

# Evaluation and comparison of SSR, ISSR, and retrotransposon molecular markers in the study of genetic diversity of *Aegilops tauschii* as wild ancestor of wheat

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## Research Article

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## Abstract

Wheat is an annual plant from the cereal family, this plant is the most important agricultural plant used in the human food chain. In plant breeding programs, identifying genes resistant to biotic and non-biotic stresses is of particular importance to transfer them to crop species to increase production. The most important goal in breeding programs is the existence or creation of diversity to select superior plants. One of the ways to enrich genetic resources is to know the level of diversity in germplasm and genetic treasures, which is one of the important sources of diversity and identification of useful genes in wild relatives. In this research, the genetic diversity of 125 *Aegilops tauschii* genotypes was investigated using SSR, ISSR, and retrotransposon markers. In this research, based on the presented results and the changes in  $\Delta K$  and its maximum curve at  $K = 7$ , the number of 125 studied *Aegilops tauschii* genotypes were divided into 7 subpopulations with a probability of more than 70%. In general, the results obtained from this study showed that retrotransposon markers were better markers than SSR and ISSR in terms of useful indicators. However, the average PIC index in the other two systems was higher than retrotransposon, but they did not differ much from each other. Among the three marker systems, retrotransposons and SSRs were able to show a high level of intraspecies diversity.

## Introduction

Genetic diversity is the basis of plant breeding, one of the important components that come from natural diversity and the stability of biological systems. Evaluation of genetic diversity in crop plants is vital for plant breeding programs and the protection of genetic resources. Awareness of genetic diversity in plant species is important for selecting parents to obtain suitable hybrids and to predict the hybrid foundation, especially in products whose hybrids have commercial value (Nandakumr et al., 2004). Wheat is an annual plant from the cereal family, this plant is the most important crop plant used in the human food chain (Hedge et al. 2002). Wheat provides half of the world's food every year (Ashraf & Arzani. 2017). In plant breeding programs, the identification of genes resistant to biotic and non-biotic environmental stresses to be transferred to agricultural species to increase production is of particular importance. The most important and basic need in breeding programs is the existence or creation of diversity to select superior plants (Prasanna & Mohammadi 2003).

Climatic and geographical changes and the weakening of the genetic base of wheat have caused to face serious risks in the production of this very important plant in the human food cycle. One of the ways to deal with this condition is to use new alleles in its gene pool (Warschefsky et al. 2014). The consciousness of the level of diversity in germplasm and genetic reserves can be used to enrich genetic reserves through appropriate genes. Wild relatives are one of the important sources of diversity and identification of useful genes. The wild relatives of wheat contain useful genes for resistance to pests and diseases, drought, salinity, and even improved yield as well as adaptation to the stresses resulting from changes in weather conditions (Warschefsky et al. 2014).

Since genetic diversity is limited in crop species that have been improved, the achievement of new alleles in these species becomes less. Therefore, to solve this problem, the use of wild relatives of cultivated plants can provide a rich and diverse gene source of new alleles (Pour-Aboughadareh et al. 2018). The results of various research show that most studies of breeders in the field of wild relatives of crop species are related to the wild ancestors of wheat (Maxted et al. 2006). One of the ways to enrich genetic reserves is to know the level of diversity in germplasm and genetic vaults, which is one of the important sources of diversity and identification of useful genes in wild relatives. In wheat, the wild relatives have large amounts of useful genes to resist pests, diseases, drought, and salinity and even increase yield and adaptability (Warschefsky et al., 2014). *Aegilops* species direct the donor species of the D genome to bread wheat and play an important role in its evolution (Kilian et al, 2007).

Molecular markers are one of the most important tools for investigating genetic diversity between species. Until now, many marker systems have been used to investigate the genetic diversity in different crops and their wild relatives. Among these marker systems, SSR (microsatellite) markers are particularly important. Microsatellites have repetitive units that can be between one and six units, microsatellites are more common among eukaryotes so that at least one row of microsatellites can be seen in every ten kb pair of DNA (Daneshvar et al., 2020)

Due to the feature of the SSR marker system, this marker has been used in many plant and animal species in the assessment of genetic diversity or even in the investigation of phylogenetic relationships. ISSR markers or repetitive internal are amplified by PCR using microsatellite core sequences as primers, along with a few selected nucleotides at the 3' or 5' end as anchors within the adjacent non-repetitive regions (Li et al., 2011). ISSR markers are widely used in sequences based on polymerase chain reactions and are among the modified types of microsatellite markers. ISSR markers plant population diversity studies due to their high reproducibility, investigation and analysis of several gene loci at the same time, high diversity and accuracy, and high speed of their reproduction against low costs. ISSR markers have high polymorphism and are semi-random and have high discriminating power as well as characterizing variation between populations (Hassani Tesie et al., 2016).

Transposons or elements moving and moving along the genome are mobile pieces of DNA and can move along the genome, these pieces were first discovered in corn by Barbara McClintock. Transposon elements are in two categories, the first category is the elements that can be moved in the form of DNA and the second category is the elements that are transferred by an RNA intermediate (Pardue and Debarysh, 2003)

Transposons with LTRs exist almost throughout the plant genome, although each of these types of retrotransposons exists in separate families in a specific part of the genome. Most retrotransposons are mostly located in euchromatin regions (Deininger et al., 2003). The presence or absence of a transposon in a gene locus can be used as a molecular marker for fingerprinting genotypes, investigating genetic diversity, determining evolutionary relationships, and also in preparing linkage maps. Due to the wide distribution, present everywhere in the genome and the abundance of these mobile genetic elements, several molecular markers based on these mobile genetic elements have been introduced in recent years (Grzebrlus, 2006). Regarding the use of molecular markers in the investigation of genetic diversity in wheat and its wild relatives, there are various studies in Iran and other parts of the world. Saeedi investigated genetic diversity in populations of *Ae. Tauschii* from different regions of Iran used microsatellite markers and according to their results, a high level of genetic diversity was observed. The average amount of polymorphism in their studies was 3.55 (Saeidi et al, 2006).

In another report by Naghavi the genetic diversity between populations of *Ae. Boeoticum* was investigated using SSR, RAPD, and AFLP molecular markers (Naghavi et al, 2009).

