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Original scientific article

GENETIC DIVERSITY AND GENE-POOL OF *Aegilops tauschii* Coss. (Poaceae) BASED ON RETROTRANSPOSON-BASED MARKERS

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Genetic variation is an essential feature of biological variety for conservation methods, particularly for scarce and strictly endemic species. Iran's population genetic structure, genetic variation, and morphological variations are all unknown. Because of the therapeutic value of this species, six regional populations of *Aegilops tauschii* were studied for genetic diversity and population structure. To uncover within and among population genetic variation in this plant, we employed six inter-retrotransposon amplified polymorphism (IRAP) markers and 15 combined IRAP markers. The AMOVA test indicated a substantial genetic difference (PhiPT = 0.66, P = 0.010) among the tested populations, as well as the fact that 85 percent of overall genetic differences was related to within-population variety and 15 percent to genetic divergence across populations. The Mantel test revealed a substantial positive connection between genetic distance and geographical distance across the populations investigated. Based on (IRAP) markers, these findings suggested that regional populations of *Aegilops tauschii* are well separated.

Keywords: Gene flow, IRAP, *Aegilops tauschii*; population differentiation

INTRODUCTION

The ability to deduce developmental factors like as selection pressures and drift from spatial genetic architecture is a powerful tool (DE KORT *et al*., 2012). Because of the physical isolation of populations, little gene flow may actually enhance the degree of local diversity

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(HENDRY, 2002). However, rather than genetic differentiation, phenotypic plasticity may be a better approach to adapt genotypes to environment; specifically, increased environmental diversity supports higher degrees of plasticity (HAHN *et al*., 2012).

Approximately 8,000–10,000 years ago, a natural hybridization of tetraploid wheat with *Ae. tauschii* resulted in the development of hexaploid wheat, with *Ae. tauschii* providing several genes that increased climate tolerance and enhanced bread making quality (BELL, 1987; KIHARA, 1944; MCFADDEN and SEARS, 1946; YAMASHITA *et al.*, 1957; KERBER and TIPPLES, 1969; LAGUDAH *et al.*, 1991). This wild D-genome donor, on the other hand, has a lot more genetic variety (NAGHAVI *et al*., 2009). In comparison to the wheat D-genome, *Aegilops tauschii* has a lot of genetic diversity for diseases and abiotic resistance factors. *Ae. tauschii* is divided into two subspecies by MCFADDEN and SEARS (1946), *Ae. tauschii* subsp. *tauschii* and *Ae. tauschii* subsp. *strangulata* (Eig) Tzvel. Under the subsp. *tauschii* umbrella, four variants have been identified: var. *tauschii*, var. *meyeri* (Griseb.) Tzvel, var. *anathera* (Eig) Hammer, and var. *paleidenticulata* (Gandilyan) Hammer. The large, cylindrical spikelets of the *tauschii* subspecies are distinctive. More quadrate spikelets of similar length and breadth describe the subsp. *strangulata*. Some scientists have also identified the intermediate types (KIM *et al.*, 1992). Phenotypic categorization of subspecies, particularly variations, is difficult. As a result, phenotypic data frequently do not match well with genetic categorization (LUBBERS *et al.*, 1991; DVORAK *et al*., 1998). Due to hybridization and the development of intermediate forms, the phenotypic divides in *A. tauschii* may not always be discernible (KIHARA *et al.*, 1965; DVORAK *et al.*, 1998). This also suggests that *Ae. tauschii* morphological variations should not necessarily be utilized to predict genetic heterogeneity at the molecular level.

The subsp. *strangulata* develops mostly between Rasht and Azadshahr on the southern beaches of the Caspian Sea, whereas the subsp. tauschii grows to the eastern and western parts of this area (KERBER and TIPPLES, 1969). The D-genome source of *T. aestivum* is thought to be *Ae. tauschii* populations in Iran's southwest Caspian Sea (KERBER and TIPPLES, 1969) and neighboring mountainous regions in Azerbaijan. This is due to the waxy bloom alleles' distribution in the populations that exist in the areas (NAKAI, 1979).

