Postprint of ISSR Analysis of Genetic Diversity and Population Structure in Aegilops tauschii Germplasm

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Abstract

Aegilops tauschii has a wide distribution range and rich genetic variation, and is considered an important gene source for improving common wheat. To deeply understand the genetic diversity and population structure of *Aegilops tauschii* germplasm from different sources, this study used ISSR molecular markers to conduct genetic diversity and population structure analysis on 56 accessions of *Aegilops tauschii* germplasm. The results showed that: (1) Sixteen ISSR primers detected a total of 170 polymorphic loci, with each ISSR primer producing 3-18 polymorphic bands, averaging 10.63; the polymorphism information content (PIC) ranged from 0.17 to 0.85, with an average of 0.67. (2) Comparison of genetic diversity among four *Aegilops tauschii* populations revealed that the Central Asian population had the highest level of genetic diversity (He = 0.2254, I = 0.3557), with relatively low gene flow among populations (Nm = 1.6386). (3) At a genetic similarity coefficient of approximately 0.67, the clustering results grouped six accessions from Tajikistan and two from Turkmenistan into one cluster (Group 2); the other 48 accessions formed a large cluster (Group 1), which could be further divided into three subgroups, indicating that *Aegilops tauschii* germplasm materials from the same origin tended to cluster together. (4) Population structure analysis divided the 56 *Aegilops tauschii* accessions into five populations, among which germplasm materials from Iran in West Asia (Population V) had relatively consistent genetic backgrounds with low admixture; further analysis of Q values for each population revealed that germplasm materials in Population IV had relatively complex sources of genetic relationships and the richest genetic diversity. These research results can provide important reference for phylogenetic relationship analysis and germplasm diversity conservation of *Aegilops tauschii*, and lay a foundation for its scientific utilization and evolutionary studies.

Full Text

Genetic Diversity and Population Structure of *Aegilops tauschii* Germplasm Based on ISSR Analysis

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Abstract: *Aegilops tauschii*, widely distributed and rich in genetic variation, is considered an important gene source for improving common wheat. To understand the genetic diversity and population structure of *A. tauschii* from different origins, this study analyzed 56 *A. tauschii* accessions using ISSR molecular markers. The results showed: (1) Sixteen ISSR primers detected a total of 170 polymorphic loci, with each primer producing 3–18 polymorphic bands (average 10.63). Polymorphism information content (PIC) ranged from 0.17 to 0.85, with an average of 0.67. (2) Comparison of genetic diversity among four *A. tauschii* populations revealed that Central Asian populations exhibited the highest level of genetic diversity (He = 0.2254, I = 0.3557), with relatively low gene flow among populations (Nm = 1.6386). (3) At a genetic similarity coefficient of approximately 0.67, cluster analysis grouped six accessions from Tajikistan and two from Turkmenistan into one cluster (Group 2), while the remaining 48 accessions formed a large cluster (Group 1). Group 1 could be further divided into three subgroups, with a tendency for accessions from the same origin to cluster together. (4) Population structure analysis divided the 56 accessions into five populations. Accessions from Iran in West Asia (Population V) showed relatively consistent genetic backgrounds with low admixture. Further analysis of Q-values revealed that Population IV had relatively complex genetic relationships and the most abundant genetic diversity. These findings provide an important reference for elucidating genetic relationships, conserving germplasm diversity, and laying a foundation for the scientific utilization and evolutionary research of *A. tauschii*.

Keywords: *Aegilops tauschii*, genetic diversity, cluster analysis, population structure, ISSR analysis

Introduction

Aegilops tauschii Coss. (DD, 2n = 2x = 14), also known as goatgrass, belongs to the tribe Triticeae and genus *Aegilops*, and is considered the D-genome donor of common wheat (*Triticum aestivum*, AABBDD, 2n = 6x = 42) (Lagudah et al., 1991; Dvorak et al., 2012). *Aegilops tauschii* is primarily distributed across central Eurasia, with its center of origin along the southern coast of the Caspian Sea and Azerbaijan. From there, it spread eastward across the Kopet Mountains

in Turkmenistan to Xinjiang and the middle Yellow River region of China, and westward through valleys in southeastern Turkey to central Syria (Lubbers et al., 1991). Previous studies have demonstrated that the D-genome of *A. tauschii* harbors rich genetic variation for stress resistance (Abbas et al., 2021), disease resistance (Olson et al., 2013), and quality traits (Hsam et al., 2001), making it an important germplasm resource for wheat improvement (Lagudah et al., 1991; Dvorak et al., 2012; Zhao et al., 2019; Gao et al., 2021).