In another study, the genetic diversity between different populations of *Ae. tauschii* was investigated using microsatellite markers, in this study using 19 SSR markers, a total of 231 alleles were identified and the average number of each allele for each locus was 13.94. Also, according to the genetic similarity coefficients and the dendrogram obtained from cluster analysis, it was shown that there is a significant level of genetic diversity within the investigated populations (Tahernezhad et al, 2010).

Pour-Aboghadaresh investigated the genetic diversity between 103 different populations of *Aegilops* using two markers CDBP and SCoT (Pour-Aboghadaresh et al, 2019). Investigating the genetic diversity of 20 wheat genotypes using *T. aestivum*, *Ae. Tauschii*, *Ae. Speltoides*, *Ae. crasa*, and *Ae. cylindrica* with ISSR marker showed a high level of genetic diversity within the species (Mirzakhani et al, 2012). The existence of a high level of genetic diversity within the wild relatives of wheat in Iran has doubled the importance of studying these resources, and it seems that the evaluation and attention to these valuable natural resources will create a new way to benefit from them in wheat breeding programs (Etrminan et al., 2019). The purpose of this research is to investigate the amount of genetic diversity in some populations of *Aegilops tauschii* and also to investigate the capability of the marker systems used in the separation and grouping of the investigated populations.

## Materials and Methods

In this study, 125 species of *Aegilops tauschii* whose information is given in Table 1 were used. 30 microsatellite markers (SSR), 30 ISSR markers, and 9 markers based on retrotransposons were used to investigate the genetic diversity between genotypes. DNA extraction was done by CTAB method (Saghai et al., 1944). To determine the quantity and quality of genomic DNA extracted by this method, spectrophotometry and 0.8% agarose gel were used, respectively. Also, to make the concentration of all stock DNAs the same to use them in a polymerase chain reaction (PCR), DNAs extracted with a certain ratio of sterile water obtained from the formula  $C1V1 = C2V2$ , were diluted. SSR, ISSR, and retrotransposon primers were selected by reviewing sources and searching genetic databases and articles. The criterion for selecting markers in this research was the review of related sources, their location on the map with maximum genomic coverage, and the degree of marker diversity (markers with high polymorphism or with many alleles) in previous studies. The temperature and reaction program of the PCR cycle was carried out as described in Table 5. One PCR master mix kit from Gene Direx Company was used to perform PCR. Based on the clarity of the bands, the separation of the amplified products was done using 3% agarose gel, and after photographing them, the band patterns were scored as the presence of a band (number one) and the absence of a band (number zero). Based on the data obtained from molecular markers, genetic diversity statistics, including polymorphic information content, Nei genetic diversity, and Shannon's index were calculated using POPGENE32 software version 1.31. Molecular variance analysis was performed to separate the total genetic diversity into diversity within and between populations using Gen ALE software. The genetic distance matrix of populations was calculated based on the Dice coefficient and the dendrogram was prepared using the Neighbor-joining (NJ) algorithm. The exact classification of the populations into appropriate subpopulations and the detection of mixed populations was done using Structure 4.2. v3 software (Pritchard et al., 2000). In this analysis, the number of 10 independent replications is considered for each of the possible subpopulations. The number of K also varied between 1 and 10.

## Result

### Usefulness of markers

The average indices obtained for the primers used are shown in Table No. 6. The primers used produced a suitable banding pattern. Knowing about the ability of marker systems to show the level of polymorphism in the investigated samples is one of the primary tasks for choosing the type of marker system in programs based on the molecular modification. Based on this, polymorphic information content (PIC), allele frequency and number of observed alleles, Shannon's index, number of effective alleles, and Nei's diversity index were calculated separately for each of the markers. In this study, the highest PIC value for the ISSR marker system was with an average of 0.332, and in this marker system, the highest PIC value was related to the UBC824 marker with an average of 0.383. Primers whose PIC values are between 0.25 and 0.5 are useful, and those that are less than 0.25 contain little useful information (Ditta et al., 2018). According to the results, the highest MI was for retrotransposon markers with an average of 0.378, and in this marker system, the highest MI for the LTR1061-UBC834 marker was 0.434. The numerical range of the Shannon index coefficient was the highest in retrotransposon markers with an average of 0.345, and in this marker system, the highest Shannon index was for the LTR1061-UBC834 marker with an amount of 0.457.

The calculation of the Nei genetic diversity index as one of the most important indices for evaluating genetic diversity among cultivars and populations showed that this index was the highest among retrotransposon markers with an average of 0.334. UBC834 was 0.325. The number of polymorphic alleles was the highest in retrotransposon markers with an average of 1.538 and for the LTR1061-UBC834 marker with an average of 1.735. The number of effective alleles was the highest with an average of 1.417 for the retrotransposon and the LTR1061-UBC834 marker with an average of 1.577. Based on these results, the retrotransposon marker system is the best marker system to investigate the diversity of *Ae. tauschii*.

### Genetic structure of populations

In this research, based on the presented results and the changes in  $\Delta K$  and its maximum curve at  $K = 7$  (Figs. 1 and 2), the 125 studied *Aegilops tauschii* genotypes were divided into 7 subpopulations with a probability of more than 70% (Fig. 2). Decomposing the population structure into subpopulations makes it possible to identify mixed masses. The creation of a phenomenon called gene flow can be one of the factors in the creation of populations that are known as mixed among the populations of different regions. In some studies, the existence of such mixed populations is considered the result of hybridization between two subspecies (Saeidi et al., 2006). In a study by Sohail on 81 genotypes of *Ae. tauschii* collected from different geographical areas by DaRT markers, they observed the degree of genetic similarity in the range of 0.61 to 0.99 (Sohail et al, 2012).

# Molecular analysis of variance

To calculate the amount of intra- and inter-group variances of the populations, molecular variance analysis was performed (Table No. 7). The results of molecular variance analysis, based on the markers used, separated the genetic diversity observed in the studied genotypes into two parts of interspecies and intraspecies diversity. The results estimated the inter-group variance based on ISSR, SSR, and retrotransposon markers as 31%, 17%, and 7% and the intra-group variance as 69%, 83%, and 93% respectively. These results showed that the amount of difference and genetic diversity between *Aegilops tauschii* populations was higher than the type of diversity within the species. High genetic diversity within the subpopulations shows the genetic differences of individuals within the populations resulting from duplicated gene locations, and this issue shows the heterogeneity within the subpopulations. Polyploidy, physical mixing of seeds, transformation, and different crossings between cultivars can be factors of high heterogeneity within subpopulations (Nouri et al., 2021).

