

Article

Distribution, Genetic Diversity and Population Structure of *Aegilops tauschii* Coss. in Major Wheat-Growing Regions in China

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Abstract: *Aegilops tauschii* Coss. is known as a noxious grass weed seriously affecting wheat quality and yield. To investigate its present occurrence in wheat fields and the potential genetic diversity of the grass weed in China, a field survey covering major wheat production regions was conducted during 2017–2019. Seeds of different *Ae. tauschii* populations collected from the survey were analyzed with Simple Sequence Repeats (SSRs) technique. Results showed that *Ae. tauschii* was occurring in each of the provinces surveyed with varied occurrence frequency ranging from 0.91% in Sichuan Province to 92.85% in Henan Provinces. Eighty alleles with size ranging from 98 bp to 277 bp were detected from the 192 collected *Ae. tauschii* populations with 17 SSR markers. *Ae. tauschii*, in this study, exhibited a moderately high level of genetic diversity, high differentiation, deficient heterozygosity and limited gene flow. Compared with other provinces, Hubei populations possessed relatively low genetic diversity. Dendrogram analysis showed that genetic distance did not seem to be related to geographic distribution. Additionally, STRUCTURE analysis suggested that *Ae. tauschii* populations in wheat fields of China can be divided into three groups, which was further supported by cluster analysis. Among the three groups, solely 7% of the total variation was detected, whereas the majority variation (67%) occurred among different populations within same group. Undoubtedly, such information will help us to better understand population relationships and spread of *Ae. tauschii* in China and will provide a new perspective for its integrated management.

Keywords: *Aegilops tauschii* Coss.; frequency of occurrence; microsatellite marker; genetic diversity; population structure



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1. Introduction

Aegilops tauschii Coss., an alien invasive species, is widely distributed in major wheat production areas of China during recent years. It is reported that *Ae. tauschii* occurred in eight wheat-growing provinces of China by 2007, with a damage area of approximately 3.3×10^5 ha [1]. As a member of the Poaceae family, *Ae. tauschii* is a self-pollinated, annual diploid plant [2]. *Ae. tauschii* has extremely strong producibility, and an individual plant can produce 10–40 tillers, with 100–800 seeds, on average [1,3]. Usually, *Ae. tauschii* emerges from September to November and matures from May to June of the following year, competing with wheat for sunlight, water and fertilizer [4]. It can cause a 50–80% wheat yield loss when its ears are up to 457 m^{-2} [1]. As a donor of wheat D genome, *Ae. tauschii* has high homology with wheat in genetic background, and only a few selective herbicides are available for this weed. In addition, the upper spikelets of *Ae. tauschii* are easily detached from the rachis and fall into the soil before reaching complete maturity, while the basal spikelets remain in the rachis, spreading with wheat seeds. The distribution

of weeds is closely related to its monitoring and management. Descriptive studies of the genetic diversity in weed populations can also be extremely important, because they provide essential background for further focused research, such as understanding the ability of weeds to adapt to different environments and the impact of herbicide selection on weed populations [5,6]. It is generally believed that *Ae. tauschii* originated from Azerbaijan and the southern coastal region of the Caspian Sea [7]. From its origin, it spread westward to central Syria through the valleys of mountainous southeastern Turkey and eastward to western China through the Kopet Dag Mountains of Turkmenistan [7]. As early as 1955, *Ae. tauschii* was first spotted in Henan Province, central China. Before the 1990s, *Ae. tauschii* only sporadically infested wheat in the provinces of Henan, Shanxi and Shaanxi [8]. Since the 2000s, it escaped from control management and experienced a rapid expansion to new habitats. However, the precise distribution and occurrence states of *Ae. tauschii* in wheat fields in China are obscure.

Although the genetic diversity of *Ae. tauschii* in China has been described previously, the materials in this research were focused on the populations from provinces of Henan, Shaanxi and Xinjiang [9,10]. Nevertheless, the distribution range of the species is gradually expanding across the wheat fields in China, particularly in recent years. Thus, more populations from a wider range of locations are required to comprehensively evaluate its genetic diversity and understand its spread in China.

Plant phenotypes and their biochemical or molecular markers can be used to evaluate their genetic diversity. Molecular markers play an increasingly important role in estimating genetic distance, due to their predictable genetic basis and abilities to distinguish specific segments of DNA or their products. Furthermore, it is less susceptible to environmental fluctuations, compared with morphological features [11]. Among that, Simple Sequence Repeats (SSRs) are tandemly repeated motifs of 1–6 nucleotides per unit that are widely spread throughout a genome. Compared with other molecular markers, SSRs are more preferable, due to their high polymorphism, reproducibility, high mutation rates and relative scoring ease [12–14]. It is reported that *Ae. tauschii* genome contains a large number of SSRs [15]. Recently, Yang et al. [5] used SSR markers to assess the genetic diversity of *Commelina communis*, a hard-to-control weed in summer crops. In another study, microsatellite markers were also used to evaluate the genetic diversity of *Olea europaea* and *Eleusine coracana* L. [16,17].

Currently, the latest information regarding the occurrence and infestation of *Ae. tauschii* in China is not well known. It is necessary to comprehensively understand its genetic diversity and population structure. The aims of this study were to (i) ascertain the occurrence state and infestation of *Ae. tauschii* in wheat fields in China, (ii) assess its genetic diversity and population structure and (iii) clarify the genetic relationships among different populations.

2. Materials and Methods

2.1. *Aegilops tauschii* Coss. Field Survey in China

The survey was conducted in 12 major wheat production provinces, municipalities and autonomous regions (Anhui, Beijing, Hebei, Henan, Hubei, Jiangsu, Shaanxi, Shandong, Shanxi, Sichuan, Tianjin, Xinjiang; hereafter referred to as “province”) during 2017–2019. The assessments in different provinces were carried out when wheat was at the ripening stage. Detailed survey time is shown in Table 1. County was considered as the basic survey unit, and all counties with wheat planting in each province were surveyed. The sampling locations were randomly selected within 10-km intervals, and within each location, three representative sites of 1 ha wheat field were identified. Sites were surveyed by two people walking in an inverted “W” pattern for approximately 200 m, and the presence or absence of *Ae. tauschii* was recorded. In addition, random countywide surveys were conducted by Plant Protection and Quarantine Stations of each county as supplement data of the local occurrence state of *Ae. tauschii*. The occurrence frequency of *Ae. tauschii* in each province = counties with *Ae. tauschii* infestation/the total counties surveyed in the province.