The collection and evaluation of core germplasm form the foundation for germplasm utilization, innovation, and crop breeding. Studying the genetic diversity and population structure of donor species not only clarifies their phylogenetic relationships but also provides a theoretical basis for their rational and efficient conservation and utilization (Mourad et al., 2020). Genetic diversity in *A. tauschii* has been extensively investigated using morphological, physiological, seed storage protein, isozyme, and molecular markers (William et al., 1993; Ghasemzade et al., 2008; Mahjoob et al., 2021). Among these, molecular markers are recognized as efficient and flexible tools for studying genetic diversity and population structure because they are not limited by phenotypic conditions and can be applied at early developmental stages (Abouzied et al., 2013). Lubbers et al. (1991) analyzed 25 loci using RFLP in 102 *A. tauschii* accessions from different origins, finding the greatest variation in accessions from the Caspian Sea region, followed by Afghanistan, with the least variation in Turkey and Pakistan, further supporting a southern Caspian Sea origin. Kong et al. (1998) used RAPD markers to analyze genomic DNA polymorphism in two *A. tauschii* subspecies, showing that *Aegilops tauschii* ssp. *tauschii* exhibited significantly higher polymorphism than *Aegilops tauschii* ssp. *strangulata*. Pestsova et al. (2000) employed SSR markers to assess genetic diversity in 113 *A. tauschii* accessions, revealing greater diversity in Caucasian populations than in Central Asian populations, and classified them into two major groups corresponding to geographical distribution.

Inter-simple sequence repeat (ISSR) is a dominant molecular marker technology that offers high polymorphism, reproducibility, stability, and operational simplicity. It has been widely applied in genetic diversity studies of wheat and its wild relatives, including Egyptian wheat (Abdel-Lateif & Hewedy, 2018), durum wheat (*Triticum durum*) (Aslan-Parviz et al., 2020), barb goatgrass (*Aegilops triuncialis*) (Khodaee et al., 2021), and barley (*Hordeum vulgare*) (Zhang et al., 2020). However, few studies have investigated *A. tauschii* genetic diversity using ISSR markers. Li et al. (2017) used nine ISSR markers to study 75 Chinese *A. tauschii* accessions, dividing them into two groups (Yellow River Basin and Xinjiang) and identifying five unique Yellow River Basin types. Further research suggests that the Yellow River Basin population originated directly from Iran or southern Turkmenistan (Wei et al., 2008; Su et al., 2020). The D-genome of common wheat derives from a limited group of *A. tauschii*, but polyploidization and evolutionary bottlenecks have led to an increasingly narrow genetic base. Compared with the A and B genomes, the D-genome of wheat exhibits particularly scarce genetic diversity. To expand the D-genome using *A. tauschii*

from different sources, it is essential first to elucidate the genetic diversity of *A. tauschii* germplasm. Therefore, this study employed 16 ISSR markers to investigate genetic diversity and population structure in 56 *A. tauschii* accessions from different origins, aiming to understand their genetic relationships and provide a theoretical foundation for germplasm conservation and scientific utilization.

1.1 Experimental Materials

This study utilized 56 *Aegilops tauschii* accessions obtained from the National Genetic Resources Program (NPGS) and maintained at the Wheat Center of Henan Institute of Science and Technology. The collection included 23 accessions from West Asia (18 from Iran, 2 each from Turkey and Azerbaijan, and 1 from Georgia), 26 from Central Asia (11 from Afghanistan, 9 from Turkmenistan, and 6 from Tajikistan), 3 from South Asia (Pakistan), 1 from East Asia (China), and 3 of unknown origin.