The goals of this work were to use the inter-retrotransposon amplified polymorphism (IRAP) approach to explore genetic diversity across *Aegilops tauschii* cultivars/populations with diverse geographical origins, and to use IRAP markers to detect genetic variation among and within materials.

MATERIALS AND METHODS

Plant materials

During the months of July-August 2016, 85 individuals from six natural populations of *Aegilops tauschii* were sampled in Iran's East Azerbaijan, Mazandaran, and Guilan provinces (Table 1, Fig 1). Fresh leaves from 5-8 individuals in each population were collected and dried in Silica Gel right away (Table 1). For the accurate species identification (*Aegilops tauschii*), many sources were employed (KIHARA, 1944; MCFADDEN and SEARS, 1946; YAMASHITA *et al*., 1957). Table 1 lists the sampling locations in detail. The vouchers were placed in the Islamic Azad University's Science and Research Branch's herbarium in Tehran, Iran (IAUH).

Table 1. Populations studied their locality and ecological features

No	Subspecies	Locality	Latitude	Longitude	Altitude (m)
Pop1	ssp. *strangulata*	Mazandaran, Amol to Sari	36°52'37'	52°23' 92'	122
Pop2	ssp. *strangulata*	Gilan, Lahijan	37°50′03″	49°24′28″	-6
Pop3	ssp. *strangulata*	Mazandaran, Ramsar	36°20′07″	50° 52′08″	13
Pop4	ssp. *tauschii*	Gilan, Bandar-e Anzali	37°07′02″	49°44′32″	-18
Pop5	ssp. *tauschii*	Azarbaijan, Arasbaran, Kolaleh	38°57′22″	46°28′31″	1010
Pop6	ssp. *tauschii*	Azarbaijan, Arasbaran, Kolaleh	38°07′24″	46° 59′06″	1108

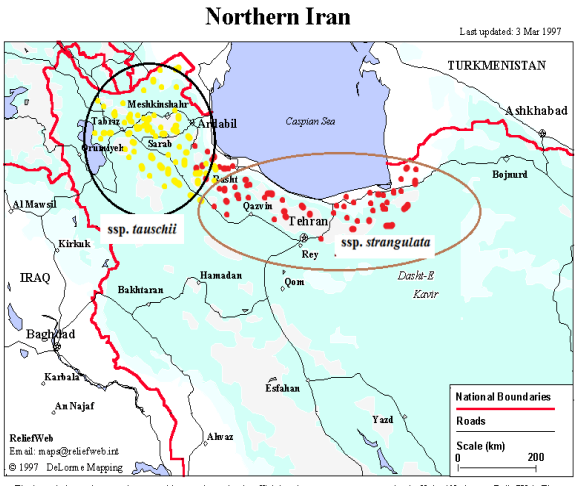

Fig. 1. Distribution map of the studied populations. (Population numbers are according to Table 1).

DNA extraction and IRAP assay

Fresh leaves were collected at random from 5-10 plants in each of the groups investigated. Silica gel powder was used to dry them. To extract genomic DNA, the CTAB activated charcoal procedure was applied (ESFANDANI-BOZCHALOYI *et al.*, 2019).

Table 2. IRAP primers based on SMYKAL et al. (2011) study

IRAP	Sequence (5′-3′)
GU735096	ACCCCTTGAGCTAACTTTTGGGGTAAG
GU980589	AGCCTGAAAGTGTTGGGTTGTCG
GU929878	GCATCAGCCTGGACCAGTCCTCGTCC
GU735096	CACTTCAAATTTTGGCAGCAGCGGATC
GU929877	TCGAGGTACACCTCGACTCAGG
GU980590	ATTCTCGTCCGCTGCGCCCCTACA

Data analyses

Molecular analyses

Binary characters were assigned to the IRAP profiles acquired for each sample. Nei's gene heterogeneity (H), Shannon data index (I), number of functional alleles, and polymorphism percentage were all calculated (WEISING *et al.*, 2005; FREELAND *et al*., 2011).

