only one accession per species for the current study data.
Table 4: Table Estimated genetic variation parameters for different *Aegilops* and *Triticum* species.

<table>
<thead>
<tr>
<th>Populations</th>
<th>Ploidy level</th>
<th>Genome</th>
<th>No. of collection</th>
<th>No. Bands</th>
<th>Freq. &gt;= 5%</th>
<th>Percentage of Polymorphic Loci %</th>
<th>Heterozygosity</th>
<th>SE of Mean Heterozygosity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. aestivum</em></td>
<td>6x</td>
<td>ABD</td>
<td>2</td>
<td>81</td>
<td>3.60</td>
<td>0.015</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td><em>T. boeoticum</em></td>
<td>2x</td>
<td>A</td>
<td>11</td>
<td>100</td>
<td>37.41</td>
<td>0.115</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td><em>T. dicoccoides</em></td>
<td>4x</td>
<td>AB</td>
<td>2</td>
<td>80</td>
<td>5.76</td>
<td>0.024</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td><em>T. dicoccon</em></td>
<td>4x</td>
<td>AB</td>
<td>1</td>
<td>78</td>
<td>0.00</td>
<td>0.000</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td><em>Ae. cylindrica</em></td>
<td>4x</td>
<td>CD</td>
<td>7</td>
<td>114</td>
<td>46.04</td>
<td>0.131</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td><em>Ae. triuncialis</em></td>
<td>4x</td>
<td>UC</td>
<td>14</td>
<td>122</td>
<td>62.59</td>
<td>0.172</td>
<td>0.016</td>
<td></td>
</tr>
<tr>
<td><em>Ae. ovida</em></td>
<td>4x</td>
<td>MU</td>
<td>4</td>
<td>90</td>
<td>17.27</td>
<td>0.061</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td><em>Ae. candata</em></td>
<td>2x</td>
<td>c</td>
<td>2</td>
<td>95</td>
<td>17.99</td>
<td>0.074</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td><em>Ae. lorentii</em></td>
<td>4x</td>
<td>UM</td>
<td>2</td>
<td>103</td>
<td>29.50</td>
<td>0.122</td>
<td>0.016</td>
<td></td>
</tr>
<tr>
<td><em>Ae. kotschyi</em></td>
<td>4x</td>
<td>SU</td>
<td>2</td>
<td>86</td>
<td>16.53</td>
<td>0.069</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td><em>Ae. umbellulata</em></td>
<td>2x</td>
<td>U</td>
<td>1</td>
<td>78</td>
<td>0.00</td>
<td>0.000</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td><em>Ae. tauschii</em></td>
<td>2x</td>
<td>D</td>
<td>2</td>
<td>76</td>
<td>15.53</td>
<td>0.054</td>
<td>0.010</td>
<td></td>
</tr>
</tbody>
</table>

Discussion:
Assessments of genetic diversity among and within the wild relatives of bread wheat is an important step for more efficient management and use of this crop, and it is an initiative step in any improvement programs (Begna 2021). In the current study genetic diversity was studied at the inter and intra-specific level of different genome-bearing species of *Aegilops* and *Triticum* complex using SCoT marker. Studying the extent of genetic distance and their relatedness to *Triticum* could play an important role in broadening the cultivated wheat gene pool (Jabari et al. 2023). The genetic resources stored in wild relatives of crops are potential sources of new genetic diversity to be introduced into wheat breeding programs (Sharma et al. 2021). *Aegilops* and wild wheat species have a potential interest to be useful allele sources in the improvement of *T. aestivum* (Huertas-García et al. 2021; Rakszegi et al. 2020). Many physiological, productivity and quality performances of cultivated wheat could be modified via the contribution of *Aegilops* species (Kumar et al. 2019; Qiu et al. 2022). There are few molecular studies that have investigated genetic diversity and phylogenetic relationships of the members of the genome-bearing species of *Triticum*. To our knowledge, this is the first report on genetic diversity among *Aegilops* and *Triticum* species in the Kurdistan region using the SCoT marker.

DNA analyses employing SCoT markers were established in the current study as an efficient and inexpensive technique to evaluate the extent of genetic diversity in *Aegilops* and *Triticum*. Among the obtained bands, 92.80% were polymorphic. A high level of polymorphism was observed among the accessions using SCoT markers. This result confirms the effectiveness of such markers used as a suitable DNA tool to study the genetic diversity in wheat and their wild relatives (Pour-Aboughadareh et al. 2018). This marker technology was able to reveal a high level of polymorphism (83-100%), which is close to the results obtained previously (El-Esawi et al. 2022). The number of alleles per locus varied, ranging from 5 to 9 alleles. A higher number of alleles per marker were obtained when SCoT markers were applied to *Ae.
triuncialis in Iran by Khodaee et al. (2021). All SCoT markers here are considered informative to show a high level of genetic diversity.