Table 1. The occurrence frequency of *Aegilops tauschii* Coss. and populations used for genetic diversity analysis in each province.

Province	Year	Month	Occurrence Frequency (%)	Number of Populations
Shaanxi	2017	5–6	56.96	43
Henan	2017	5–6	92.85	30
Anhui	2017	5	8.57	2
Jiangsu	2017	5	14.00	2
Xinjiang	2017	8	1.47	1
Hubei	2017	5	3.17	2
Sichuan	2017	4–5	0.91	0
Shandong	2018	6	87.07	58
Hebei	2018	6	75.24	43
Shanxi	2018	6	11.76	8
Beijing	2019	6	25.00	1
Tianjin	2019	6	12.50	2
Total	—	—	—	192

2.2. Collection of *Aegilops tauschii* Coss. Seeds

Above surveyed sites with serious *Ae. tauschii* infestation were selected to estimate spike density. On each selected site, five 1-m² quadrats were randomly chosen in which to count the spike number. Sites with *Ae. tauschii* density over 100 spikes m⁻² were determined as those from which to collect seeds, and the seeds were randomly collected from at least 500 individual plants and pooled as a population. In total, 192 *Ae. tauschii* populations were obtained (Table 1 and Table S1). In 2019, seeds of each population were planted in pots containing a 3:1 (v/v) mixture of soil and organic fertilizer. The pots were kept in the greenhouse (25/20 °C, day/night) with a 12-h photoperiod of 320 μmol m⁻²s⁻¹ and watered as necessary to maintain the optimum soil moisture. Ten plants from each population were marked, and the leaf tissue of them were sampled and then stored at −80 °C.

2.3. DNA Extraction

One hundred milligrams of leaf tissue were ground into a fine powder in liquid nitrogen for each population. DNA was isolated using a Tiangen DNasecure Plant Kit (catalogue no. DP 320; Tiangen Biotech Co., Ltd., Beijing, China). The quality of DNA was determined visually using 1% agarose gels. To ensure consistent quantities among the samples, DNA solution was diluted to 50 ng μL⁻¹ after the concentration was measured, using a Nanodrop 2000 (Thermo Scientific, Waltham, MA, USA).

2.4. SSR Primer Pairs Screening

More than 60 microsatellite primers isolated and mapped from the bread wheat D genome [18,19] were used for PCR amplification. In total, 17 primer pairs with specific amplicons were selected as candidate primers (Table 2). The PCR mixture contained 50 ng genomic DNA, 0.5 μL forward primer and reverse primer (1.0 × 10⁻⁸ mol L⁻¹), 12.5 μL 2× Taq PCR Master Mix (catalogue no. KT 201; Tiangen, Beijing, China) and 10.5 μL ddH₂O to a total volume of 25 μL. Amplification began with a denaturation step for 4 min at 94 °C, followed by 32 cycles of 94 °C for 30 s, the annealing temperature of each primer for 30 s and 72 °C for 30 s and a final extension at 72 °C for 40 min. PCR products were evaluated with 1.8% agarose gels.

Table 2. Characteristics of 17 Simple Sequence Repeat (SSR) markers used for evaluating genetic diversity of 192 *Aegilops tauschii* Coss. populations from China.

Primer Name	Primer Sequence (5'-3') ¹	Tm (°C)	5' Modify	Allele Size (bp)
<i>Xgdm153-5D</i>	F-TATAGGCAAATTAATTAAGACG R-ATCTTTATGTGAGTACACTGC	50.3	<i>hex</i>	208–277
<i>Xgwm314-3D</i>	F-AGGAGCTCCTCTGTGCCAC R-TTCGGGACTCTCTTCCCTG	61.1	<i>fam</i>	160–181
<i>Xgwm295-7D</i>	F-GTGAAGCAGACCCACAACAC R-GACGGCTGCGACGTAGAG	60	<i>fam</i>	232–254
<i>Xgwm102-2D</i>	F-TCTCCCATCCAACGCCTC R-TGTTGGTGGCTTGACTATTG	58	<i>hex</i>	138–147
<i>Xgdm111-1D</i>	F-CACTCACCCCAAACCAAAGT R-GATGCAATCGGGTCGTTAGT	55.3	<i>fam</i>	186–193
<i>Xgdm129-4D</i>	F-GAGCAGGCAGCAGCTAGC R-TGCATCATCATCGGTCAAGT	60	<i>fam</i>	101–111
<i>Xgwm165-4D</i>	F-TGCAGTGGTCAGAGTTTTCC R-CTTTCTTTCAGATTGCGCC	55.3	<i>hex</i>	189–193
<i>Xgwm157-2D</i>	F-GTCGTGCGGGTAAGCTTG R-GAGTGAACACACGAGGCTTG	56.7	<i>hex</i>	98–100
<i>Xgwm383-3D</i>	F-ACGCCAGTTGATCCGTAAAC R-GACATCAATAACCGTGGATGG	60	<i>hex</i>	215–230
<i>Xgdm33-1D</i>	F-GGCTCAATTCAACCGTTCTT R-TACGTTCTGGTGGCTGCTC	60	<i>hex</i>	122–204
<i>Xgwm182-5D</i>	F-TGATGTAGTGAGCCCATAGGC R-TTGCACACAGCCAAATAAGG	58	<i>fam</i>	148–171
<i>Xgwm539-2D</i>	F-CTGCTCTAAGATTCATGCAACC R-GAGGCTTGTGCCCTCTGTAG	58.5	<i>fam</i>	98–106
<i>Xgwm469-6D</i>	F-CAACTCAGTGCTCACACAACG R-CGATAACCACTCATCCACACC	57	<i>hex</i>	151–205
<i>Xgwm55-6D</i>	F-GCATCTGGTACTAGCTGCC R-TCATGGATGCATCACATCCT	56.9	<i>hex</i>	124–136
<i>Xgdm126-1D</i>	F-TCCATCATATCCGTAGCACA R-CGTGGTTGATTCAGGAGGT	54.1	<i>fam</i>	178–216
<i>Xgwm205-5D</i>	F-CGACCCGGTTCACCTCAG R-AGTCGCCGTTGTATAGTGCC	57.1	<i>fam</i>	126–138
<i>Xgwm583-5D</i>	F-TTCACACCCAACCAATAGCA R-TCTAGGCAGACACATGCCTG	57.5	<i>hex</i>	138–166

¹ F, the forward primer; R, the reverse primer.

