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Genetic relationships between populations of *Aegilops tauschii* Coss. (Poaceae) using SCoT molecular markers

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Abstract. The genus Aegilops has an important potential utilization in wheat improvement because of its resistance to different biotic and abiotic stresses and close relation with the cultivated wheat. Aegilops tauschii grows in Iran, westward to Turkey and eastward to Afghanistan and China with a distribution center in the south of Caspian Sea. In spite of its very good biochemical characterization, the knowledge about the DNA variability is very limited and no DNA markers were used to analyses the genomic variability of the populations, up to date. In the present study, genetic diversity of 117 Aegilops tauschii, individuals nine populations were studied using 10 Start Codon Targeted (SCoT) markers. High polymorphic bands (96.33%), polymorphic information content (0.48) and allele number (1.024) showed SCoT as a reliable marker system for genetic analysis in Aegilops tauschii. At the species, the percentage of polymorphic loci [P] was 66.30%, Nei's gene diversity [H] was 0.35, Shannon index [I] was 0.33 and unbiased gene diversity [UHe] was 0.37. Genetic variation within populations (59%) was higher than among populations (41%) based on analysis of molecular variance (AMOVA). We used SCoT molecular marker for our genetic investigation with the following aims: 1— Investigate genetic diversity both among and with date Aegilops tauschii, 2—Identify genetic groups within these nine populations Aegilops tauschii, and 3—produce data on the genetic structure of date Aegilops tauschii populations. The results obtained revealed a high within-population genetic variability.

Keywords: *Aegilops tauschii*, genetic admixture, gene flow, genetic structure, SCoT.

INTRODUCTION

Genetic variability description specifies differences among individuals or populations of the same species and serves as a very good tool for plant breeding and conservation programmes (Karasakal et al, 2020a, 2020b; Huang et al, 2021; Hou et al, 2021, Guo et al, 2021). Different types of DNA markers have been applied in evaluation of genetic diversity of different plants, considering also the effects of the plant growing environment and

developmental stage (BI *et al*, 2021; Cheng *et al*, 2021; Khayatnezhad and Gholamin, 2020, 2021a, 2021b).

Crop wild relatives (CWRs) are valuable plant genetic resources (PGR) owing to high affinity to crops, including crop progenitors, to improve several properties of crops following the yield improvement or stability, pest or disease resistance, etc. They appear as a tangible genetic diversity that has been experimentally used for several centuries (Maxtend et al. 2015). However, the development in the biotechnological methodologies also allowed the transfer of genes from CWR species as the valuable reservoirs of genetic diversity to improve the crops (Hajjar and Hodgkin, 2007). Iran is located in Middle Eastern center of cultivated plants that are considered in the higher ranks in terms of conservation priorities for CWRs in the world (Saydi and Mehrabian, 2019). Besides, there are several wild relatives of cereals (e.g., *Triticum, Hordeum, Aegilops, secale*, etc.) (Bor, 1970) that show high potentials to improve the cereals.

There exist 22 *Aegilops* and five *Triticum* species in three ploidy levels consisting of diploid (2n=2x=14), tetraploid (2n=4x=28), and hexaploid (2n=6x=42) cytotypes (Van Slageren, 1994). Iran has been known as the main distribution center of wheat's ancestors and the associated compositions of *Triticum* and *Aegilops* as the richest wheat gene pool detected in this region.

Many agronomically valuable characteristics including the bread making quality (Orth and Bushuk, 1973), cold hardiness (Marcussen et al., 2014), and salt tolerance (Schachtman et al., 1992) are governed by D genome. *Aegilops tauschii* grows in Iran, westward to Turkey and eastward to Afghanistan and China with a distribution center in the south of Caspian Sea.

The natural hybridization of tetraploid wheat and *Ae. tauschii* about 8,000–10,000 years ago led to the formation of hexaploid wheat, with *Ae. tauschii* contributing many genes that extended the climatic adaptation and improved the bread making quality (Lagudah et al., 1991). However, much greater genetic diversity is present in this wild donor of D-genome. *Aegilops tauschii* harbors considerable genetic diversity for diseases and abiotic resistance factors relative to the wheat D-genome. Hammer (1980) classified *Ae. tauschii* into two subspecies, *A. tauschii* subsp. *tauschii* and *A. tauschii* subsp. *strangulata* (Eig) Tzvel. Four varieties were identified under subsp. *tauschii* including var. *tauschii*, var. *meyeri* (Griseb.) Tzvel, var. *anathera* (Eig) Hammer, and var. *paleidenticulata* (Gandilyan) Hammer. The typical subspecies *tauschii* is characterized by elongated, cylindrical spikelets. The subsp. *strangulata* is characterized by more quadrate spikelets with equal length and width. The intermediate forms have also been identified by some scientists (Kim et al., 1992). The phenotypic classification of the subspecies, especially the varieties, is challenging. Therefore, the phenotypic data often poorly correlate with genetic classification (Lubbers et al., 1991). The phenotypic divisions in *A. tauschii* may not always be distinguishable due to the hybridization and, as a result, the occurrence of intermediate forms (Dvorak et al., 1998). Furthermore, this indicates that the morphological variations of *Ae. tauschii* should not always be used to predict the genetic variability at the molecular level.