Since PIC is the probability of a primer detecting polymorphism between individuals, it would provide a scale, that helps to determine the effectiveness of primers used in the fingerprinting process (Pour-Aboughadareh et al. 2017). In the current study, the estimation of this level of PIC for SCoT markers (0.56 on average) states the markers with a strong discrimination power for studying genetic diversity (Jabari et al. 2023). Hence, they considered an efficient and informative to reveal genetic diversity in the accessions of *Aegilops* and *Triticum* possess various ploidy levels in the Kurdistan region of Iraq. For a further indication of the diversity of markers used, the marker index (MI) was estimated for all the primers (Ahmad et al. 2022; Nisar and Hussain 2022). The MI values were found to be reasonably high (3.7 on average). The MI obtained here was in accordance with results conducted by Bokaei et al. (2023) for some *Aegilops* species based on SCoT markers. Results of the current MI indicate better ability, higher efficiency and potency of the SCoT markers in future investigations on *Aegilops* and *Triticum* species (Shaban et al. 2022). In addition, a strong positive correlation was observed between MI and PIC value (r=0.87, r²=0.76, p= <0.0001), relying on one of these two parameters could linearly represent the other. The marker informativeness indices PIC and MI establish a good discriminating power of these primers, suggesting a high efficiency of this DNA marker to assess genetic relationships among the wild relatives of wheat crops. Previous studies are in agreement with the current result in evidencing the power of SCoT markers to detect genetic diversity in a wide range of species and recognizing populations of the same species (Abouseada et al. 2023; Pour-Aboughadareh et al. 2017).

In the cluster analysis, all the accessions belonging to wild species of *Triticum* boeoticum, *T. dicoccoides* and *T. dicoccon*, posing ‘A’ genome in share with *Triticum*, are all clustered in group 1. The clustering of these species within the first clade parallel to *T. aestivum* is in agreement with their biological classification, and they are known as a closer wild relative to bread wheat (Pour-Aboughadareh et al. 2017). The presence of genetic diversity within *Triticum* species bearing A, B or D genomes is of considerable interest, are known as the main gene pool of domesticated wheat, and a source of useful alleles for wheat breeding purposes (Pour-Aboughadareh et al. 2021). *Aegilops* is considered the most successfully used genus in the tribe Triticeae to conduct wide crosses in wheat improvement programs (Gong et al. 2014). This fact is true for the *Ae. tauschii* clustered in the first group of *Triticum* clustering, posing the D genome constitution and being a diploid ancestor of *T. aestivum* (Wang et al. 2021). It could be used to widen the genetic base and would serve as a bridge to transfer desirable traits into modern wheat varieties and to elicit adoption estimates (Aberkane et al. 2020; Gaurav et al. 2022). The accessions of *Ae. tauschii* are clustered with *Triticum* genera group. A similar trend was observed for the genetic relationship of *Aegilops* and *Triticum* by Pour-Aboughadareh et al. (2018) to be clustered consistently based on their genomic structure being *Ae. tauschii* a diploid ancestors of *T. aestivum*. Accessions 4 and 6, belonging to *T. dicoccoides*, were clustered very close to *T. aestivum*. Same as for accession 25 (*T. dicoccon*) to cluster close to *T.
aestivum. Reducing the genetic diversity in wheat resulted from domestication bottleneck, inevitable the
investigation and incorporation of such wild relatives into the breeding program (Yadav et al. 2023).

Accessions in each group showed a considerable amount of diversity. A total of 22 accessions were
clustered into group 2. Group 2-a covered all Ae. cylindrica. Although Ae. tauschii is the putative donor of
the D genome for Ae. cylindrica, the D genome of tetraploid cytotypes of Ae. cylindrica was found to be
slightly different from D genome of diploid Ae. tauschii, and made part of the second group. In other
words, Ae. cylindrica did not cluster with other D-genome species of the studied accessions. This
difference may be caused by maternal species involved in hybridization events to produce polyploid Ae.
cylindrica species (Goryunova et al. 2004). The same sub-group covered all the accessions of Ae.
triuncialis except for accession 31. This sub-group also involved accessions of Ae. caudata (13 and 24),
being progenitor male parents of Ae. triuncialis (Thomas and Bebeli 2010). Also gathering of Ae. caudata
and Ae. cylindrica together in this subgroup indicates their similarities, as Ae. caudata is the putative donor
of C genome also for Ae. cylindrica (Kimber and Zhao 1983). Being Ae. caudata (C) as a donner parent of
each of Ae. cylindrica (DC) and Ae. triuncialis (UC), is supporting their possibility for their accession to be
clustered together within the same subgroup.