1.2 DNA Extraction

Genomic DNA was extracted from young leaves of the 56 *A. tauschii* accessions using the 2×CTAB method described by Yan et al. (2002). DNA concentration and purity were assessed using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, USA).

1.3 ISSR-PCR Amplification and Analysis

Based on Khodaee et al. (2021), 16 ISSR primers with clear amplification and good repeatability were selected for genetic diversity analysis of the 56 accessions (synthesized by Shanghai Biological Engineering Technology Service Co., Ltd.) .

The ISSR-PCR reaction was performed in a 20 L volume containing 10 L 2×Taq PCR StarMix (Beijing Kangrun Chengye Biotechnology Co., Ltd.), 6 L ddH_2O, 2 L ISSR primer (10 pmol · L^{-1}), and 2 L DNA template (50–60 ng · L^{-1}). The amplification program consisted of initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 30 s, 53.7–60.5°C (primer-dependent) for 45 s, and 72°C for 2 min; followed by a final extension at 72°C for 7 min, and storage at 4°C. PCR products were separated on 1.2% denaturing agarose gels with D2000 bp DNA ladder (Tiangen Biotech Co., Ltd.) as a size standard. Gels were visualized, photographed, and documented.

1.4 Data Analysis

ISSR amplification profiles were scored as binary data: presence of a band at the same migration position was recorded as 1, absence as 0, and unclear bands as 999, creating a binary data matrix. PowerMarker 3.2.5 (Liu & Muse, 2005) was used to calculate polymorphism information content (PIC). Popgene 1.32

(Yeh et al., 1999) was employed to compute observed number of alleles (Na), effective number of alleles (Ne), Nei' s gene diversity index (He), Shannon' s information index (I), total gene diversity (Ht), gene diversity within populations (Hs), gene diversity among populations (Dst), genetic differentiation coefficient (Gst), and gene flow (Nm). Ntsys 2.1 (Rohlf, 2000) was used for unweighted pair group method with arithmetic mean (UPGMA) cluster analysis based on simple matching coefficients (SM).

Data were converted to Structure 2.3.4 format for population genetic structure analysis. K-values from 2 to 20 were tested with 10 independent runs per K. The optimal K-value was determined using Structure Harvester (Earl & Vonholdt, 2012), and genetic structure diagrams were constructed. Q-values, representing the probability that an accession' s variation originated from a given population, were calculated to analyze genetic composition.

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2 Results and Analysis

2.1 ISSR Primer Polymorphism in 56 *Aegilops tauschii* Accessions

Sixteen ISSR primers amplified 175 clear and stable bands across the 56 accessions. ISSR-13 produced the most bands (19), while ISSR-23 and ISSR-28 produced the fewest (3 bands each), with an average of 10.94 bands per primer. The number of polymorphic bands ranged from 3 to 18, averaging 10.63, yielding a polymorphic loci percentage of 97.14%. PIC values ranged from 0.17 (ISSR-28) to 0.85 (ISSR-5 and ISSR-27), with a mean of 0.67. Twelve primers (75%) exhibited PIC values above the average. According to Botstein et al. (1980), PIC values are classified as high (>0.50), moderate (0.25–0.50), or low (<0.25). In this study, all primers except ISSR3 (PIC = 0.49), ISSR7 (PIC = 0.19), ISSR23 (PIC = 0.39), and ISSR28 (PIC = 0.17) showed high polymorphism information content.

2.2 Genetic Diversity and Differentiation Among 56 *Aegilops tauschii* Accessions

Genetic diversity parameters (Na, Ne, He, and I) were highest in Central Asian populations, followed by West Asian, other, and South Asian populations . This indicates that Central Asian *A. tauschii* populations possess the highest genetic diversity, while South Asian and other populations show relatively lower diversity. Further analysis of population differentiation revealed that total gene diversity (Ht) was 0.2360, with moderate genetic differentiation among the four populations (Gst = 0.2338). Gene diversity within populations accounted for 0.7662 of the total (Hs/Ht), with moderate gene flow (Nm = 1.6386), suggesting that most genetic variation resides within populations rather than among them .