2.5. SSR Detection

The selected candidate primers were used for amplification of all samples for a second time. This time, each forward primer was modified with fluorophores (*hex* or *fam*) at the 5' ends. PCR products with different fluorophores were mixed and analyzed by an ABI PRISM 3730xl DNA Sequencer with GS500 (Applied Biosystems, Foster city, USA) as an internal size standard. Alleles of each locus were determined by GeneMarker (version 2.2.0).

2.6. Data Analyses

To generate a binary data matrix for further analysis, each allele was scored as 0 when absent and 1 when present. The effective number of alleles (N_e), the observed number of alleles (N_a), the observed heterozygosity (H_o), the expected heterozygosity (H_e), Shannon's information index (I), the proportion of differentiation among populations (F_{st}),

Nei's gene diversity (H) [20], Wright's fixation index ($F = 1 - H_o/H_e$) and the gene flow ($Nm = [(1/Fst) - 1]/4$) were calculated by the POPGENE 32-bit version program. Polymorphism information content (PIC) was calculated according to

$$PIC = 1 - \sum P_{ij}^2, \quad (1)$$

where P_{ij} was the frequency of the j th genotype at the i th loci. Pairwise, genetic similarity among different populations was calculated based on the Dice similarity coefficient, and then a cluster was generated with the unweighted pair group method using arithmetic means (UPGMA) by NTSys 2.10e and MEGA 7.0 software (Tokyo, Japan). Under a Bayesian model, the analysis of population structure was carried out using STRUCTURE 2.3.4 [21]. The K value was set from 2 to 10. Under an admixture model using correlated allele frequencies, each K value was run ten times, independently. The iterations of a burn-in period and Markov chain Monte Carlo (MCMC) steps were set to 100,000 and 200,000, respectively. The most appropriate K value was determined with Structure Harvester. Analysis of molecular variance (AMOVA) was performed using GenAlex 6.1 to further investigate the population structure of *Ae. tauschii*. GenAlex 6.1 was also used to obtain, pairwise, F_{st} and Nm among different genetic groups based on the results of population structure analysis.

3. Results

3.1. The Distribution and Occurrence of *Aegilops tauschii* Coss. in China

Although all the surveyed provinces had *Ae. tauschii* infestations, the incidences were different among these provinces. *Ae. tauschii* was most commonly found in Henan, Hebei and Shandong provinces, with occurrence frequency over 70%. In Henan Province, *Ae. tauschii* occurred in all counties except eight counties in Xinyang. No *Ae. tauschii* plants were found in 19 counties belonging to Yantai, Qingdao, Weihai and Rizhao of Shandong Province. Except for 25 counties in Zhangjiakou, Chengde and Tangshan of Hebei Province, infestations of *Ae. tauschii* with wheat fields were observed in other counties. In addition, in Shaanxi Province, *Ae. tauschii* infested wheat fields in over half of counties (56.96%). However, Shanxi, Jiangsu, Beijing and Tianjin exhibited relatively low occurrence frequency of *Ae. tauschii* (10–40%). Among 16 surveyed districts in Beijing, *Ae. tauschii* was only found in Haidian, Tongzhou, Fangshan and Daxing. In Shanxi Province, the weed was mainly found in the counties located at the south of wheat planting, such as Hongtong, Huozhou and Linyi counties. By contrast, Hubei, Anhui, Sichuan and Xinjiang provinces showed very low infestations of *Ae. tauschii* (<10%). For example, in Hubei Province, *Ae. tauschii* was only found in Zaoyang and Xiangzhou counties among 63 surveyed counties (Table 1).

3.2. Polymorphism of the SSR Markers

In total, 80 polymorphic alleles were amplified using 17 SSR markers, with size varying from 98 bp to 277 bp (Table 2). Among 192 *Ae. tauschii* populations, the observed number of alleles (N_a) in different loci ranged from 2 to 11, with an average of 4.706. The mean value of the effective number of alleles (N_e) was 1.684, varying from 1.011 to 3.534. The highest value of Shannon's information index (I) was 0.032, and the lowest value was 1.385. The ranges of the expected heterozygosity (H_e) and observed heterozygosity (H_o) were from 0.010 to 0.719 (mean = 0.285) and 0 to 1 (mean = 0.181), respectively. The average of gene diversity based on Nei's gene diversity (H) was 0.284, with a range from 0.010 to 0.717. Polymorphic information content (PIC), with a mean of 0.284, varied from 0.010 to 0.717 (Table 3).

Table 3. PCR amplification results and genetic parameters of microsatellite loci ¹.