RESULTS

Populations' genetic diversity

Table 3 shows the genetic diversity characteristics obtained in six regional populations of *Aegilops tauschii*. Mazandaran, Amol to Sari (population No. 1, ssp. *strangulata*) had the greatest percentage polymorphism (43.75 percent), indicating a high value for gene diversity (0.42). & Shanon, index of information (0.49). Population Azarbaijan, Arasbaran, Kolaleh (No.5, ssp. *tauschii*) has the lowest proportion of polymorphism (11.53 percent) and Shanon, information index (0.20), and He has the lowest percentage of polymorphism (11.53 percent) (0.22).

Table 3. Genetic diversity parameters in the studied populations Aegilops tauschii

Pop	Na	Ne	I	He	UHe	%P
Pop1	0.341	1.338	0.49	0.42	0.41	43.75%
Pop2	0.155	1.077	0.377	0.34	0.32	35.05%
Pop3	0.299	1.057	0.24	0.23	0.34	41.26%
Pop4	0.554	1.055	0.32	0.25	0.38	23.53%
Pop5	0.411	1.048	0.20	0.22	0.25	11.53%
Pop6	0.215	1.021	0.25	0.38	0.32	40.15%

(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)

Population genetic differentiation

Gst analysis (0.657, p = 0.001) and AMOVA (PhiPT = 0.66, P = 0.010) indicated significant differences across the analyzed groups (Table 4). It also indicated that within-population genetic variety accounted for 25% of overall genetic variability, whereas among-population genetic difference accounted for 75%. The results of pairwise AMOVA showed a substantial difference between the groups examined. Furthermore, after 999 permutations, we obtained high values for the Hedrick standardized fixation index (G'st = 0.657, P = 0.001) and the Jost differentiation index (D-est = 0.886, P = 0.001). These findings suggest that *Aegilops tauschii* regional populations are genetically distinct from one another.

Table 4. Analysis of molecular variance (AMOVA) of the studied species

Source	df	SS	MS	Est. Var.	%	*ΦPT*
Among Pops	68	226.576	55.317	33.011	75%	
Within Pops	52	77.767	77.539	10.120	25%	75%
Total	120	301.342		43.613	100%	

df: degree of freedom; SS: sum of squared observations; MS: mean of squared observations; EV: estimated variance; *Φ*PT: proportion of the total genetic variance among individuals within an accession, ($P < 0.001$).

Populations' genetic affinity

In the NJ tree, two large clusters emerged (Fig. 2). The first major cluster includes two sub-clusters: the Gilan population, Bandar-e Anzali (pop. No. 4, ssp. *tauschii*), is different and stays separated from the other populations by a significant distance. The remaining populations from ssp. *tauschii* and ssp. *strangulate*, which exhibited close genetic affinity, constituted the second sub-cluster. Only ssp. *tauschii* was found in the second main cluster, which was distant from the other investigated populations and joined them at a large distance.

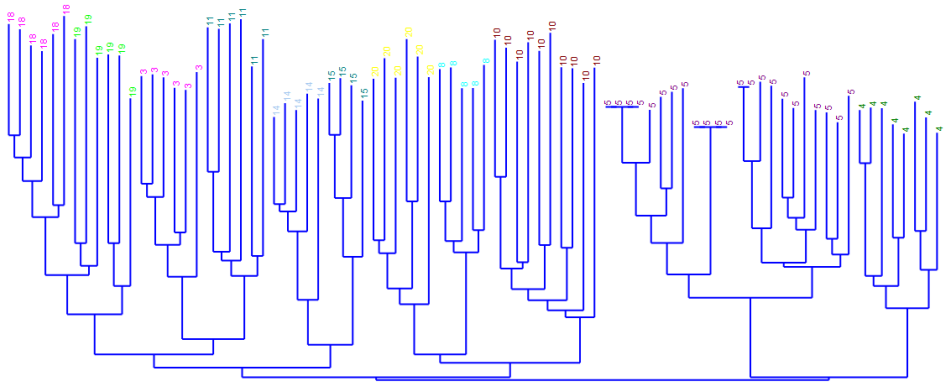

Fig.2. NJ clustering of populations in *Aegilops tauschii* based on IRAP data. (Population numbers are according to Table 1).