The subsp. *strangulata* grows mainly on the southeastern shores of the Caspian Sea between Rasht and Azadshahr, whereas subsp. *tauschii* is distributed to the east and west of this area (Aghaei et al., 2008). The *Ae. tauschii* populations in the southwest of the Caspian Sea in Iran (Aghaei et al. 2008) and nearby mountainous areas in Azerbaijan are believed to be the D-genome source of *T. aestivum*. This is because of the distribution of the waxy bloom alleles in the populations occurring in the regions (Tsunewaki, 1966). The evaluation of esterase isozymes also provided the support in the southwest of the Caspian Sea, Iran as the origin of *T. aestivum* (Nakai 1979). In addition to the *Ae. tauschii* populations found in the south of Caspian Sea and throughout the Alborz mountains, some special populations of this species are also found in Fars, Hormozgan and Kerman provinces in the southern Iran.

In recent years, a novel marker system termed start codon targeted (SCoT) markers was developed by Collard and Mackill (Collard and Mackill 2009) based on the short-conserved region flanking the start codon (ATG) in plant genes. SCoT employs long primers (18-mers), and can generate polymorphisms that are reproducible. It is considered as a dominant marker system, requiring no prior sequence information, and the polymorphism is correlated to functional genes and their corresponding traits. Other excellent characteristics include their simplicity of use, high polymorphism, the use of universal primers, low cost and gene targeted markers. This technique has been successfully used to assess genetic diversity and structure (Collard and Mackill 2009; Ma et al, 2021; Peng et al, 2021; Si et al., 2021; Sun et al., 2021; Miao et al 2018; Zou et al, 2019; Wang et al 2020; Xiaolong et al, 2021; Hou et al, 2021), construct DNA fingerprints, identify QTLs, and analyze differential gene expression and screen stress tolerance genes. The present study is the first attempt to use SCoT markers to assess the level of genetic diversity of *Aegilops tauschii* which were collected from the wild populations. The main objectives of this study were to

assess the genetic diversity and genetic relationship of *Aegilops tauschii* in Iran. These results could benefit *Aegilops tauschii* germplasm collection, conservation and future breeding.

MATERIALS AND METHODS

Plant materials

A total of 117 individuals were sampled representing nine natural populations of *Aegilops tauschii* in East Azerbaijan, Alborz, Mazandaran, Guilan, Golestan, and Ardabil Provinces of Iran during July-Agust 2018 (Table 1). For morphometric and SCoT analysis we used 117 plant accessions (four to eleven samples from each populations) belonging to nine different populations with different eco-geographic characteristics were sampled and stored in -20 till further use. More information about geographical distribution of accessions are in Table 1. Different references were used for the correct identification of species (*Aegilops tauschii*).

Environmental variables

In this experiment, the data regarding climate variables included elevation, and geographic data (latitude and longitude), and this data was determined at each site using an electronic GPS. The climate variable data of mean annual temperature, mean maximum temperature (°C), mean minimum temperature (°C), annual rainfall (mm), number of frost days were downloaded from http://www.worldclim.org. (Table 1).

DNA extraction and SCoT-PCR amplification

Fresh leaves were used randomly from four to eleven plants in each of the studied populations. These were dried by silica gel powder. CTAB activated charcoal protocol was used to extract genomic DNA. The quality of extracted DNA was examined by running on 0.8% agarose gel. A total of 25 SCoT primers developed by Collard and Mackill (2009), 10 primers with clear, enlarged, and rich polymorphism bands were chosen (Table 2). PCR reactions were carried in a 25µl volume containing 10 mM Tris-HCl buffer at pH 8; 50 mM KCl; 1.5 mM MgCl2; 0.2 mM of each dNTP (Bioron, Germany); 0.2 µM of a single primer; 20 ng genomic DNA and 3 U of *Taq* DNA polymerase (Bioron, Germany). The thermal program was carried out with an initial denaturation for 1 min at 94°C, followed by 40 cycles in three segments: 35 s at 95°C, 40s at 47°C and 55s at 72°C. Final extension was performed at 72°C for 5 min. The amplification products were observed by running on 1% agarose gel, followed by the ethidium bromide staining. The fragment size was estimated by using a 100 bp molecular size ladder (Fermentas, Germany).

Data analyses

Morphological studies

In total 19 morphological (19 quantitative) characters were studied. Four to twelve samples from each population were randomly studied for morphological analyses (Table 2). Morphological characters were first standardized (Mean = 0, Variance = 1) and used to establish Euclidean distance among pairs of taxa (Podani, 2000). For grouping of the plant specimens, The UPGMA

Table 1. Populations studied, their locality and ecological features of *Ae. tauschii* in this study.

Pop No.	Subspecies	Locality	No. of collected accessions	Mean maximum temperature (°C)	Mean minimum temperature (°C)	Annual rainfall (mm)
1	ssp. *strangulata*	Gilan, Lahijan	10	40.12	-18.12	325
2	ssp. *strangulata*	Mazandaran; Chalous	9	35.55	-20.34	378
3	ssp. *strangulata*	Mazandaran, Kandovan	18	41.34	-10.34	377
4	ssp. *strangulata*	Gorgan, Ramian	16	39.14	-17.55	390
5	ssp. *strangulata*	Mazandaran, Behshahr	12	36.88	-11.23	320
6	ssp. *tauschii*	Alborz, Asara	19	32.55	-22.45	334
7	ssp. *tauschii*	Ardabil, Fandoghlou	10	30.44	-18.66	229
8	ssp. *tauschii*	Azarbaijan, Arasbaran, Kolaleh	13	32.88	-11.66	210
9	ssp. *tauschii*	Azarbaijan, Arasbaran, Kaleybar	10	20.44	-25.66	478

Table 2. Evaluated morphological characters in *Ae. tauschii* species.