2.3 Cluster Analysis of 56 *Aegilops tauschii* Accessions

UPGMA cluster analysis based on ISSR data distinguished 52 of the 56 accessions (92.86%), with pairwise genetic similarity coefficients (GS) ranging from 0.64 to 0.94 [Figure 1: see original paper]. At a genetic similarity coefficient of approximately 0.67, six accessions from Tajikistan (PI662084, PI662095, PI662105, PI662106, PI662112, PI662116) and two from Turkmenistan (PI662076, PI662078) formed a distinct cluster (Group 2), while the remaining 48 accessions formed a large cluster (Group 1). At a coefficient of approximately 0.70, Group 1 subdivided into three subgroups: Subgroup 1 comprised 26 accessions from eight countries, with accessions from the same origin clustering together (e.g., Turkmenistan: PI662065, PI662066, PI662067, PI662068, PI662069, PI662070, PI662072; Afghanistan: CIae3, CIae4, CIae5, CIae6, PI220331, PI220641, PI220642, PI317392, PI317394, PI317398, PI511366). Subgroup 2 primarily contained accessions from Iran (CIae8, CIae9, CIae10, CIae13, CIae15, CIae16, CIae17, CIae18, CIae19, CIae20, CIae21, PI268210, PI276985, PI511368, PI511378, PI511379, PI511382), except for three accessions (Turkey: PI486269; unknown: PI330489, PI369627). Subgroup 3 included accessions from Azerbaijan (PI349037, PI428564). Notably, unknown accession CIae51 clustered with Afghan PI220642, while unknown accessions PI330489 and PI369627 clustered with Iranian PI276985, suggesting Afghan origin for CIae51 and Iranian origin for PI330489 and PI369627. The cluster analysis effectively distinguished most accessions, demonstrating significant genetic differences among the germplasm and confirming the effectiveness of ISSR markers for *A. tauschii* analysis.

2.4 Genetic Structure Analysis of 56 *Aegilops tauschii* Accessions

Structure analysis based on ISSR data identified K = 5 as the optimal number of populations [Figure 2: see original paper], dividing the 56 accessions into five genetic populations [Figure 3: see original paper]. Population V, primarily comprising accessions from Iran in West Asia, showed a relatively consistent genetic background with low admixture, while other populations exhibited higher intermixing, particularly Population IV. The analysis revealed that accessions from the same geographic origin tended to cluster together, consistent with the UPGMA results.

Q-value distribution across populations showed that 15 accessions (26.8%) had Q-values below 0.6, while 50.0% and 42.9% of accessions had Q-values above 0.8 and 0.9, respectively , indicating that most germplasm had relatively pure genetic backgrounds. However, comparing Q-values above 0.8 across populations revealed that Population V had the highest proportion (83.3%), while Population IV had the lowest (26.7%). These results indicate that Population IV possesses relatively complex genetic relationships and the richest genetic diversity.

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3 Discussion

3.1 Genetic Diversity Analysis of *Aegilops tauschii* Using ISSR Markers

Molecular markers play a crucial role in biodiversity and evolutionary studies, and ISSR is a PCR-based DNA polymorphism detection technology. In this study, 16 ISSR markers analyzed 56 *A. tauschii* accessions, revealing 97.14% polymorphic loci, Na = 1.9771, Ne = 1.3875, He = 0.2428, I = 0.3826, and within-population genetic diversity of 0.1809. In comparison, Li et al. (2017) used nine ISSR markers to study 75 Chinese *A. tauschii* accessions (from Xinjiang, Henan, and Shaanxi), reporting 89.31% polymorphic loci, Na = 1.9934, Ne = 1.4715, He = 0.2734, I = 0.4152, and within-population genetic diversity of 0.2367. Except for the percentage of polymorphic loci, all other parameters were slightly lower in our study population (primarily from West and Central Asia) compared with the Chinese population, suggesting that genetic diversity increased as *A. tauschii* spread eastward from West and Central Asia to Xinjiang and the middle Yellow River region, with partial differentiation occurring within Chinese populations. Comparisons with ISSR studies in other species, such as *Aegilops triuncialis* (average 9.50 polymorphic bands, PIC = 0.30; Khodaee et al., 2021), durum wheat (average 10.00 polymorphic bands, PIC = 0.42; Aslan-Parviz et al., 2020), and barley (average 8.313 polymorphic bands; Zhang et al., 2020), indicate that *A. tauschii* exhibits relatively high ISSR polymorphism and PIC values, likely attributable to the broad geographic origins of the germplasm used in this study.