The high genetic diversity within *tauschii* sub-populations can be caused by the origin of each of the studied samples from different geographical areas, and due to their adaptation to the conditions of their habitat, they may be different from each other in terms of genetic content. This difference itself can cause a high level of polymorphism within the species. Concerning the studied populations of *Aegilops tauschii*, the most significant reason for the high variance within the group can be due to the mixing of seeds during reproduction, the small size of the studied populations, or the lack of starting compounds for complete coverage of the genome, and as a result, the lack of complete evaluation of genotypes from The ratio of genetic diversity in the whole genome.

## Genetic diversity and related indicators

According to the analysis of molecular variance based on the markers used for the studied population, it was divided into two parts, the diversity within and between species (Fig. 3). The research results showed a significant amount of diversity within *Ae. tauschii* was investigated by the markers used, but retrotransposon markers estimated the most diversity within the species. These markers also had the highest markers of indicator usefulness. Differences in genetic traits, changes in environmental conditions, and natural selection can be factors that differentiate populations. Random processes such as migration and mutation can also cause genetic differentiation between subpopulations (Putman and Carbone, 2014). In various studies, the genetic diversity of cultivated and wild species and wild relatives of wheat has been evaluated, in Ahmadi study on the subject of screening wheat germplasms under stress conditions, they showed that *Aegilops* has more diversity and more resistance to stress (Ahmadi et al., 2018).

## Grouping of genotypes

The grouping of the studied genotypes was done based on the studied markers (SSR, ISSR, and retrotransposons), and using structure software and using dissimilarity matrices, and the studied genotypes were divided into 7 groups (Figure No. 2 and Table No. 8).

## Conclusion

In general, the results obtained from this study showed that retrotransposon primers were better markers than SSR and ISSR in terms of useful markers. However, the average PIC index in the other two systems was higher than retrotransposon, but they did not differ much from each other. Among the three marker systems, retrotransposons and SSRs were well able to show a high level of intraspecific variation. The results obtained from this study showed that the studied populations were classified into seven groups, which can be considered for the selection of parents in breeding programs to regulate distant crosses. On the other hand, it will be useful for the management of germplasm resources in the gene bank to prevent the maintenance of the same genetic background. The indices of genetic diversity of the markers used in this research showed a high genetic diversity within the species, this result represents the gene flow and high *Fst* among the species. In addition, the high genetic indices as well as the high level of gene flow between subpopulations can indicate the presence of high allelic diversity in each subpopulation. Therefore, the protection and potential use of this germplasm source in wheat breeding are recommended.

## Declarations

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### Competing Interests

*"The authors have no relevant financial or non-financial interests to disclose."*

### Author Contributions

*"All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by all authors. The first draft of the manuscript was written by [Mehran Falaknaz] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript."*