1	Spike length (mm)SL
2	Number of spikelets per spike NSp
3	Spikelet length (mm) SpL
4	Spikelet Width SpW
5	Length of upper glumes LUG
6	Width of upper glumes WUG
7	Length of upper lemas LUL
8	Width of upper lemasWUL
9	Length of lower glumes LLG
10	Width of lower lemas WLG
11	Width of upper lemas WLL
12	Number of Awner spikelets NAP
13	Longest awns of the upper glumes LAUG
14	Shortest awns of the upper glumes SAUG
15	Middel awns of the upper glumes MAUG
16	Awns number on lower glumes ANLG
17	Awns number on second glumes ANSG
18	Awns number on third glumes TNTG
19	Awns number on forth glumes TNTG

(Unweighted paired group using average) and Ward (Minimum spherical characters) as well as ordination methods of MDS (Multidimensional scaling) were used (Podani, 2000). PAST version 2.17 (Hammer *et al.* 2012) was used for multivariate statistical analyses of morphological data.

Molecular analyses

Excel 2013 was used to calculate the total number of bands (TNB), the number of polymorphic bands (NPB), and the percentage of polymorphic bands (PPB). The polymorphism information content (PIC) of SCoT primers was determined using POWERMARKER v3.25. Binary characters (presence = 1, absence = 0) were used to encode SCoT bands and used for further analyses. Parameter like Nei's gene diversity (H), Shannon information index (I), number of effective alleles, and percentage of polymorphism (P% =number of polymorphic loci/number of total loci) were determined (Freeland *et al.* 2011).

Shannon's index was calculated by the formula: H' = -Σpiln pi. Rp is defined per primer as: Rp = ∑ Ib, were "Ib" is the band informativeness, that takes the values of 1-(2x [0.5-p]), being "p" the proportion of each genotype containing the band. The percentage of polymorphic loci, the mean loci by accession and by population, UHe, H' and PCA were calculated by GenAlEx 6.4 software. Nei's genetic distance among populations was used for Neighbor Joining (NJ) clustering and Neighbor-Net networking (Freeland *et al.* 2011; Huson & Bryant 2006). Mantel test checked the correlation between geographical and genetic distances of the studied populations (Podani, 2000). These analyses were done by PAST ver. 2.17 (Hammer *et al.* 2012), DARwin ver. 5 (2012) and SplitsTree4 V4.13.1 (2013) software.

AMOVA (Analysis of molecular variance) test (with 1000 permutations) as implemented in GenAlex 6.4 (Peakall & Smouse, 2006), and Nei's Gst analysis as implemented in GenoDive ver.2 (2013) were used to show genetic difference of the populations. Moreover, populations' genetic differentiation was studied by G'ST est = standardized measure of genetic differentiation (Hedrick, 2005), and D_est = Jost measure of differentiation.

To assess the population structure of the *Aegilops tauschii* accessions, a heuristic method based on Bayesian clustering algorithms were utilized. The clustering method based on the Bayesian-model implemented in the software program STRUCTURE (Falush et al. 2007) was used on the same data set to better detect population substructures. This clustering method is based on an algorithm that assigns genotypes to homogeneous groups, given a number of clusters (K) and assuming Hardy-Weinberg and linkage equilibrium within clusters, the software estimates allele frequencies in each cluster and population memberships for every individual (Pritchard et al. 2000). The number of potential subpopulations varied from two to ten, and their contribution to the genotypes of the accessions was calculated based on 50,000 iteration burn-ins and 100,000 iteration sampling periods. The most probable number (K) of subpopulations was identified following Evanno et al. (2005). In K-Means clustering, two summary statistics, pseudo-F, and Bayesian Information Criterion (BIC), provide the best fit for k.

Gene flow (Nm) which were calculated using POPGENE (version 1.31) program. Gene flow was estimated indirectly using the formula: Nm = 0.25(1 - FST)/FST. In order to test for a correlation between pair-wise genetic distances (FST) and geographical distances (in km) between populations, a Mantel test was performed using Tools for Population Genetic Analysis (TFPGA; Miller, 1997) (computing 999 permutations). This approach considers equal amount of gene flow among all populations.

RESULTS

SCoT polymorphisms

Twenty-five SCoT primers were tested with four *Aegilops tauschii* accessions as DNA templates; all prim-

Table 3. SCoT primers used for this study and the extent of polymorphism.

Primer name	Primer sequence (5'-3')	TNB	NPB	PPB	PIC
SCoT-1	CAACAATGGCTACCACCA	14	13	95.74%	0.67
SCoT-3	CAACAATGGCTACCACCG	13	12	92.31%	0.54
SCoT-6	CAACAATGGCTACCACGC	17	17	100.00%	0.47
SCoT-11	AAGCAATGGCTACCACCA	11	9	96.89%	0.43
SCoT-14	ACGACATGGCGACCACGC	13	12	95.81%	0.34
SCoT-15	ACGACATGGCGACCGCGA	12	12	100.00%	0.47
SCoT-16	CCATGGCTACCACCGGCC	13	12	92.31%	0.34
SCoT-17	CATGGCTACCACCGGCCC	11	11	100.00%	0.57
SCoT-18	ACCATGGCTACCACCGCG	9	9	88.89%	0.33
SCoT-19	GCAACAATGGCTACCACC	17	17	100.00%	0.49
Mean		14	13	96.33%	0.48
Total		139	131		