The plant specimens of each analyzed subspecies were not clustered together as a consequence of these findings, demonstrating that the subspecies are not demarcated by IRAP molecular markers. Consequently, this research backs up our morphological findings. In these groups, the Mantel test revealed a strong connection between genetic distance and geographical distance ($r = 0.88$, $P = 0.001$) after 5000 permutations. As a result, populations that are further

apart geographically have less gene flow, resulting in isolation by distance (IBD) in *Aegilops tauschii*.

Populations genetic structure

The presence of two genetic groups is shown when K = 2. Evanno test on STRUCTURE analysis found a large peak at k = 2, which yielded a similar result. Both of these studies found that *Aegilops tauschii* populations are genetically stratified.

Using a k = 2 STRUCTURE plot, the genetic differences between populations 4-6 (differently colored) and 1-3 were highlighted. However, it revealed genetic similarity between populations 2-3. (similarly colored). For all IRAP loci, the mean Nm = 0.22 was found, indicating little gene flow across populations and supporting genetic differentiation as demonstrated by K-Means and STRUCTURE studies. The results of the population assignment test backed with Nm's findings, indicating that there was no considerable gene flow between these groups. Nevertheless, a reticulogram based on the lowest square approach (Figure not included) found several common alleles between populations 1 and 5, as well as populations 3 and 6, as well as populations 2 and 5. Because these populations were put near to one other, this result agrees with the grouping we got with the PCoA diagram. These common alleles form a relatively small percentage of the genomes in these populations, as indicated by the STRUCTURE plot based on the admixture model, and all of these data concur in indicating a high degree of genetic stratification among *Aegilops tauschii* populations. A total of 88 IRAP bands (loci) were acquired, with 25 of them being private. There were 1-6 private bands in populations 1 and 5-6.

DISCUSSION

In genetic and breeding research, population genetics analyses are crucial. They give data on genetic diversity levels, genetic variability partitioning within and across populations, inbreeding and outcrossing, effective population size, and population bottlenecks (ELLIS and BURKE, 2007; JI *et al*., 2020; YIN *et al.*, 2021). The use of molecular markers has considerably aided population genetic research. Among the various Salicornia accessions, these markers have been utilized to identify possibly unique genotypes (ESFANDANI-BOZCHALOYI, *et al*. 2017a, 2017b, 2017c, 2017d). Randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), inter simple sequence repeat (ISSR), and inter-retrotransposon amplified polymorphism (IRAP) have all been used to measure genetic variation and relationships in cultivars and landraces in recent years (ESFANDANI-BOZCHALOYI, *et al*. 2018a, 2018b, 2018c, 2018d). The majority of plant genomes are made up of transposable elements, mainly retrotransposons. Their replication produces genetic variation, making them a valuable source of molecular markers (SMYKAL *et al.*, 2011; MA *et al.*, 2021; PENG *et al.*, 2021; SI *et al*., 2021). By amplifying the segments of DNA between two retrotransposons, the inter-retrotransposon amplified polymorphism (IRAP) approach shows insertional polymorphisms. It's been utilized in a number of genetic diversity investigations (BI *et al*., 2021; CHENG *et al*., 2021; ZHENG *et al*., 2021; ZHU *et al.*, 2021; JIA *et al.*, 2021).

The genotypes were subjected to cluster analysis using the UPGMA method, according to LUBBERS *et al.* (1991). Durum wheat was in a different class in this group, but subsp. There was no separation between *strangulata* and subsp. *tauschii*. The morphological analyses and

geographical locations of the *Ae. tauschii* accessions did not support this categorization. In fact, no subspecies or geographical locations were used to classify the animals. There was no significant grouping based on the accessions' or subspecies' geographic location, which is consistent with our findings.