The second subgroup (Group 2-b) covered all the accessions of Ae. ovata, Ae. kotschyi, with an accession
from each species of Ae. columnaris and Ae. umbellulata. All the species here are sharing ‘U’ genome
from diploid and tetraploid genomes, which confirms the stability of the U genome as the pivotal genome
(Thomas and Bebeli 2010). Grouping Ae. Kotschyi with its male parent (Ae. umbellulata) in the current
study is parallel and in agreement with what was found previously by applying ISSR markers (Han-Yu et
al. 2006). The last group (3) covered only two species of Ae. lorentii. The species comprised both U and M
genome chromosomes and their study would be useful when applying in wide crosses for wheat
improvement (Schneider et al. 2005).

Based on the cluster analysis of SCoT markers, the grouping of the most accessions tends to behave in their
attributed species classification with the exception of an accession belonging to Ae. triuncialis (No. 31) that
clustered in different groups from the other accessions of its belonging species. This result might refer to
the reason that, Ae. triuncialis included ancestral variation from different parental lineages and therefore
might have originated multiple times (Vanichanon et al. 2003), and the genomes present in the species
accessions have sufficiently diverged during evolution to be distinguished by the current SCOT markers.
The current marker system is not able to confirm the described different subspecies of this taxon,
inadequate intraspecific classification of this species could be one reason for this species distortion.

To further dissect the relationships among species, principal coordinate analysis (PCoA) was performed,
based on the dissimilarity matrix of the molecular data, to explain half of the total variation of the
accessions. All the accessions formed 3 distinct groups, corresponding to UPGMA cluster. Considering the
results pattern obtained of PCoA and cluster analysis it is indicated that there is no clear relationship
between genetic divergence and geographical origins of the accessions. The current patterns of differences between accessions could be expected for wild species, indicating that the species belonging to *Aegilops* and *Triticum* taxa represent a large gene pool with a significant level of diversity in their species. They would have a potential interest in utilization in wheat improvement for biotic, abiotic and quality improvement (Khodaee et al. 2021).

Results of AMOVA analysis revealed a higher distribution of genetic variation among studied species as compared to individuals within species. This result is in contrast to what was obtained from analyses of genetic variation among *Triticum* species (Pour-Aboughadareh et al. 2017) and *Aegilops* (Pour-Aboughadareh et al. 2020) using SCoT markers. Covering two genera of *Aegilops* and *Triticum* in the current study could be a reason for less genetic variation within the studied populations (biological species) compared to the variation among the species populations. The current distribution of genetic variation between and within populations studied here is operated from the extent of gene flow between them, which depends on the size and the degree of isolation and the rate of exchanging pollen grains between these populations (Scheepens et al. 2012). This thought is also supported by Nei Genetic Distance for the population studied based on molecular data analysis, to indicate the higher genetic distance between the populations of *Aegilops* and *Triticum* taxa more than the distance among the accession within the taxon (Table 5).

Table 5: Pairwise Population Matrix of Nei Genetic Distance for the population studied based on Molecular data analysis

<table>
<thead>
<tr>
<th>Accession</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 T. aestivum</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 T. boeoticum</td>
<td>0.087</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 T. dicoccoides</td>
<td>0.117</td>
<td>0.184</td>
<td>0.068</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 T. dicocon</td>
<td>0.273</td>
<td>0.215</td>
<td>0.292</td>
<td>0.377</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Ae. cylindrica</td>
<td>0.314</td>
<td>0.231</td>
<td>0.342</td>
<td>0.399</td>
<td>0.049</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Ae. triuncialis</td>
<td>0.543</td>
<td>0.424</td>
<td>0.519</td>
<td>0.559</td>
<td>0.184</td>
<td>0.190</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 Ae. ovata</td>
<td>0.367</td>
<td>0.272</td>
<td>0.418</td>
<td>0.487</td>
<td>0.091</td>
<td>0.063</td>
<td>0.227</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 Ae. caudata</td>
<td>0.403</td>
<td>0.375</td>
<td>0.433</td>
<td>0.492</td>
<td>0.252</td>
<td>0.214</td>
<td>0.380</td>
<td>0.254</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 Ae. lorentii</td>
<td>0.538</td>
<td>0.438</td>
<td>0.514</td>
<td>0.541</td>
<td>0.230</td>
<td>0.247</td>
<td>0.046</td>
<td>0.280</td>
<td>0.442</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 Ae. kotschyi</td>
<td>0.615</td>
<td>0.461</td>
<td>0.589</td>
<td>0.644</td>
<td>0.254</td>
<td>0.269</td>
<td>0.118</td>
<td>0.297</td>
<td>0.442</td>
<td>0.133</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 Ae. umbellulata</td>
<td>0.601</td>
<td>0.541</td>
<td>0.589</td>
<td>0.604</td>
<td>0.241</td>
<td>0.259</td>
<td>0.174</td>
<td>0.308</td>
<td>0.479</td>
<td>0.233</td>
<td>0.290</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>12 Ae. columnaris</td>
<td>0.069</td>
<td>0.059</td>
<td>0.108</td>
<td>0.190</td>
<td>0.266</td>
<td>0.298</td>
<td>0.496</td>
<td>0.360</td>
<td>0.383</td>
<td>0.516</td>
<td>0.540</td>
<td>0.604</td>
<td>0.000</td>
</tr>
</tbody>
</table>