TNP: total number of bands; NPB: number of polymorphic bands; PPB: percentage of polymorphic bands; PIC: polymorphism information content.

ers produced amplification products, and only primers showing clear and reproducible band patterns were selected for further analysis. Ten primers were then chosen for species identification and phylogenetic analysis. As shown in Table 3, all 10 primers used for SCoT analysis. The gel electrophoresis pattern obtained using primer SCoT-14 is illustrated in Figure 1. A total of 139 fragments were obtained, and 131 of the fragments were polymorphic. The number of polymorphic fragments for each SCoT primer ranged from 9 (SCoT-18, 11) to 17 (SCoT-19,6), with an average of 13. The percentage of polymorphic fragments was from 88.99% to 100.00%, with an average of 96.33% polymorphism. Polymorphism information content (PIC) values were 0.33 to 0.67, with an average of 0.48. The number of different alleles was 1.024 at the species (Table 4). These results

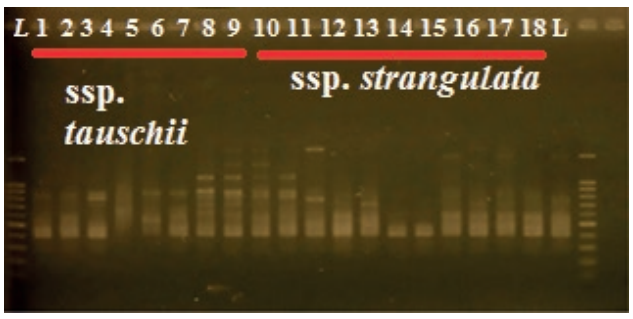

Figure 1. Electrophoresis gel of studied ecotypes from DNA fragments produced by SCoT-19(Population numbers according to Table 1).

indicated that a high level of polymorphism could be detected among *Aegilops tauschii* accessions using SCoT markers.

Populations genetic diversity

Genetic diversity parameters determined in nine geographical populations of *Aegilops tauschii* are presented in Table 4. The percentage of polymorphic loci (*P*) and Nei's gene diversity (*H*) were important parameters for measuring the level of genetic diversity. In Table 4, the genetic diversity parameters of the nine populations are shown. The highest value of percentage polymorphism (64.30%) was observed in Gilan, Lahijan (population No.1) which shows high value for gene diversity (0.35) and Shanon information index (0.33). Population Alborz, Asara (No.6) has the lowest value for percentage of polymorphism (42.15%) and the lowest value for Shanon, information index (0.15), and He (0.18).

Population genetic differentiation

AMOVA (PhiPT = 0.789, P = 0.010), revealed significant difference among the studied populations (Table 5). It also revealed that, 59% of total genetic variability was due to within population diversity and 41% was due to among population genetic differentiation.

Moreover, pair-wise AMOVA revealed significant genetic difference almost among all the studied populations. These results indicate that *Aegilops tauschii* population are genetically differentiated and we can use such genetic difference in future breeding programs of this valuable plant species.

Table 4. Genetic diversity parameters in the studied populations *Ae. tauschii* (N = number of samples, Na = Number of different alleles, Ne = number of effective alleles, I= Shannon's information index, He = gene diversity, UHe = unbiased gene diversity, P%= percentage of polymorphism, populations).

Pop	N	Na	Ne	I	He	UHe	%P
Pop1	10	0.288	1.024	0.33	0.35	0.37	64.30%
Pop2	9	0.499	1.067	0.18	0.271	0.24	49.26%
Pop3	18	0.261	1.024	0.192	0.26	0.28	43.15%
Pop4	16	0.555	1.021	0.29	0.29	0.28	43.53%
Pop5	12	0.431	1.088	0.23	0.22	0.29	57.53%
Pop6	19	0.255	1.021	0.15	0.18	0.12	42.15%
Pop7	10	0.258	1.029	0.231	0.28	0.27	45.38%
Pop8	13	0.452	1.089	0.18	0.29	0.25	45.05%
Pop9	10	0.333	1.006	0.31	0.27	0.26	43.23%
Mean		0.355	1.024	0.23	0.284	0.252	45.91%

Table 5. Analysis of molecular variance (AMOVA) of the studied species.

Source	df	SS	MS	Est. Var.	%	*ΦPT*
Among Pops	22	333.576	30.327	6.082	41%	41%
Within Pops	40	587.767	9.530	5.230	59%	
Total	62	888.342		11.513	100%	

The pairwise comparisons of 'Nei genetic identity' among the studied populations *Aegilops tauschii* (Table not included) have shown a higher a genetic similarity (0.91) between populations Mazandaran; Chalous (ssp. *strangulata*; pop. No 2) and Mazandaran, Kandovan (ssp. *strangulate*; pop. No 3), while the lowest genetic similarity value (0.733) occurs between Mazandaran, Kandovan (ssp. *strangulate*; pop. No.3) and Azarbaijan, Arasbaran, Kaleybar (ssp. *tauschii*; pop. No. 9).