There was no significant categorization according to geographical areas or subspecies in the SSR marker research by SAEIDI *et al.* (2006). LUBBERS *et al.* (1991), PESTSOVA *et al.* (2000), and SAEIDI *et al.* (2006) all reported on Iran's great genetic diversity. The highest amount of variety in *Ae. tauschii* may be found in Iran's north (South of Caspian Sea). *Ae. tauschii* also has a lot of genetic variety based on physical features, indicating that the Iranian genepool has a lot of promise for this species. The ISSR data was unable to distinguish between the *tauschii* and *strangulata* subspecies. This might be owing to the *tauschii* and strangulate subspecies being classified separately. Indeed, gene flow between the two subspecies in Iran has the potential to reduce genetic divergence between them.

In addition, KIHARA *et al*. (1965) discovered intermediate and hybrid subspecies. By examining a highly conserved section of ribosomal DNA in Aedes tauschii subspecies, KIM *et al*. (1992) were unable to identify ssp. *strangulata* genotype from ssp. *Tauschii* genotype. The morphological trait-based categorization did not match the SSR marker-based and geographical region-based classification. According to VOJDANI and MEYBODI (1993), there is no link between morphological and ecogeographic diversity.

Many investigations have shown that morphological diversity-based division does not correspond to genetic division. As a result, the *Ae. tauschii* genepool occurs in close proximity to the *strangulata* genepool, and the genetic categorization differs from the morphological classification. Gene flow is negatively related to gene differentiation, although it is critical for population evolution and occurs between populations via pollen and seeds (SONG *et al.*, 2010). The observed gene flow (Nm) among *Ae. tauschii* subspecies in this study was 0.11, indicating modest genetic divergence among *Ae. tauschii* subspecies. One of the key origin places for A*e. tauschii*, according to research by LUBBERS *et al.* (1991) and PESTSOVA *et al.* (2000), is the southwest of the Caspian Sea. As a result, research on Iranian *Ae. tauschii*, particularly in the south of the Caspian Sea, as well as the discovery of genetic variety, are extremely useful in breeding efforts. This is because the major origin location of *Ae. tauschii*, where bread wheat has originated, is located south of the Caspian Sea (LUBBERS *et al*., 1991; PESTSOVA *et al*., 2000).

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GENETIČKI DIVERZITET I GENSKI FOND *Aegilops tauschii* Coss. (Poaceae) NA OSNOVU MARKERA ZASNOVANIH NA RETROTRANSPOZONIMA

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Izvod

Genetički diverzitet je jedan aspekt biološke raznovrsnosti koji je izuzetno važan za strategije očuvanja, posebno retkih i usko endemičnih vrsta. Nema informacija o njegovoj populacijskoj genetičkoj strukturi, genetskoj raznolikosti i morfološkoj varijabilnosti u Iranu. Zbog medicinskog značaja ove vrste, sprovedena je studija genetske varijabilnosti i strukture populacija proučavanjem šest geografskih populacija *Aegilops tauschii*. Stoga smo koristili šest markera inter-retrotransposon pojačanog polimorfizma (IRAP) i 15 kombinovanih IRAP markera da bismo otkrili unutar i među populacijsku genetsku raznovrsnost kod ove biljke. AMOVA test je proizveo značajnu genetsku razliku (PhiPT = 0,66, P = 0,010) među proučavanim populacijama i takođe je otkrio da je 85% ukupne genetske varijabilnosti posledica unutar populacijske raznolikosti, dok je 15% posledica genetske diferencijacije među populacijama. Mantel test je pokazao signifikantnu pozitivnu korelaciju između genetske udaljenosti i geografske udaljenosti ispitivanih populacija. Ovi rezultati su pokazali da su geografske populacije *Aegilops tauschii* dobro diferencirane na osnovu IRAP markera.

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