3.2 Population Genetic Structure of *Aegilops tauschii*

Mizuno et al. (2010) and Sohail et al. (2012) analyzed the genetic structure of geographically diverse *A. tauschii* germplasm, identifying two lineages: L1 (belonging to *Aegilops tauschii* ssp. *tauschii*) and L2 (belonging to *Aegilops tauschii* ssp. *strangulata*). They suggested that only a few L2 lineage accessions contributed to the origin of common wheat' s D-genome, while the non-contributing L1 lineage harbors abundant genetic variation. Wang et al. (2013) further subdivided these lineages geographically into western (W) and eastern (E) sublineages: L1W in eastern Turkey, Armenia, Azerbaijan, and western Iran; L1E from central Iran to western China; and L2W and L2E in regions from Armenia to Azerbaijan and from southern Azerbaijan to the northern Iranian Caspian coast, respectively.

Pestsova et al. (2000) used SSR markers to analyze 113 *A. tauschii* accessions, finding the highest diversity in Caucasian populations (Georgia, Armenia, and Dagestan, Russia) and the lowest in Central Asian populations (Uzbekistan and Turkmenistan). In contrast, our comparison of four populations revealed the highest genetic diversity in Central Asian accessions (Afghanistan, Turkmenistan, and Tajikistan; He = 0.2254, I = 0.3557), contradicting Pestsova et al. (2000). This discrepancy may be attributed to greater variation among

Afghan and Tajikistani accessions in our study. Wang et al. (2010) used SSR markers to cluster 78 *A. tauschii* accessions into six major groups at a similarity coefficient of 0.77, while Sohail et al. (2012) used DArT markers to divide 81 accessions into three groups (A/B/C), with Group C primarily from Iran. Our cluster analysis at a similarity coefficient of ~0.67 divided the 56 accessions into two major groups (Group 1 and Group 2), with Group 1 further subdivided into three subgroups, while Structure analysis identified five populations. Although these methods differed in the number of groups, both demonstrated that accessions from the same origin tended to cluster together, supporting the conclusion that genetic relationships correlate with geographic origin (Wang et al., 2010; Sohail et al., 2012). Additionally, only 15 accessions (26.8%) had Q-values below 0.6, indicating that most germplasm has relatively pure genetic backgrounds, with variation primarily occurring within populations or geographic regions. Thus, gene exchange and recombination within populations during dispersal appear to be the main drivers of differentiation among *A. tauschii* populations.

3.3 Conservation Biology Implications of *Aegilops tauschii*

Natural genetic variation is one of the most important fundamental resources in plant biology (Koornneef et al., 2004). Common wheat originated from natural hybridization between tetraploid wheat and *A. tauschii* followed by chromosome doubling, a process that excluded substantial genetic variation from the donor species during domestication and modern breeding (Dubcovsky & Dvorak, 2007; Dvorak et al., 2012). Synthetic wheat, created by mimicking this evolutionary process, serves as an important bridge for reintroducing donor genetic variation and holds great potential for wheat breeding (Hao et al., 2019). However, Waines (1998) reported that many *Aegilops* species habitats have been destroyed or are under threat, particularly in the eastern Mediterranean coastal region. Therefore, understanding *A. tauschii* population structure is crucial for genetic diversity conservation. This study' s analysis of genetic diversity and population structure in 56 *A. tauschii* accessions from diverse origins clarifies their phylogenetic relationships and enables selection of representative accessions from each population or region for conservation, providing a foundation for future research and utilization of natural genetic variation in *A. tauschii*.

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