According to Nei’s measures of genetic distance, the lowest genetic distance (0.047) was found between T. dicoccoides (AB) and T. aestivum (ABD). The same pattern was found between Ae. tauschii (D) and all the *Triticum* species, however, the highest genetic distance was found between Ae. umbellulata and all *Triticum* species. Our results reveal that, Ae. tauschii shares more similarities with T. aestivum (genetic distance of 0.069) after T. dicoccoides (0.047) compared to all other *Aegilops*. The results of the genetic distance matrix support the hypothesis that Ae. tauschii is the D genome ancestors of T. aestivum (Mahjoob et al. 2021; Pour-Aboughadareh et al. 2018). Also, Ae. cylindrica had the least genetic distance with each of Ae. triuncialis and Ae. caudata (0.049 and 0.091, respectively), due to their C genome sharing, as they all clustered into a subgroup of the second clade.
Among the *Triticum* genera, *Triticum boeoticum* had the highest value of heterozygosity (0.115) followed by *T. dicoccoides* and aestivum (Table 4 and Fig. 4), while among the *Aegilops* *Ae. triuncialis* had the highest heterozygosity of 0.172, followed by *Ae. cylindrica* (0.131). This pattern is also parallel to the number of bands frequency (\( \geq 5\% \)) and also for polymorphic loci, giving the values of 122 and 62.59\%, respectively, for *Ae. triuncialis*. This result also confirms the higher gene pool of *Aegilops* compared to that of *Triticum* (Wang et al. 2022). The beneficial wild alleles present in *Triticum* and *Aegilops* related species could be introduced into the elite cultivars through interspecific hybridization as a promising approach to enlarge the genetic diversity of cultivated bread wheat (Kishii 2019).

![Fig 4: band pattern of the SCoT markers across the accessions’ populations](image)

**Fig 4: band pattern of the SCoT markers across the accessions’ populations**

**Conclusion:**

Results of the current study indicated that the *Aegilops* and *Triticum* species, endemic to Fertile Crescent, have a remarkable level of genetic diversity in the Kurdistan region of Iraq. The potential power of SCoT markers for analyzing the genetic relationships among *Aegilops* and wheat germplasm was established. According to the UPGMA clustering pattern and PCoA biplot, the *Aegilops* and *Triticum* accessions were clustered based on their genomic constitution and coherent with their taxonomic classification. Analysis of molecular variance (AMOVA) indicated that the amount of genetic diversity within species was more than among them. Detected species of high levels of genetic variation could provide an available source of potentially useful variation for wheat improvement based on the available gene pool. This molecular technique seems to allow reliable information and will improve strategies for the effective collection and conservation of wheat germplasm. The genetic diversity estimated by the SCoT markers would offer important information in the management of germplasm resources for *Aegilops* and wild wheat germplasm, to discover novel and functional alleles to be integrated into the modern cultivars.

**Conflict of interest:** The authors declare that they have no conflict of interest.
Acknowledgements: The author would like to thank Kurdistan Botanical Foundation (KBF) for facilitating the collection of some of the accessions used in the study. The author is also grateful to Dr. Azad Rastagar at HKS Herbarium, Forests and Rangelands Research Department, Kurdistan Agricultural and Natural Resources, Research and Education Center, AREEO, Sanandaj, Iran, for his effort in the classification of the collected accessions. Special thanks also due to Dr Heydar Azizi at Guilan University in Iran for his contribution to analyzing part of the study data.

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