### Data Availability

## References

- Ahmadi J, Pour-Aboughadareh A, Fabriki-Ourang S (2018) Screening wild progenitors of wheat for salinity stress at early stages of plant growth: insight into potential sources of variability for salinity adaptation in wheat. *Crop Pasture Sci* 69, 649–58.
- Arzani A, Ashraf M (2017) Cultivated ancient wheats (*Triticum* spp.): A potential source of health-beneficial food products. *Compr Rev Food Sci Food Saf* 16, 477-488.
- Daneshvar Z, Omid M, Etminan A, Ebrahimi A. (2020) Assessment of genetic diversity and population structure in two wild wheat species *Aegilops cylindrica* and *Ae. crassa* using microsatellite (SSR) markers. *Modern Genetics journal*. 15 (3) :267-276
- Deininger P L, Moran J V, Batzer M A, Kazazian H H (2003) Mobile element and mammalian genome evolution. *Current Opinion in Genetic & Development*. 9130: 651-658.
- Ditta A, Zhou Z, Cai X (2018) Assessment of genetic diversity, population structure, and evolutionary relationship of uncharacterized genes in a novel germplasm collection of diploid and allotetraploid *Gossypium* accessions using EST and genomic SSR markers. *Int J of Mol Sci* 19, 2401.
- Etminan A, Pour-Aboughadareh A, Mehrabi AA, Shooshtari L, Ahmadi-Rad A, Moradkhani H (2019) Molecular characterization of the wild relatives of wheat using CAAT-box derived polymorphism. *Plant Biosyst* 153, 398-405.
- Etminan A, Pour-Aboughadareh A, Mohammadi R (2018) Applicability of CAAT box derived polymorphism (CBDP) markers for analysis of genetic diversity in durum wheat. *Cereal Res Commun* 46, 1–9.
- Grzebelus, D (2006) Transposon insertion polymorphism as a new source of molecular markers. *J. Fruit and Ornament. Plant Research*, 1: 21-29.
- Hassani Tesie S F, Samizadeh Lahiji H, Shoaie Deilami M (2015). Assessment of Genetic Diversity among and Within Different Types of Tobacco (*Nicotiana tabacum* L.) Using IRAP and REMAP Markers. *Journal of Crop Breeding* Vol. 7, No. 16. 1-9
- Hedge S G, Valkoun J, Waines J G (2002) Genetic diversity in wild and weedy *Aegilops*, *Amblyopyrum*, and *secale* species a preliminary survey. *Crop Science* 42:608-614.
- Kilian B, Ozkan H, Deusch O, Effgen Sm Brandolini A, Kohl J, Martin W, Salamini F (2007) Independent wheat B and G genome origins in outcrossing *Aegilops* progenitor haplotypes. *Molecular Biology Evolution* 24:217–227.
- Li X, Wei Y, Moore K J, Michaud R, Viands D R, Hansen J L, Acharya A, Brummer E C (2011) Association mapping of biomass yield and stem composition in a tetraploid alfalfa breeding population. *Plant genome*, 1: 24-35.
- Maxted N, Ford-Lloyd BV, Jury SL, Kell SP, Scholten MA (2006) Towards a definition of a crop wild relative. *Biodiversity and Conservation* 15:2673–2685
- Mohammadi SA, Prasanna BM (2003) Analysis of genetic diversity in crop Plants-Salient statistical tools and considerations. *Crop Sci* 43, 1235-1248
- Moradkhani H, Pour-Aboughadareh AR, Mehrabi AA, Etminan A (2012) Evaluation of genetic relationships of *Triticum-Aegilops* species possessing D genome in different ploidy levels using microsatellites. *Int J Agri Crop Sci* 23, 1746–1751.
- Naghavi MR, Hajikaram M, Taleei AR, Jafar Aghaee M (2010) Microsatellite analysis of genetic diversity and population genetic structure of *Aegilops tauschii* Coss. In Northern Iran. *Genet Res Crop Evol* 57, 423-430.
- Naghavi MR, Maleki M, Alizadeh H, Pirseiedi M, Mardi M (2009) An assessment of genetic diversity in wild diploid wheat *Triticum boeoticum* from west of Iran using RAPD, AFLP and SSR markers. *Journal of Agricultural Science and Technology* 11:585–598.
- Nandakumr N, Singh A, Sharma K, Mohapara R K, Prabhu T K V, Zaman F U (2004) Molecular fingerprinting of hybrids and assessment of genetic purity of hybrid seeds in rice using microsatellite markers. *Euphytica*. (136): 257-26
- Nouri A, Etminan A, Golabadi M, Mehrabi AA, Rezaee A (2021) Molecular variation and genetic relationships among *Aegilops tauschii* accessions based on CAAT-box derived polymorphism. *Agricultural Biotechnology Journal* 13 (1), 57-74.
- Pardue ML, Debarysh PG (2003) Retrotransposons provide evolutionarily robust non-telomerase mechanism to maintain telomers. *Annual Review of genetic*. Vol(37):485-493
- Pour-Aboughadareh A, Ahmadi J, Mehrabi AA Etminan A, Moghaddam M (2018) Insight into the genetic variability analysis and relationships among some *Aegilops* and *Triticum* species, as genome progenitors of bread wheat, using SCoT markers. *Plant Biosystem* 152:694–703.
- Pour-Aboughadareh A, Etminan A, Shooshtari L, Maleki-Tabrizi N (2019) Comparative assessment of SCoT and CBDP markers for investigation of genetic diversity existing in different *Aegilops* species. *Agric Biotechnol J* 11 (4), 153-174. (In Persian)
- Pritchard J K, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data||, *Genetics*, 2: 945-959.
- Putman AI, Carbone I (2014) Challenges in analysis and interpretation of microsatellite data for population genetic studies. *Ecol Evol* 4, 4399–4428.
- Saeidi H, Rahiminejad MR, Vallian S, Heslop-Harrison JS (2006) Biodiversity of diploid D-genome *Aegilops tauschii* Coss. In Iran measured using microsatellites. *Genetic Resources and Crop Evolution* 53:1477–1484
- Saghai Maroof M A, Biyashev R M, Yang G P, Zhang Q, Allard R W (1994) Extraordinarily polymorphic microsatellite DNA in barley species diversity chromosomal location. and population dynamics. *Proceeding of national Academy of Sciences*, 12: 5466-5570.
- Tahernezhad Z, Zamani MJ, Solouki M, Zahravi M, Imamjomeh AA, Jafaraghaei M, Bihamta MR (2010) 'Genetic diversity of Iranian *Aegilops tauschii* Coss. using microsatellite molecular markers and morphological traits. *Molecular Biology Reports* 37:3413–3420.
- Warschefsky E, Penmetza RV, Cook DR, von Wettberg EJB (2014) Back to the wilds: tapping evolutionary adaptations for resilient crops through systematic hybridization with crop wild relatives. *American Journal of Botany* 101:1791–1800



Table 1  
Information and collection location of *Aegilops tauschii* genotypes investigated in this research