Primer Name	<i>Na</i>	<i>Ne</i>	<i>Ho</i>	<i>He</i>	<i>H</i>	PIC	<i>I</i>	<i>F</i>	<i>Fst</i>	<i>Nm</i>
<i>Xgdm153-5D</i>	4	1.021	0.005	0.021	0.021	0.021	0.069	0.748	0.874	0.036
<i>Xgwm314-3D</i>	7	3.534	0.609	0.719	0.717	0.717	1.385	0.151	0.575	0.185
<i>Xgwm295-7D</i>	7	1.851	0.010	0.461	0.460	0.460	0.852	0.977	0.989	0.003
<i>Xgwm102-2D</i>	3	1.320	0.005	0.244	0.243	0.243	0.460	0.979	0.989	0.003
<i>Xgdm111-1D</i>	3	1.042	0.000	0.041	0.041	0.041	0.113	1.000	1.000	0.000
<i>Xgdm129-4D</i>	3	1.021	0.000	0.021	0.021	0.021	0.065	1.000	1.000	0.000
<i>Xgwm165-4D</i>	2	1.587	0.000	0.371	0.370	0.360	0.557	1.000	1.000	0.000
<i>Xgwm157-2D</i>	2	1.011	0.000	0.010	0.010	0.010	0.033	1.000	1.000	0.000
<i>Xgwm383-3D</i>	5	2.656	0.912	0.625	0.624	0.623	1.102	−0.462	0.269	0.679
<i>Xgdm33-1D</i>	11	2.641	0.010	0.623	0.621	0.621	1.239	0.983	0.992	0.002
<i>Xgwm182-5D</i>	5	1.328	0.000	0.248	0.247	0.257	0.487	1.000	1.000	0.000
<i>Xgwm539-2D</i>	2	1.011	0.000	0.010	0.010	0.010	0.032	1.000	1.000	0.000
<i>Xgwm469-6D</i>	10	2.786	1.000	0.643	0.641	0.644	1.214	−0.560	0.220	0.886
<i>Xgwm55-6D</i>	6	2.596	0.505	0.616	0.615	0.616	1.118	0.178	0.589	0.174
<i>Xgdm126-1D</i>	5	1.094	0.010	0.086	0.086	0.086	0.231	0.879	0.939	0.016
<i>Xgwm205-5D</i>	2	1.011	0.000	0.010	0.010	0.010	0.033	1.000	1.000	0.000
<i>Xgwm583-5D</i>	3	1.105	0.005	0.095	0.095	0.095	0.219	0.945	0.973	0.007
Mean	4.706	1.683	0.181	0.285	0.284	0.284	0.542	0.695	0.848	0.117
Total	80	—	—	—	—	—	—	—	—	—

¹ *Na*, Observed number of alleles; *Ne*, Effective number of alleles; *Ho*, Observed heterozygosity; *He*, Expected heterozygosity; *H*, Nei's gene diversity; PIC, Polymorphism information content; *I*, Shannon's information index; *F*, Wright's fixation ($F = 1 - Ho/He$); *Fst*, Proportion of differentiation among populations; *Nm*, Gene flow ($Nm = [(1/Fst) - 1]/4$).

Wright's fixation index (*F*), a measure of heterozygotic deficiency or excess, varied between −0.560 and 1.000, with an average of 0.695 per marker. Two SSR loci (*Xgwm383-3D* and *Xgwm469-6D*, $F < 0$) demonstrated excess heterozygosity, while the other 15 loci ($F > 0$) displayed deficient heterozygosity, revealing deficient heterozygosity for the 17 SSR markers. The proportion of differentiation among populations (*Fst*) and gene flow (*Nm*) ranged from 0.220 to 1.000 (mean = 0.848) and 0.000 to 0.886 (mean = 0.117), respectively, indicating a high level of differentiation among populations and low gene flow in *Ae tauschii* (Table 3).

3.3. Comparative Genetic Diversity Analysis of *Aegilops tauschii* Coss. from Different Provinces

Ae. tauschii from different provinces displayed variable levels of genetic diversity (Figure 1). The *Na* varied from 1.118 to 3.059 and averaged 1.845, while the *Ne* ranged from 1.118 to 1.653, with a mean of 1.410. The PIC, *Ho*, *He* and *I* per province ranged from 0.059 to 0.276 (mean = 0.184), 0.118 to 0.294 (mean = 0.185), 0.078 to 0.294 (mean = 0.223) and 0.082 to 0.497 (mean = 0.303), respectively. The population differentiations in Henan, Shandong, Shaanxi and Hebei provinces were relatively high. Additionally, the lowest genetic diversity indexes (*Na*, *Ne*, *Ho*, *He*, *I* and PIC) were observed in Hubei Province, among the studied provinces.

3.4. Cluster Analysis

Based on the Dice similarity coefficient, a dendrogram was constructed with UPGMA (Figure 2). The value of the Dice similarity coefficient ranged from 0.31 to 1.00. When the Dice similarity coefficient was 0.75, 192 *Ae. tauschii* populations could be mainly divided into four clusters. Some clusters included populations from different provinces, while some populations from the same province were dispersed into different clusters. Cluster 1 contained most populations from Shandong and Hebei, approximately half of populations from Henan and very few populations from Shaanxi. Hubei and Tianjin populations were scattered into this cluster as well. Cluster 2 included Jiangsu and Shanxi populations, together with the majority of Shaanxi populations and a few populations from Shandong and Hebei. Very few populations from Henan were also dispersed into cluster 2. The remaining populations from Henan and Shaanxi, as well as a few pop-

ulations from Shandong and Hebei, formed cluster 3, and cluster 4 only contained the Xinjiang population.