Populations genetic affinity

UPGMA dendrogram and Neighbor-Net network produced similar results therefore only UPGMA dendrogram is presented and discussed (Figure 2). Two major clusters were formed in the UPGMA tree (Fig. 2). The first major cluster contained two sub-clusters: the population of Azarbaijan, Arasbaran, Kolaleh (pop. No. 8, ssp. *tauschii*) is distinct and remains separate from the other populations with a great distance and comprises the first sub-cluster. The second sub-cluster was formed by the other populations from ssp. *tauschii*, which showed close genetic affinity. The second major cluster contained only ssp. *strangulate,* which separated from the other studied populations and joined the others with a great distance. These results show that the plant specimens of each studied subspecies were not grouped together, indicating that the subspecies are delimited based on the SCoT molecular markers. Therefore, this result confirms our morphology results. The Nm analysis by Popgene software also produced mean Nm= 0.734, which is considered a very low value of gene flow among the studied species. Mantel test after 5000 permutations produced significant correlation between genetic distance and geographical distance in these populations (r = 0.348, P = 0.001). Therefore, the populations that are geographically more distant have less amount of gene flow, and we have isolation by distance (IBD) in *Aegilops tauschii.* This result was similar to the result of the STRUCTURE analysis at $K = 2$.

The principal coordinate analysis (PCoA) (Figure 3) for 9 populations of *Aegilops tauschii* revealed that the populations 1 -5 (ssp. *strangulate*), as well as populations 6 -9 (ssp. *tauschii*) are separated from the other populations and also show closer genetic affinity. The results of PCoA were the same from the other cluster analyses as shown above.

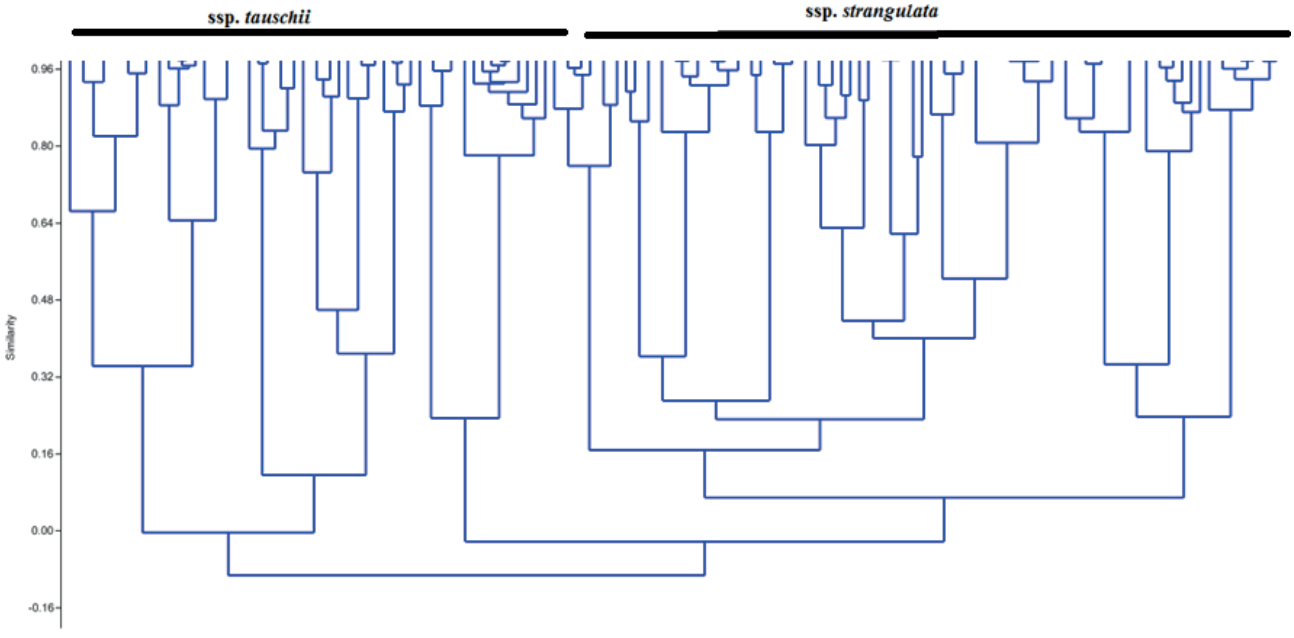

Figure 2. UPGMA tree of populations in *Ae. tauschii* based on SCoT molecular markers, (Population numbers are according to Table 1).