| No | Genebank Code | Province                         | No | Genebank Code | Province                       | No  | Genebank Code | Province                    |
|----|---------------|----------------------------------|----|---------------|--------------------------------|-----|---------------|-----------------------------|
| 1  | IUGB-00020    | Iran                             | 43 | IUGB-00305    | Iran/unknown                   | 85  | TN-01-0699    | Iran/Golestan               |
| 2  | IUGB-00039    | Iran/Ardabil-Sarein Road         | 44 | IUGB-00306    | Tajikistan                     | 86  | TN-01-0804    | Iran/unknown                |
| 3  | IUGB-00051    | Iran/unknown                     | 45 | IUGB-00307    | Iran/unknown                   | 87  | TN-01-0836    | Iran/Mazandaran             |
| 4  | IUGB-00080    | Azerbaijan                       | 46 | IUGB-00308    | Azerbaijan                     | 88  | TN-01-0945    | Iran/unknown                |
| 5  | IUGB-00107    | Iran/Salman Shahr                | 47 | IUGB-00309    | Iran/unknown                   | 89  | TN-01-1005    | Iran/unknown                |
| 6  | IUGB-00108    | Iran/Gilan- Kooch Esfahan Road   | 48 | IUGB-00310    | Iran/unknown                   | 90  | TN-01-1559    | Iran/unknown                |
| 7  | IUGB-00141    | Iran/ unknown                    | 49 | IUGB-00311    | Sweden                         | 91  | TN-01-1695    | Iran/unknown                |
| 8  | IUGB-00143    | Iran/ unknown                    | 50 | IUGB-00312    | Iran/unknown                   | 92  | TN-01-1745    | Iran/Semnan                 |
| 9  | IUGB-00144    | Iran/ unknown                    | 51 | IUGB-00313    | Iran/unknown                   | 93  | TN-01-1770    | Iran/unknown                |
| 10 | IUGB-00151    | Tajikistan                       | 52 | IUGB-00314    | Azerbaijan                     | 94  | TN-01-1970    | Iran/unknown                |
| 11 | IUGB-00157    | Iran/Ardabil-Sarein Road         | 53 | IUGB-00315    | Iran/unknown                   | 95  | TN-01-1772    | Iran/unknown                |
| 12 | IUGB-00164    | Iran/Sadraddin Village           | 54 | IUGB-00325    | Iran/Karaj-Chaloos             | 96  | TN-01-2115    | Iran/unknown                |
| 13 | IUGB-00193    | Iran/ Astara-Ardabil Road        | 55 | IUGB-00362    | Armenia                        | 97  | TN-01-2120    | Iran/unknown                |
| 14 | IUGB-00196    | Iran/Ahar-Kelibar                | 56 | IUGB-00365    | Iran/Mazandaran-Amol           | 98  | TN-01-2207    | Iran/unknown                |
| 15 | IUGB-00198    | Iran/Ramsar                      | 57 | IUGB-00366    | Iran/Ghaemabad-Lahijan Village | 99  | IUGB-02054    | Iran/Rasht                  |
| 16 | IUGB-00223    | Iran/Zanjan                      | 58 | IUGB-00367    | Iran/30km Ahar-Kelaibar        | 100 | IUGB-02055    | Iran/Rasht                  |
| 17 | IUGB-00224    | Iran/ Salmanshahr-Ramsar Road    | 59 | IUGB-00369    | Iran/Gilan-Kalachay            | 101 | IUGB-02056    | Iran/Rasht                  |
| 18 | IUGB-00238    | Iran/Gilan-Koochsfehan Road      | 60 | IUGB-00370    | Iran/unknown                   | 102 | IUGB-02057    | Iran/unknown                |
| 19 | IUGB-00245    | Iran/Karaj-Chaloos,              | 61 | IUGB-00371    | Iran/unknown                   | 103 | IUGB-02058    | Iran/unknown                |
| 20 | IUGB-00247    | Iran/Karaj-Chaloos               | 62 | IUGB-00374    | Iran/Astaneh Ashrafyeh         | 104 | IUGB-02059    | Iran/unknown                |
| 21 | IUGB-00249    | Iran/Mazandaran-Amol             | 63 | IUGB-00375    | Iran/Dashte Moghan             | 105 | IUGB-02060    | Iran/Baraghan               |
| 22 | IUGB-00260    | Iran/Salmanshahr-Mazandaran Road | 64 | IUGB-00383    | Iran/Abbasabad –Ramsar         | 106 | IUGB-02061    | Iran/Rezvanshahr            |
| 23 | IUGB-00261    | Iran/Gilan-3km Astara            | 65 | IUGB-00386    | Iran/30km Ahar-Kelaibar        | 107 | IUGB-02062    | Iran/Rezvanshahr            |
| 24 | IUGB-00263    | Iran/Karaj- Chaloos Road         | 66 | IUGB-00396    | Iran/Mazandaran-Noshahr        | 108 | IUGB-02063    | Iran/Rezvanshahr            |
| 25 | IUGB-00269    | Iran/Mazandaran-Amol             | 67 | IUGB-00400    | Iran/20km Chalooos Road        | 109 | IUGB-02064    | Iran/Somesara               |
| 26 | IUGB-00273    | Iran/Rasht                       | 68 | IUGB-00401    | Iran/Noor                      | 110 | IUGB-02065    | Iran/Astara                 |
| 27 | IUGB-00274    | Iran/km5 Sarein-Ardabil Road     | 69 | IUGB-00402    | Iran/Noor                      | 111 | IUGB-02066    | Iran/Somesara               |
| 28 | IUGB-00276    | Iran/Chaloos                     | 70 | IUGB-00404    | Iran/Rasht- Talesh             | 112 | IUGB-02067    | Iran/Somesara               |
| 29 | IUGB-00279    | Iran/ Galoogah Bandar Behshahr   | 71 | IUGB-00405    | Iran/Karaj-Chaloos             | 113 | IUGB-02068    | Iran/Somesara               |
| 30 | IUGB-00289    | Iran/10km Ahar-Tabriz Road       | 72 | IUGB-00429    | Iran/unknown                   | 114 | IUGB-02069    | Iran/rasht-Feiz mahaleh     |
| 31 | IUGB-00290    | Afghanistan                      | 73 | IUGB-01746    | Iran/unknown                   | 115 | IUGB-02070    | Iran/rasht-Feiz mahaleh     |
| 32 | IUGB-00291    | Turkmenistan                     | 74 | KC-50006      | Iran/unknown                   | 116 | IUGB-02071    | Iran/rasht-Feiz mahaleh     |
| 33 | IUGB-00292    | Azerbaijan                       | 75 | KC-50037      | Iran/unknown                   | 117 | IUGB-02072    | Iran/Gilan-Ponel Road       |
| 34 | IUGB-00293    | Japan                            | 76 | KC-50084      | Iran/Azarbayjan sharghi        | 118 | IUGB-02073    | Iran/Foman                  |
| 35 | IUGB-00295    | Turkey                           | 77 | KC-50133      | Iran/Khorasan                  | 119 | IUGB-02074    | Iran/Karaj                  |
| 36 | IUGB-00296    | Armenia                          | 78 | KC-50136      | Iran/Khorasan                  | 120 | IUGB-00205    | Iran/Ardabil-Hayran Village |

| No | Genebank Code | Province     | No | Genebank Code | Province                | No  | Genebank Code | Province                    |
|----|---------------|--------------|----|---------------|-------------------------|-----|---------------|-----------------------------|
| 37 | IUGB-00297    | Iran/unknown | 79 | TN-01-0312    | Iran/Azarbayjan gharbi  | 121 | IUGB-00205    | Iran/Golestan-Aliabad_Ketol |
| 38 | IUGB-00298    | Iran/unknown | 80 | TN-01-0369    | Iran/Azarbayjan sharghi | 122 | IUGB-00205    | Iran/Ardabil-Sarein Road    |
| 39 | IUGB-00299    | Afghanistan  | 81 | TN-01-0562    | Iran/Semnan             | 123 | IUGB-00205    | Iran/Astara                 |
| 40 | IUGB-00300    | Iran/unknown | 82 | TN-01-0563    | Iran/Gilan              | 124 | IUGB-00205    | Iran/Harsin-Noorabad Road   |
| 41 | IUGB-00302    | Afghanistan  | 83 | TN-01-0569    | Iran/Gilan              | 125 | IUGB-00205    | Iran/unknown                |
| 42 | IUGB-00303    | Turkey       | 84 | TN-01-0667    | Iran/Azarbayjan sharghi |     |               |                             |