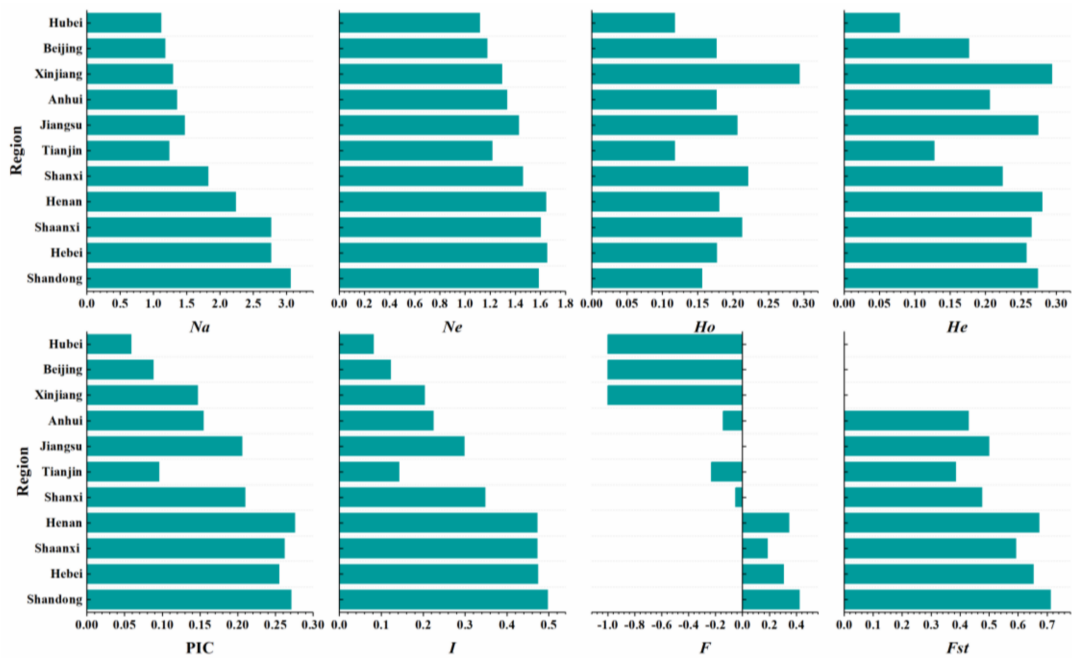


Figure 1. Comparative genetic diversity analysis of *Aegilops tauschii* Coss. from different provinces. Abbreviations are as shown in Table 3.

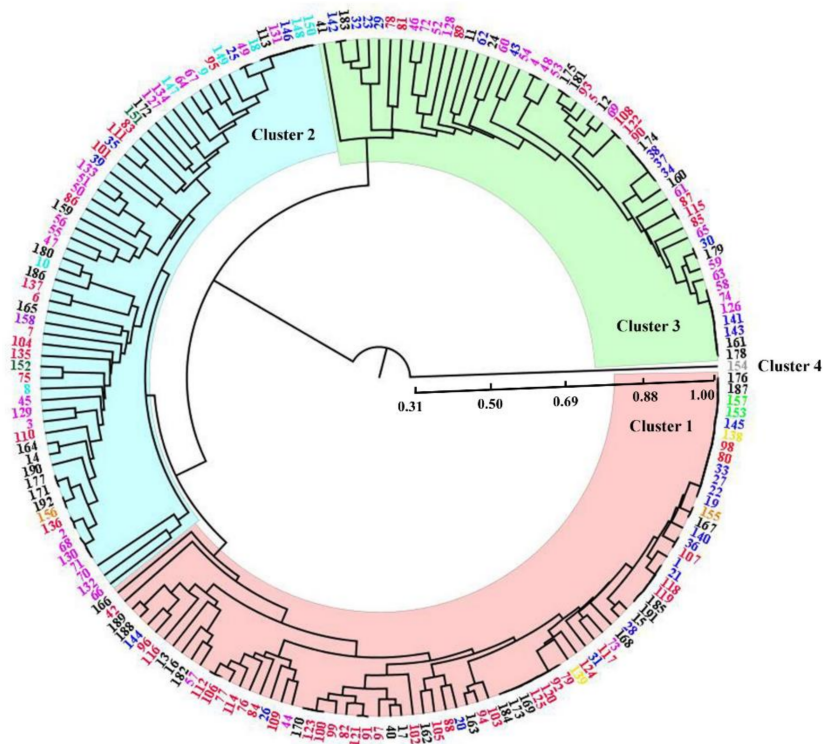


Figure 2. Dendrogram of the 192 *Aegilops tauschii* Coss. populations based on 17 SSR markers. The population codes are colored by geographic locations. The branches are colored by genetic distance. The population codes by province: Hebei, black; Shandong, red; Henan, blue; Shanxi, aqua; Shaanxi, fuchsia; Tianjin, yellow; Anhui, orange; Hubei, green; Jiangsu, dark green; Beijing, violet; Xinjiang, gray. The branches by genetic cluster: cluster 1, pink; cluster 2, aqua; cluster 3, green; cluster 4, white (population code referred to Table S1).

3.5. Population Structure Analysis

To illustrate the underlying population structure of the studied populations, the number of hypothetical groups for the 192 populations was evaluated by the Bayesian model-based clustering method. The structure simulation by Structure Harvester indicated that $K = 3$ is optimal (Figure 3A–D); in other words, it is rational to divide the given populations into three groups: group 1 (green), group 2 (red) and group 3 (purple) (Figure 3E). The result was supported by UPGMA analysis, except that populations 13, 116, 166, 188 and 189, belonging to cluster 1, and populations 72 and 128, belonging to cluster 3, were collected into group 2. Additionally, Xinjiang population belonging to cluster 4 was included in group 2 as well.