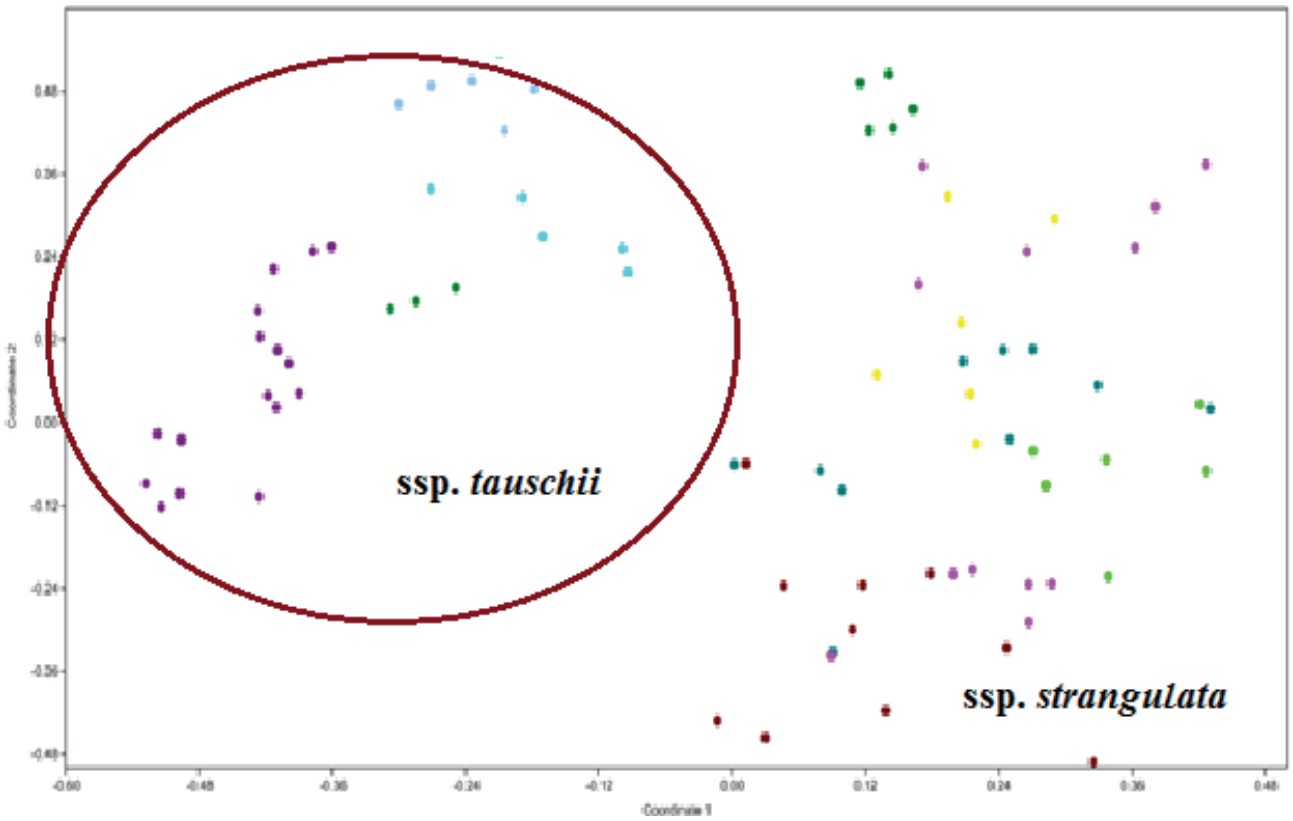

Figure 3. PCoA plot of populations in *Ae. tauschii* based on SCoT molecular markers, (Population numbers are according to Table 1).

Populations genetic structure

The number of genetic groups was determined by two methods of 1—K-Means clustering which is based on the maximum likelihood approach, and 2—Evanno test which is based on STRUCTURE analysis and is a Bayesian approach based method. K-Means clustering, based on pseudo-F and BIC (Bayesian Information Criterion) recognized 2 and 4 genetic groups, respectively. This is in agreement with AMOVA result, showing significant genetic difference among date populations of *Ae. tauschii*.

Evan test based on delta k (Figure 4) identified the optimum number of genetic groups 2. We performed STRUCTURE analysis based on k = 2, to identify the genetic groups (Figure 5). In the plot of k = 2, the populations Mazandaran, Kandovan; Gorgan, Ramian and Azarbaijan, Arasbaran, Kolaleh (pop. No 3,4,8) (red colored) are placed in the first genetic group, while the other populations of *Ae. tauschii* formed the second genetic group. These different genetic groups may be used in future breeding and hybridization programs of Iranian date *Ae. tauschii*.

The mean Nm = 0.734 was obtained for all SCOT loci, which indicates high amount of gene flow among

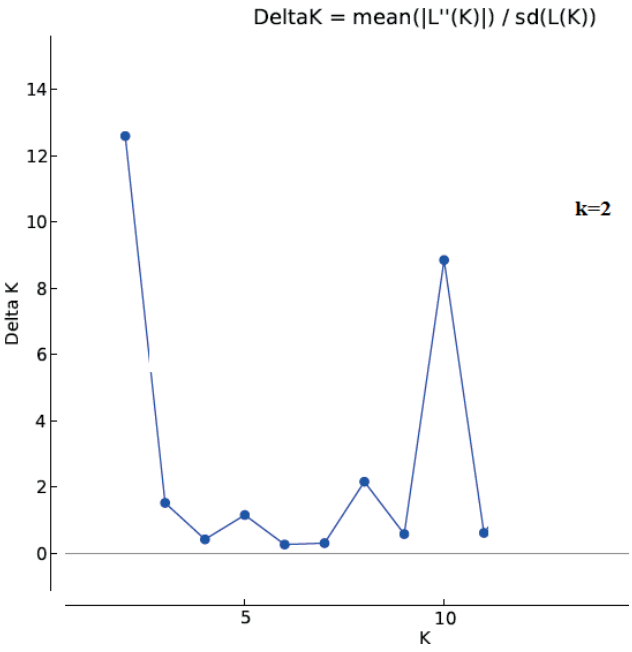

Figure 4. Delta k plot of Evanno's test based on STRUCTURE analysis.