Table 2  
SSR markers used in the research

| No | Marker  | Chromosome | Type of marker | Sequence (5– 3)                   | No | Marker  | Chromosome | Type of marker | Sequence (5– 3)                      |
|----|---------|------------|----------------|-----------------------------------|----|---------|------------|----------------|--------------------------------------|
| 1  | Xgwm33  | 1D         | Left           | GGA GTC ACA CTT GTT<br>TGT GCA    | 16 | Xgwm165 | 4D         | Left           | TGC AGT GGT CAG<br>AGT TTT CC        |
|    |         |            | Right          | CAC TGC ACA CCT AAC<br>TAC CTG C  |    |         |            | Right          | CTT TTC TTT CAG ATT<br>GCG CC        |
| 2  | Xgwm642 | 1D         | Left           | ACG GCG AGA AGG TGC<br>TC         | 17 | Xgwm654 | 5D         | Left           | TGC TGA TGT TGT<br>AAG AAG GC        |
|    |         |            | Right          | CAT GAA AGG CAA GTT<br>CGT CA     |    |         |            | Right          | TGC GTC AGA TAT GCC<br>TAC CT        |
| 3  | Xgwm337 | 1D         | Left           | CCT CTT CCT CCC TCA<br>CTT AGC    | 18 | Xgwm16  | 5D         | Left           | GCT TGG ACT AGC<br>TAG AGT ATC ATA C |
|    |         |            | Right          | TGC TAA CTG GCC TTT<br>GCC        |    |         |            | Right          | CAA TCT TCA ATT CTG<br>TCG CAC GG    |
| 4  | Xgwm106 | 1D         | Left           | CTG TTC TTG CGT GGC<br>ATT AA     | 19 | Xgwm271 | 5D         | Left           | CAA GAT CGT GGA<br>GCC AGC           |
|    |         |            | Right          | AAT AAG GAC ACA ATT<br>GGG ATG G  |    |         |            | Right          | AGC TGC TAG CTT<br>TTG GGA CA        |
| 5  | Xgwm539 | 2D         | Left           | CTG CTC TAA GAT TCA<br>TGC AAC C  | 20 | Xgwm174 | 5D         | Left           | GGG TTC CTA TCT<br>GGT AAA TCC C     |
|    |         |            | Right          | GAG GCT TGT GCC CTC<br>TGT AG     |    |         |            | Right          | GAC ACA CAT GTT CCT<br>GCC AC        |
| 6  | Xgwm30  | 2D         | Left           | ATC TTA GCA TAG AAG<br>GGA GTG GG | 21 | Xgwm358 | 5D         | Left           | AA CAG CGG ATT TCA<br>TCG AG         |
|    |         |            | Right          | TTC TGC ACC CTG GGT<br>GAT        |    |         |            | Right          | TCC GCT GTT GTT<br>CTG ATC TC        |
| 7  | Xgwm320 | 2D         | Left           | CGA GAT ACT ATG GAA<br>GGT GAG G  | 22 | Xgwm565 | 5D         | Left           | GCG TCA GAT ATG CCT<br>ACC TAG G     |
|    |         |            | Right          | ATC TTT GCA AGG ATT<br>GCC C      |    |         |            | Right          | AGT GAG TTA GCC<br>CTG AGC CA        |
| 8  | Xgwm102 | 2D         | Left           | TCT CCC ATC CAA CGC<br>CTC        | 23 | Xgwm55  | 6D         | Left           | GCA TCT GGT ACA CTA<br>GCT GCC       |
|    |         |            | Right          | TGT TGG TGG CTT GAC<br>TAT TG     |    |         |            | Right          | TCA TGG ATG CAT CAC<br>ATC CT        |
| 9  | Xgwm341 | 3D         | Left           | TTC AGT GGT AGC GGT<br>CGA G      | 24 | Xgwm469 | 6D         | Left           | CAA CTC AGT GCT CAC<br>ACA ACG       |
|    |         |            | Right          | CCG ACA TCT CAT GGA<br>TCC AC     |    |         |            | Right          | CGA TAA CCA CTC ATC<br>CAC ACC       |
| 10 | Xgwm383 | 3D         | Left           | ACG CCA GTT GAT CCG<br>TAA AC     | 25 | Xgwm325 | 6D         | Left           | TTT CTT CTG TCG TTC<br>TCT TCC C     |
|    |         |            | Right          | GAC ATC AAT AAC CGT<br>GGA TGG    |    |         |            | Right          | TTT TTA CGC GTC AAC<br>GAC G         |
| 11 | Xgwm2   | 3D         | Left           | CTG CAA GCC TGT GAT<br>CAA CT     | 26 | Xgwm121 | 7D         | Left           | TCC TCT ACA AAC AAA<br>CAC AC        |
|    |         |            | Right          | CAT TCT CAA ATG ATC<br>GAA CA     |    |         |            | Right          | CTC GCA ACT AGA<br>GGT GTA TG        |
| 12 | Xgwm114 | 3D         | Left           | ACA AAC AGA AAA TCA<br>AAA CCC G  | 27 | Xgwm111 | 7D         | Left           | TCT GTA GGC TCT CTC<br>CGA CTG       |
|    |         |            | Right          | ATC CAT CGC CAT TGG<br>AGT G      |    |         |            | Right          | ACC TGA TCA GAT CCC<br>ACT CG        |
| 13 | Xgwm608 | 4D         | Left           | ACA TTG TGT GTG CGG<br>CC         | 28 | Xgwm44  | 7D         | Left           | GTT GAG CTT TTC<br>AGT TCG GC        |
|    |         |            | Right          | GAT CCC TCT CCG CTA<br>GAA GC     |    |         |            | Right          | ACT GGC ATC CAC TGA<br>GCT G         |
| 14 | Xgwm609 | 4D         | Left           | GCG ACA TGA CCA TTT<br>TGT TG     | 29 | Xgwm295 | 7D         | Left           | GTG AAG CAG ACC<br>CAC AAC AC        |

| No | Marker  | Chromosome | Type of marker | Sequence (5– 3)                   | No | Marker  | Chromosome | Type of marker | Sequence (5– 3)                |
|----|---------|------------|----------------|-----------------------------------|----|---------|------------|----------------|--------------------------------|
|    |         |            | Right          | GAT ATT AAA TCT CTC<br>TAT GTG TG |    |         |            | Right          | GAC GGC TGC GAC<br>GTA GAG     |
| 15 | Xgwm624 | 4D         | Left           | TTG ATA TTA AAT CTC<br>TCT ATG TG | 30 | Xgwm350 | 7D         | Left           | ACC TCA TCC ACA TGT<br>TCT ACG |
|    |         |            | Right          | AAT TTT ATT TGA GCT<br>ATG CG     |    |         |            | Right          | GCA TGG ATA GGA<br>CGC CC      |