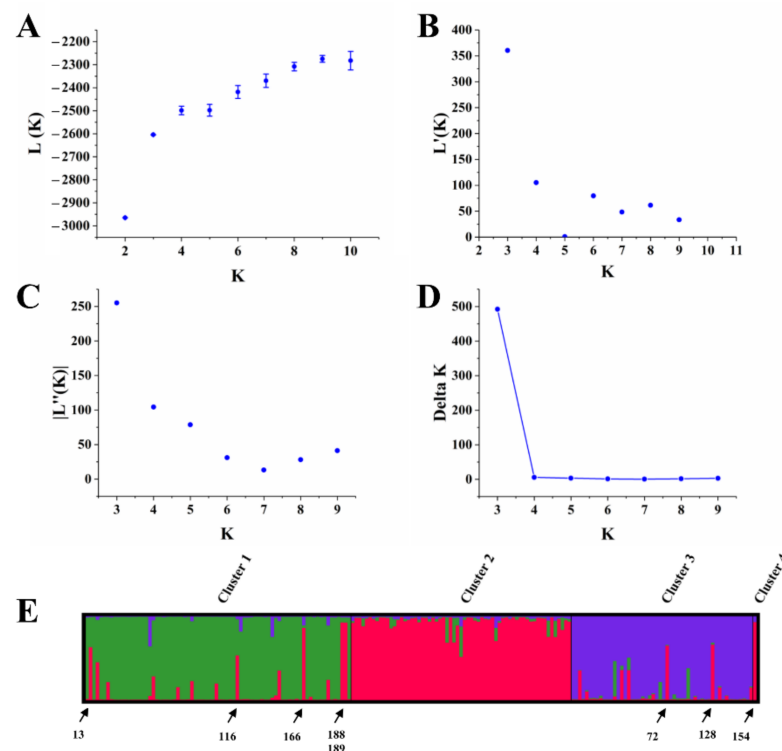


Figure 3. STRUCTURE analysis of the 192 *Aegilops tauschii* Coss. populations. (A) Plot of mean likelihood $L(K)$ and variance per K value. (B) Plot of mean rate of change of the likelihood distribution $L'(K)$ per K value. (C) Plot of absolute value of the second order rate of change of the likelihood distribution $|L''(K)|$ per K value. (D) Plot of Delta K value; Delta $K = \text{mean}(|L''(K)|)/\text{sd}(L(K))$ per K value. (E) Genetic relationships of the 192 *Ae. tauschii* populations using STRUCTURE analysis. Each individual is represented by a thin vertical line, which is partitioned into K colored segments that represent the individuals' estimated membership fractions in K clusters.

The AMOVA analysis showed that variance occurring among the three hypothetical groups accounted for 7% of the total variation. Majority variations (67%) occurred among different populations within same group, and 26% was attributed to variation within populations (Table 4). Additionally, the lowest mean value of F_{st} and highest mean value of N_m were detected between group 2 and group 3 (Table 5), indicating that there were markedly low genetic differentiation and high gene flow between group 2 and group 3. However, a reverse phenomenon was observed between group 1 and group 3.

Table 4. Analysis of molecular variation (AMOVA) among *Aegilops tauschii* Coss. groups using 17 SSR primers.

Source of Variation	Degree of Freedom (<i>df</i>)	Sum of Squares	Mean of Squares	Estimate Variance	Percentage of Variation (%)
Among groups	2	130.464	65.232	0.439	7
Among populations within groups	189	1832.106	9.694	4.071	67
Within populations	192	298	1.552	1.552	26
Total	383	2260.57	6.062	6.062	100

Table 5. Pairwise proportion of differentiation among populations (*F_{st}*, below the diagonal) and gene flow (*N_m*, above the diagonal) of *Aegilops tauschii* Coss. among different groups.

Group	1	2	3
1	0	2.721	2.362
2	0.084 ** ¹	0	8.264
3	0.096 **	0.029 **	0

¹ **, Significant difference at 0.01 probability.

4. Discussion

By 2007, it was reported that *Ae. tauschii* was mainly distributed in Hebei, Shanxi, Chongqing, Shandong, Jiangsu, Henan, Inner Mongolia and Shaanxi provinces in China [1]. Our field survey showed that the grass weed further spread into Anhui, Sichuan, Hubei, Xinjiang, Beijing and Tianjin during recent years. The varied occurrence frequency of *Ae. tauschii* in the surveyed provinces indicated different infestation levels across wheat fields in China. Thus, effective control of *Ae. tauschii* is urgently needed. The survey was conducted during 2017–2019. *Ae. tauschii* is easily spread across wheat fields, due to the limited effective herbicides against it, extensive ecology adaptability and high propagation coefficient [3,22]. Therefore, the present occurrence frequency of *Ae. tauschii* may be higher than our survey results.

Given the expanding distribution ranges and serious infestations, the genetic diversity and population structure of *Ae. tauschii* from surveyed sites were studied to understand its population relationships and spreading across wheat fields in China. SSR markers are generally considered as effective tools to explain genetic diversity and population structure [16,17,23]. Among the strictly screened SSR markers, five markers were highly informative, with PIC > 0.5; three markers were reasonably informative, with 0.25 < PIC < 0.5; and nine markers were minimally informative, with PIC < 0.25, according to a criterion published previously [24]. The mean value of PIC per marker was between 0.25 and 0.5, indicating a reasonably discriminating and indicative property of the selected markers. Therefore, these markers are reliable in illuminating the population structure and assessing the genetic diversity of *Ae. tauschii*. In another study, however, the PIC value (0.55) for *Ae. tauschii* was higher than the present results, which may be explained by the different microsatellite markers and *Ae. tauschii* populations used in the two studies [10].

Eighty alleles were collectively identified by the 17 SSR markers, with a mean of 4.706 alleles per marker. This observation was largely similar to previous studies [25–27]. Beyond that, the mean values of *H* and *I* implied moderately high genetic diversity in *Ae. tauschii*, which was strongly supported by a high level of genetic variance detected in other research [9,10]. Genetic diversity plays an instrumental role in the capability to survive in different environments, as well as in the population evolution [16,28]. Unquestionably, the high genetic diversity of *Ae. tauschii* would intensify the difficulties of managing it. Furthermore, Hubei populations, with narrow genetic backgrounds, are closely related to each other. Genetic diversity partly depends on the species' evolutionary history [28]. The invasion of *Ae. tauschii* to wheat fields in Hubei Province was reported recently [3]. Therefore, it was assumed that the low genetic diversity of Hubei populations was related with its short history of infestation; in contrast, fewer populations found from the province may also be responsible for this phenomenon.

In the dendrogram, genetic distance did not seem to be related to geographic distribution, since some populations, especially those collected from different regions of China, were not grouped in a way that exactly relied on geographic location in the cluster analysis. That may be attributed to the long-distance seed distribution mediated by harvesting activities [1].