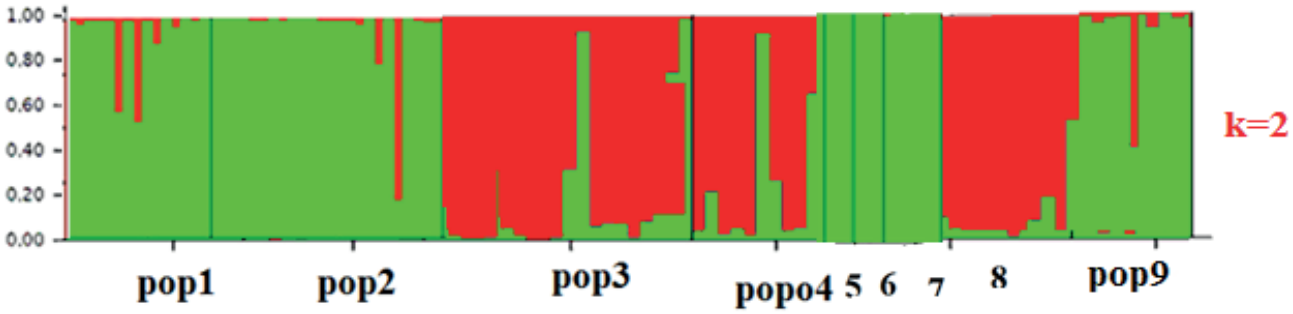

Figure 5. STRUCTURE plot of *Ae. tauschii* populations based on k = 2, Numbers are according to Table 1.

the populations and supports genetic stratification as indicated by K-Means and STRUCTURE analyses. This result is in agree with grouping we obtained with PCoA plot, as these populations were placed close to each other. As evidenced by STRUCTURE plot based on admixture model, these shared alleles comprise very limited part of the genomes in these populations and all these results are in agreement in showing high degree of genetic stratification within *Aegilops tauschii* populations.

Morphometric analyses

In present study we used 117 plant accessions (four to eleven samples from each populations) belonging to nine different populations. In order to determine the most variable characters among the taxa studied, PCA analysis has been performed. It revealed that the first three factors comprised over 63% of the total variation. In the first PCA axis with 40% of total variation, such characters as spikelet length (mm) SpL, middel awns of the upper glumes and awns number on forth glumes have shown the highest correlation (> 0.7), number of awner spikelets NAP; shortest awns of the upper glumes; 1st internode length (cm) IL1and 2nd internode length (cm)IL2 were characters influencing PCA axis 2 and 3, respectively.

Different clustering and ordination methods produced similar results therefore, PCA plot of morphological characters are presented here (Figure 6). The result showed morphological difference/ divergence among most of the studied populations. This morphological difference was due to quantitative characters only. For example, character (Length of upper glumes LUG), separated population No. 1-4, character (Width of upper lemasWUL) separated population No. 6-9.

A consensus tree was obtained for both SCOT and morphological trees (no shown), to reveal the populations that are diverged based on both morphological and molecular features. Interesting enough, it showed divergence of almost all populations at molecular level as well as morphological characteristics.

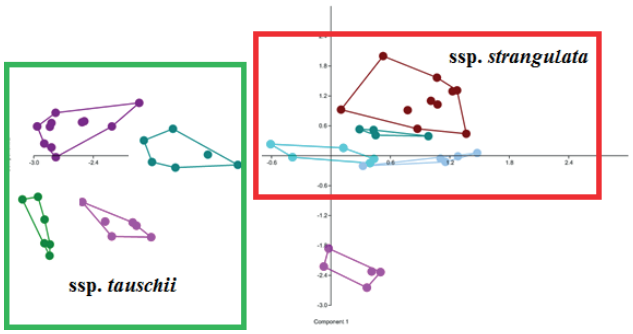

Figure 6. PCA plot of *Ae. tauschii* populations based on morphological characters. Numbers are according to Table 1.

DISCUSSION

The existing genetic variability of the individual species within and among the populations is connected to this species ability to mirror the short- and long-term specific regimes of their living habitats (Ren and Khayatnezhad 2021; Khayatnezhad and Nasehi 2021; I et al., 2021; Jia et al, 2021). The analysis of the distribution of the genetic variability patterns specific for landscape and ecological parameters is valuable for identification of the taxa most vulnerable to the anthropogenic impacts (Amedi et al 2020; Das et al 2021; Gutierrez-Pacheco et al 2021). The coupling of ecological and genetic data will provide the most suitable background for preserving the ability of the biota to respond the rapid environmental changes (Sun and Khayatnezhad 2021; Tao *et al*, 2021; Wang *et al*, 2021; Xu *et al*., 2021; Yin *et al*., 2021; Zhang *et al,* 2021). The literature reports the following basic factors influencing the distribution of genetic vari-

ation: habitat specify, plant-insect interactions, connectivity and disturbance, dispersal ability, species lifespan, reproductive rates and existing genetic diversity (Gholamin and Khayatnezhad, 2020a; 2020b, 2020c). Genetic diversity when analysed by neutral markers does not correspond to the adaptive ability of plant populations, but these types of markers are very useful for the interpretation of the past landscapes, refugia and gene flow (Brandvain et al., 2014). That is, why the selected genes or markers of active parts of plant genomes are used to interprete the plant genome response to the changes to the local climate and environment (Hoffman & Willi 2008; Hindersah et al 2021; Jordaan & Rooyen et al. 2021; Lucena et al. 2021; Mieso & Befa et al. 2020). Molecular-based population genetic data are very useful for determining the ecological and habitat events in the past and for detection of patterns of the recent genetic divergence. This can be achieved using different types DNA markers (Davey and Blaxter, 2010). SCoT markers are novel molecular markers that target the translation initiation site and preferentially bind to genes that are actively transcribed. These primers have been shown to exhibit relatively high levels of polymorphism [Collard and Mackill 2009]. It was more informative than IRAP and ISSR for the assessment of diversity of plants [Collard and Mackill 2009].