Table 3  
ISSR markers used in the research

| No                                                                                               | Marker  | Sequence (5– 3)    | No | Marker  | Sequence (5– 3)       |
|--------------------------------------------------------------------------------------------------|---------|--------------------|----|---------|-----------------------|
| 1                                                                                                | ISSR 7  | GAGAGAGAGAGAGAGAG  | 16 | UBC 840 | GAGAGAGAGAGAGAGAYT    |
| 2                                                                                                | ISSR 9  | CTCTCTCTCTCTCTCTG  | 17 | UBC 841 | GAGAGAGAGAGAGAGAYC    |
| 3                                                                                                | UBC 808 | AGAGAGAGAGAGAGAGC  | 18 | UBC 842 | GAGAGAGAGAGAGAGAYG    |
| 4                                                                                                | UBC 809 | AGAGAGAGAGAGAGAGG  | 19 | UBC 843 | CTCTCTCTCTCTCTCTRA    |
| 5                                                                                                | UBC 811 | GAGAGAGAGAGAGAGAC  | 20 | UBC 853 | TCTCTCTCTCTCTCTCRT    |
| 6                                                                                                | UBC 813 | CTCTCTCTCTCTCTCTT  | 21 | UBC 866 | CTCCTCCTCCTCCTCCTC    |
| 7                                                                                                | UBC 812 | CTCTCTCTCTCTCTCTA  | 22 | UBC 884 | AGAGAGAGAGAGAGAG      |
| 8                                                                                                | UBC 815 | CTCTCTCTCTCTCTCTG  | 23 | UBC 886 | VDVCTCTCTCTCTCTCT     |
| 9                                                                                                | UBC 822 | TCTCTCTCTCTCTCTCA  | 24 | UBC 887 | DVDTCTCTCTCTCTCTC     |
| 10                                                                                               | UBC 823 | TCTCTCTCTCTCTCTCC  | 25 | UBC 888 | BDBCACACACACACACA     |
| 11                                                                                               | UBC 824 | TCTCTCTCTCTCTCTCG  | 26 | UBC 889 | DBDACACACACACACAC     |
| 12                                                                                               | UBC 826 | ACACACACACACACACC  | 27 | UBC 890 | VHVGTTGTGTGTGTGTGT    |
| 13                                                                                               | UBC 834 | AGAGAGAGAGAGAGACYT | 28 | UBC 891 | HVHTGTGTGTGTGTGTGT    |
| 14                                                                                               | UBC 835 | AGAGAGAGAGAGAGACYC | 29 | UBC 899 | CATGGTGTGGTCATTGTTCCA |
| 15                                                                                               | UBC 836 | AGAGAGAGAGAGAGACYA | 30 | UBC 900 | ACTTCCCCACAGGTTAACACA |
| R: (A or G), Y: (C or T), V: (A or G or C), D: (A or G or T), B: (T or G or C), H: (A or T or C) |         |                    |    |         |                       |

Table 4  
Retrotransposon markers used in the research

| No | Marker           | Sequence (5– 3)                                      |
|----|------------------|------------------------------------------------------|
| 1  | LTR1061          | AGAGGGGAATGTGGGGGTTTCC                               |
| 2  | LTR2116          | CGAACCTGGGTAAACTTCGTGTC                              |
| 3  | Sukkula          | GATAGGGTCGCATCTTGGGCGTGAC                            |
| 4  | LTR1061- LTR2116 | AGAGGGGAATGTGGGGGTTTCC<br>CGAACCTGGGTAAACTTCGTGTC    |
| 5  | LTR106- Sukkula  | AGAGGGGAATGTGGGGGTTTCC<br>GATAGGGTCGCATCTTGGGCGTGAC  |
| 6  | LTR2116- Sukkula | CGAACCTGGGTAAACTTCGTGTC<br>GATAGGGTCGCATCTTGGGCGTGAC |
| 7  | LTR1061-UBC 834  | AGAGGGGAATGTGGGGGTTTCC<br>AGAGAGAGAGAGAGACYT         |
| 8  | LTR2116-UBC 834  | CGAACCTGGGTAAACTTCGTGTC<br>AGAGAGAGAGAGAGACYT        |
| 9  | Sukkula-UBC 834  | GATAGGGTCGCATCTTGGGCGTGAC<br>AGAGAGAGAGAGAGACYT      |

Table 5  
PCR thermal cycling program for markers

| No | Stage                                                                    | temperature (Celsius)     | Time                   |
|----|--------------------------------------------------------------------------|---------------------------|------------------------|
| 1  | Denaturation of genomic DNA                                              | 94                        | 4 minutes              |
| 2  | Denaturation of genomic DNA                                              | 94                        | 30 seconds to 1 minute |
| 3  | Binding the primer to the template string                                | Tm + 5                    | 30 seconds to 1 minute |
| 4  | Expansion of the new field                                               | 72                        | 4 minutes              |
| 5  | The gradient of decreasing temperature until reaching the temperature Tm | Each cycle of 0.5 degrees | —                      |
| 6  | 10 cycles of repeating steps 2 to 5                                      | —                         | —                      |
| 7  | Denaturation of genomic DNA                                              | 94                        | 30 seconds to 1 minute |
| 8  | Binding the primer to the template string                                | Tm                        | 30 seconds to 1 minute |
| 9  | Expansion of the new field                                               | 72                        | 1 minute               |
| 10 | 30 cycles of repeating steps 7 to 9                                      | —                         | —                      |
| 11 | Final expansion                                                          | 72                        | 10 minutes             |
| 12 | Maintenance stage                                                        | 4                         | 5 minutes              |