Our findings demonstrated that Xinjiang populations showed a clear separation from Yellow River Basin populations (Henan and Shaanxi), which is consistent with previous studies [9,10,29,30]. Based on the cluster analysis, a closer genetic relationship was found among some Shandong, Hebei and Henan populations, which are adjacent provinces, geographically. Historically, *Ae. tauschii* was first found in Henan Province [8]. Given that, it was speculated that Shandong and Hebei populations were introduced from Henan. Fang et al. [31] reported that transregional operation (from the South to the North in China) of combine machines in harvesting could facilitate *Ae. tauschii* seed distribution among different provinces. In the future, more attention should be paid to the spread of *Ae. tauschii* across wheat fields. For example, combine machines in harvesting should be timely cleaned in case they carry *Ae. tauschii* seeds during wheat harvesting season. Generally, Shanxi and Shaanxi populations exhibited distant genetic relationship with populations from other provinces. This might be ascribed to a long-time evolution or genetic changes associated with native climate conditions. However, a few Shandong and Hebei populations were clustered together with the above populations, which is probably related with wheat seed exchange among these provinces or seeds dispersion by Yellow River to cities located downstream. Therefore, the quality of wheat variety and irrigation water should be monitored strictly. UPGMA and STRUCTURE analysis showed that Hubei and Tianjin populations were closely related with Shandong, Hebei and Henan populations. *Ae. tauschii* was found in wheat fields in Hubei and Tianjin, recently [3]. In view of geographical locations among these provinces, it was assumed that Tianjin and Hubei populations were introduced from Hebei and Henan provinces, respectively, suggesting that the infestation of *Ae. tauschii* was gradually expanded to the north and south in China.

Cluster and admixture analyses based on the Bayesian model were regarded as a method to clarify the species' origin, to shed light on genetic relationships and to determine the gene flow among different populations [32,33]. STRUCTURE analysis suggested that the 192 *Ae. tauschii* populations in China could be divided into three groups, which was supported by UPGMA analysis. To some extent, most populations of Shandong and Hebei, which were mainly distributed in the east of *Ae. tauschii*-infested areas, were grouped together. Group 2 was mainly composed of Shanxi and Shaanxi populations spreading west of the Yellow River Basin. Group 3 was a mixture of Shandong, Henan, Hebei and Shaanxi populations. Based on that, future work to evaluate *Ae. tauschii* control strategies should be conducted in the sites representing the three groups in China. Some populations were composed of different colored segments, indicating that there was a certain degree of genetic admixture in the tested populations. This situation might be resulted from the gene flow among the populations. Human activity-mediated seed spread appeared to accelerate gene recombination and movement in different populations.

5. Conclusions

Presently, *Ae. tauschii* was found in all provinces surveyed. Shandong, Henan and Hebei provinces had very high infestations of the weed. A moderately high level of genetic diversity, high differentiation, deficient heterozygosity and limited gene flow were discovered in this species. Closer genetic relationships were found among some populations geographically located distantly, which is most likely implicated with long-distance seed distribution. The STRUCTURE and UPGMA analyses grouped the 192 populations into three main groups: Roughly, the first group was dominated by populations from the east of the Yellow River Basin, the second group involved populations mainly located at the west of the Yellow River Basin and the last group was a mixture of populations from

Henan, Shaanxi, Hebei and Shandong. These results will be helpful for the selection of representative sites to evaluate and develop control techniques and to further establish diverse management strategies aimed at different geographic areas or genetic groups of *Ae tauschii*.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agriculture11040311/s1>, Table S1: The geographic locations of 192 *Aegilops tauschii* Coss. populations used in the genetic diversity analysis.

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References

- Zhang, C.X.; Li, X.J.; Huang, H.J.; Wei, S.H. Alert and prevention of the spreading of *Aegilops tauschii*, a worst weed in wheat field. *Acta Phytotaxonomica Sin.* **2007**, *34*, 103–106.
- Sohail, Q.; Shehzad, T.; Kilian, A.; Eltayeb, A.E.; Tanaka, H.; Tssujimoto, H. Development of diversity array technology (DARt) markers for assessment of population structure and diversity in *Aegilops tauschii*. *Breed. Sci.* **2012**, *62*, 38–45. [[CrossRef](#)]
- Yu, H.Y.; Li, X.J. Distribution of *Aegilops tauschii* Coss. *China Res. Prog. J. Weed Sci.* **2018**, *36*, 1–7.
- Fang, F.; Zhang, C.X.; Huang, H.J.; Li, M.; Gao, X.; Li, Y.; Wei, S.H. The occurrence of Tausch's goatgrass (*Aegilops tauschii* Coss.) in wheat fields and its effect on wheat yield. *Acta Ecol. Sin.* **2014**, *34*, 3917–3923.
- Yang, J.; Yu, H.Y.; Li, X.J.; Dong, J.G. Genetic diversity and population structure of *Commelina communis* in China based on simple sequence repeat markers. *J. Integr. Agr.* **2018**, *17*, 2292–2301. [[CrossRef](#)]
- Zhao, S.Y.; Sun, S.G.; Dai, C.; Gituru, R.W.; Chen, J.M.; Wang, Q.F. Genetic variation and structure in native and invasive *Solidago canadensis* populations. *Weed Res.* **2015**, *55*, 163–172. [[CrossRef](#)]
- Matsuoka, Y.; Takumi, S.; Kawahara, T. Flowering time diversification and dispersal in central Eurasian wild wheat *Aegilops tauschii* Coss.: Genealogical and ecological framework. *PLoS ONE* **2008**, *3*, e3138. [[CrossRef](#)]
- Yan, J.; Yang, J.L.; Cui, N.R.; Zhong, J.P.; Dong, Y.C.; Sun, Y.Z.; Zhong, G.Y. The *Aegilops tauschii* Cosson from Yi-Li, Xinjiang, China. *Acta Agron. Sin.* **1984**, *10*, 1–8.
- Li, Y.G.; Sun, Y.Z.; Zhang, D.L.; Li, S.P. Genetic diversity of *Aegilops tauschii* accessions native to China revealed by ISSR markers. *J. Triticeae Crop.* **2017**, *37*, 30–39.
- Wei, H.; Li, J.; Peng, Z.; Lu, B.; Zhao, Z.; Yang, W. Relationships of *Aegilops tauschii* revealed by DNA fingerprints: The evidence for agriculture exchange between China and the West. *Prog. Nat. Sci.* **2008**, *18*, 1525–1531. [[CrossRef](#)]
- Staub, J.E.; Meglic, V. Molecular genetic markers and their legal relevance for cultivar discrimination: A case study in cucumber. *HortTechnology* **1993**, *3*, 291–300. [[CrossRef](#)]
- Liu, X.B.; Feng, B.; Li, J.; Yan, C.; Yang, Z.L. Genetic diversity and breeding history of Winter Mushroom (*Flammulina velutipes*) in China uncovered by genomic SSR markers. *Gene* **2016**, *591*, 227–235. [[CrossRef](#)] [[PubMed](#)]
- Zane, L.; Bargelloni, L.; Patarnello, T. Strategies for microsatellite isolation: A review. *Mol. Ecol.* **2002**, *11*, 1–16. [[CrossRef](#)] [[PubMed](#)]
- Selkoe, K.A.; Toonen, R.J. Microsatellites for ecologists: A practical guide to using and evaluating microsatellite markers. *Ecol. Lett.* **2006**, *9*, 615–629. [[CrossRef](#)]
- Luo, M.; Gu, Y.Q.; Puiu, D.; Wang, H.; Twardziok, S.O.; Deal, K.R.; Huo, N.; Zhu, T.; Wang, L.; Wang, Y.; et al. Genome sequence of the progenitor of the wheat D genome *Aegilops tauschii*. *Nature* **2017**, *551*, 498–502. [[CrossRef](#)] [[PubMed](#)]
- Pandian, S.; Satish, L.; Rameshkumar, R.; Muthuramalingam, P.; Rency, A.S.; Rathinapriya, P.; Ramesh, M. Analysis of population structure and genetic diversity in an exotic germplasm collection of *Eleusine coracana* (L.) Gaertn. using genic-SSR markers. *Gene* **2018**, *653*, 80–90. [[CrossRef](#)] [[PubMed](#)]