All of 10 primer pairs from D-genome of common wheat provided the amplification and showed a good polymorphism in *Ae. tauschii*. Totally, 150 alleles were recognized. The total number of bands per primer ranged from 9 to 20 polymorphic bands and the mean number of alleles in loci was 13.37, which did not conform to the results of Saeidi et al. (2006) who obtained these results: 7.3 mean and 4–12 range, and also according to Pestsova et al. (2000) who obtained these results: 18.8 mean and 11–25 range, which were achieved by SSR marker.

According to Nouri, et al (2021) compared the efficiency of inter-simple sequence repeat (ISSR) (as an arbitrary technique) and start codon targeted (SCoT) (as a gene-targeting technique) markers in determining the genetic diversity and population structure of 90 accessions of *Ae. tauschii*. SCoT markers indicated the highest values for polymorphism information content, marker index and effective multiplex ratio compared to ISSR markers. Their results of the analysis of molecular variance showed that the genetic variation within populations was significantly higher than among them (ISSR: 92 versus 8%; SCoT: 88 versus 12%). Furthermore, SCoT markers discovered a high level of genetic differentiation among populations than ISSRs (0.19 versus 0.05), while the amount of gene flow detected by ISSR was higher than SCoT (2.13 versus 8.62). Cluster analysis and population structure of SCoT and ISSR data divided all investigated accessions into two and four main clusters, respectively. their results revealed that SCoT and ISSR fingerprinting could be used to further molecular analysis in *Ae. tauschii* and other wild species.

In our study, genetic diversity of 117 *Aegilops tauschii,* individuals nine populations were studied using 10 Start Codon Targeted (SCoT) markers. High polymorphic bands (96.33%), polymorphic information content (0.48) and allele number (1.024) showed SCoT as a reliable marker system for genetic analysis in *Aegilops tauschii*. At the species, the percentage of polymorphic loci [*P*] was 66.30%, Nei's gene diversity [*H*] was 0.35, Shannon index [*I*] was 0.33 and unbiased gene diversity [*UHe*] was 0.37. Genetic variation within populations (59%) was higher than among populations (41%) based on analysis of molecular variance (AMOVA).

Jaaska (1981) stated that subsp. *tauschii* has a higher level of genetic variability than subsp. *strangulata*. According to Tahernezhad et al. (2009), the cluster analysis based on UPGMA algorithm was calculated for the genotypes. In this group, durum wheat was in a separate class , but subsp. *strangulata* and subsp. *tauschii* did not separate from each other. This classification did not conform to the morphological studies and geographical sites of the *Ae. tauschii* accessions. In fact, there was no classification based on subspecies or geographical regions. There was no significant grouping based on the geography of the accessions or subspecies, which conforms to our results.

In Saeidi et al.'s (2006) SSR marker study, there was also no significant grouping according to the geographical sites or subspecies. The high level of genetic diversity in Iran was reported by Lubbers et al. (1991), Pestsova et al. (2000), and Saeidi et al. (2006). The highest level of diversity in *Ae. tauschii* is seen in the North of Iran (South of Caspian Sea). Also, based on the morphological traits, there were many genetic diversities in *Ae. tauschii*, which can show the high potential of Iran genepool for this species. The ISSR data could not separate the accessions of *tauschii* and *strangulata* subspecies. This may be due to the classification of *tauschii* and *strangulate* subspecies. In fact, the gene flow occurred between the two subspecies in Iran can lead to a decrease of the genetic differentiation between them.

Also, Kihara et al. (1965) found intermediate and hybrid forms between subspecies. Kim et al. (1992) did not distinguish ssp. *strangulata* genotype from ssp. *tauschii* genotype by studying a highly conserved region of ribosomal DNA in *Ae. tauschii* subspecies. The classification based on the morphological traits did not conform to the classification according to SSR markers and

geographical regions.

Many studies showed that the division based on the morphological diversity does not conform to genetic division. Therefore, *tauschii* genepool exists around the *strangulata* genepool and the classification based on genetical information does not conform to the classification based on the morphological traits. Gene flow inversely correlates with the gene differentiation, but it is very important for the population evolution and takes place by pollen and seeds among the populations (Song *et al.,* 2010). In the present study, the detected gene flow (*N*m) among *Ae. tauschii* subspecies was 0.11, showing low genetic differentiation among *Ae. tauschii* subspecies. According to Lubbers et al. (1991) and Pestsova et al. (2000) studies, one of the important origin sites for *Ae. tauschii* is the southwest of Caspian Sea. Therefore, the study about Iranian *Ae. tauschii*, especially in the south of the Caspian Sea, and the detection of their genetic diversity are very helpful in the breeding programs. This is because the south of the Caspian Sea is the main origin site of *Ae. tauschii* where bread wheat has evolved (Lubbers et al., 1991; Pestsova et al., 2000). The study of the D-genome diversity in other D-genome containing polyploid species of the genus *Aegilops* in Iran may also lead to interesting results.

Comparison of results of this study with those based on SSR data (Saeidi et al. 2006) shows that the SSRs are suitable markers to study the genetic diversity among closely related populations, but the scot are suitable marker system to demonstrate the genetic diversity at species level, indicating the importance of choosing the suitable marker type for the analysis we need.

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