Table 6  
Comparison of marker systems in studied *Aegilops tauschii* genotypes

| Marker          | number of markers | Total number of amplified loci | The average number of amplified loci per primer | The average number of polymorphic alleles | The average number of polymorphic alleles | Average PIC | Average MI | Average Shannon index | Average genetic diversity of Nei |
|-----------------|-------------------|--------------------------------|-------------------------------------------------|-------------------------------------------|-------------------------------------------|-------------|------------|-----------------------|----------------------------------|
| SSR             | 30                | 34                             | 1.13                                            | 1,424                                     | 1,424                                     | 0.271       | 0.215      | 0.278                 | 0.196                            |
| ISSR            | 30                | 172                            | 5.73                                            | 1.339                                     | 1.339                                     | 0.332       | 0.268      | 0.232                 | 0.163                            |
| Retrotransposon | 9                 | 64                             | 7.11                                            | 1.538                                     | 1.538                                     | 0.311       | 0.378      | 0.345                 | 0.268                            |

Table 7  
Molecular variance analysis based on the markers used

| Sources of variation | Df  | Variation percentage | Est/ Var | mean square | sum of squares | Variation percentage | Est/ Var | mean square | sum of squares | Variation percentage | Est/ Var | mean square | sum of squares |
|----------------------|-----|----------------------|----------|-------------|----------------|----------------------|----------|-------------|----------------|----------------------|----------|-------------|----------------|
|                      |     | ISSR                 |          |             |                | Retrotransposon      |          |             |                | SSR                  |          |             |                |
| between populations  | 6   | 31                   | 6.69     | 120.21      | 721.257        | 7                    | 0.297    | 8.43        | 50.57          | 17                   | 1.56     | 32.46       | 194.78         |
| within populations   | 118 | 69                   | 15.22    | 15.22       | 1796.74        | 93                   | 3.77     | 3.77        | 445.05         | 83                   | 7.86     | 7.86        | 927.85         |
| Total                | 124 | 100                  | 21.92    | —           | 2518           | 100                  | 4.06     | —           | 495.63         | 100                  | 9.43     | —           | 1122.64        |

Table 8 is not available with this version.

## Figures

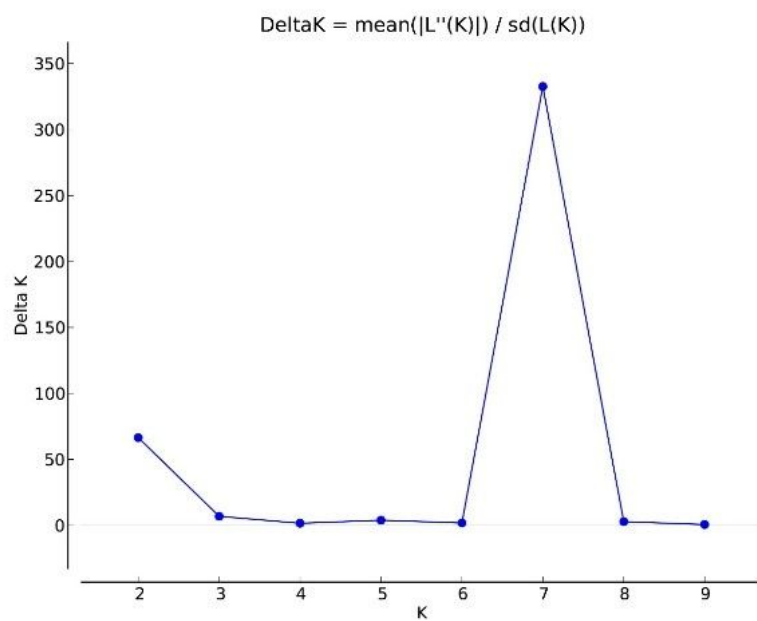


Figure 1

Two-dimensional diagram to determine the appropriate number of subpopulations in the studied *Aegilops tauschii* genotypes (K=7)

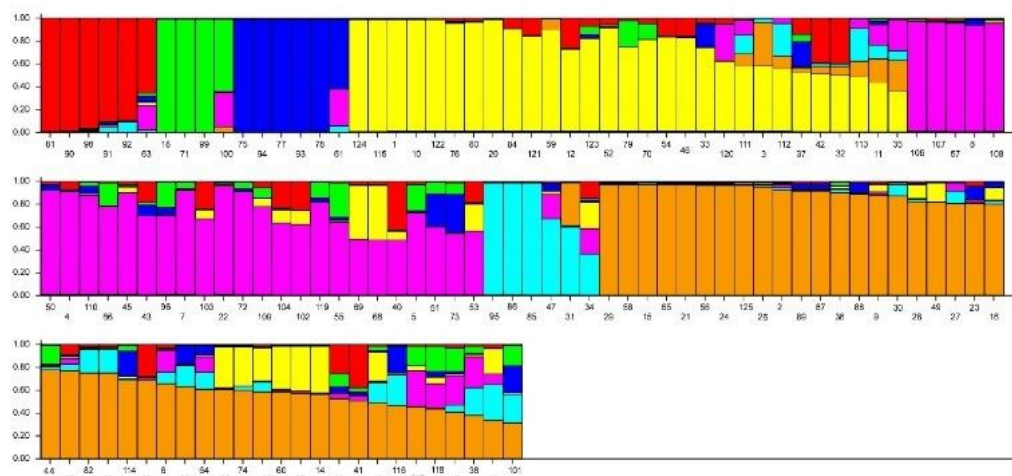


Figure 2

Bar plot diagram for 125 *Aegilops tauschii* genotypes based on SSR, ISSR, and retrotransposon markers, each color shows a subpopulation and the vertical axis shows the coefficient of belonging to each subpopulation.

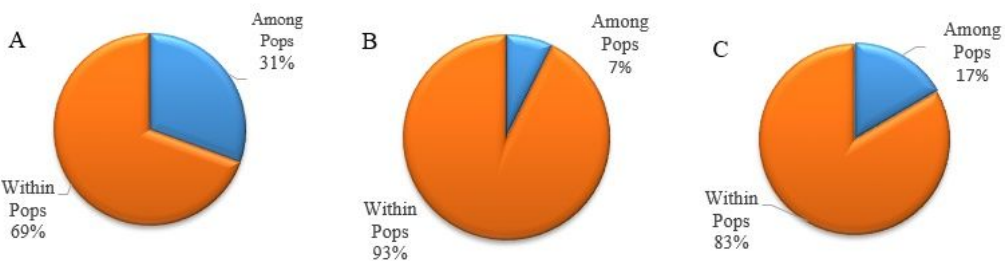


Figure 3

Estimation of genetic variance ratio between and within *Ae. tauschii* genotypes based on ISSR(A), retrotransposon(B) and SSR(C) markers.