17. Noormohammadi, Z.; Trujillo, I.; Belaj, A.; Ataei, S.; Hosseini-Mazinan, M. Genetic structure of Iranian olive cultivars and their relationship with Mediterranean's cultivars revealed by SSR markers. *Sci. Hort.* **2014**, *178*, 175–183. [[CrossRef](#)]
18. Pestsova, E.; Ganal, M.W.; Röder, M.S. Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. *Genome* **2000**, *43*, 689–697. [[CrossRef](#)]
19. Röder, M.S.; Korzun, V.; Wendehake, K.; Plaschke, J.; Tixier, M.; Leroy, P.; Ganal, M.W. A microsatellite map of wheat. *Genetics* **1998**, *149*, 2007–2023.
20. Nei, M. Analysis of gene diversity in subdivided population. *Proc. Natl. Acad. Sci. USA* **1973**, *70*, 3321–3323. [[CrossRef](#)]
21. Pritchard, J.K.; Stephens, M.; Donnelly, P. Inference of population structure using multilocus genotype data. *Genetics* **2000**, *155*, 945–959.
22. Yu, H.; Yang, J.; Cui, H.; Li, Z.; Jia, F.; Chen, J.; Li, X. Effects of plant density on tillering in the weed grass *Aegilops tauschii* Coss. and its phytohormonal regulation. *Plant Physiol. Bioch.* **2020**, *157*, 70–78. [[CrossRef](#)]
23. Tang, X.T.; Tao, H.H.; Du, Y.Z. Microsatellite-based analysis of the genetic structure and diversity of *Aleurocanthus spiniferus* (Hemiptera: Aleyrodidae) from tea plants in China. *Gene* **2015**, *560*, 107–113. [[CrossRef](#)]
24. Botstein, D.; White, R.L.; Skolnick, M.; Davis, R.W. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* **1980**, *32*, 314–331.
25. Lelley, T.; Stachel, M.; Grausgruber, H.; Vollmann, J. Analysis of relationships between *Aegilops tauschii* and the D genome of wheat utilizing microsatellites. *Genome* **2000**, *43*, 661–668. [[CrossRef](#)] [[PubMed](#)]
26. Naghavi, M.R.; Nardi, M.; Pirseyedi, S.M.; Kazemi, M.; Potki, P.; Ghaffari, M.R. Comparison of genetic variation among accessions of *Aegilops tauschii* using AFLP and SSR markers. *Genet. Resour. Crop Evol.* **2007**, *54*, 237–240. [[CrossRef](#)]
27. Saeidi, H.; Rahiminejad, M.R.; Vallian, S.; Heslop-Harrison, J.S. Biodiversity of diploid D-genome *Aegilops tauschii* Coss. in Iran measured using microsatellites. *Genet. Resour. Crop Evol.* **2006**, *53*, 1477–1484. [[CrossRef](#)]
28. Hamrick, J.L.; Didt, M.J.W. Effects of life history traits on genetic diversity in plant species. *Philos. Trans. R. Soc. Lond. Ser. B* **1996**, *351*, 1291–1298.
29. Wang, J.R.; Luo, M.C.; Chen, Z.X.; You, F.M.; Wei, Y.M.; Zheng, Y.L.; Dvorak, J. *Aegilops tauschii* single nucleotide polymorphisms shed light on the origins of wheat D-genome genetic diversity and pinpoint the geographic origin of hexaploid wheat. *New Phytol.* **2013**, *198*, 925–937. [[CrossRef](#)]
30. Lan, X.J.; Liu, D.C.; Wei, Y.M.; Yan, Z.H.; Zheng, Y.L. Diversity of esterase isozyme in *Aegilops tauschii* Cosson. *Guihaia* **2001**, *21*, 77–80.
31. Fang, F.; Gao, X.X.; Wei, S.H.; Li, Y.; Li, M.; Zhang, C.X. Occurrence and effects of *Aegilops tauschii* in China. *Acta Prataculturae Sin.* **2015**, *24*, 194–201.
32. Breton, C.; Pinatel, C.; Médail, F.; Bonhomme, F.; Bervillé, A. Comparison between classical and Bayesian methods to investigate the history of olive cultivars using SSR-polymorphisms. *Plant Sci.* **2008**, *175*, 524–532. [[CrossRef](#)]
33. Belaj, A.; Muñoz-diez, C.; Baldoni, L.; Porceddu, A.; Barranco, D.; Satovic, Z. Genetic diversity and population structure of wild olives from the North-western Mediterranean assessed by SSR markers. *Ann. Bot.* **2007**, *100*, 449–458. [[CrossRef](#)] [[PubMed](#)]