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Research Paper

Study of Genetic Diversity of Iranian Wheat through Microsatellite Marking

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Abstract

Among the most effective measures in improving crops is to investigate their genetic structures. In the present study, 25 Einkorn wheat populations from two species of Triticum boeoticum and Triticum urartu collected from west and northwest provinces of Iran were investigated using 12 pairs of microsatellite markers. 12 pairs of primers multiplied 2-14 alleles and a total of 87 alleles among 25 genotypes with mean number of 7.25 alleles for each primer pair. Polymorphism information content was obtained 0.37 to 0.92 and the mean was calculated 0.72. Cluster analysis was carried out using UPGMA method and Dice and Jaccard similarity coefficients. Based on the plotted dendrograms, the genotypes of T.boeoticum and T.urartu species showed the highest diversities in Lorestan and Kermanshah provinces, respectively. This implies Lorestan and Kermanshah as the main origins of the two mentioned species. Totally, the studied populations of Einkorn Wheat plants exhibited higher diversities in the west provinces compared to the northwest ones.

Keywords: Triticum Boeoticum; Triticum Urartu; Genetic Diversity; Microsatellite Markers

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1. Introduction

During the past decades, genetic diversity of wheat has tended to decrease as well as many other crops. Researchers believe that information and diversity of current varieties of wheat have been applied completely for improvement purposes. There are three requisites for advances in wheat improvement: first, new resources of genetic diversity to provide optimal alleles for genetic advances; second, technologies for diversity recombination and producing new genotypes; and third, technologies for identification and selecting the genotypes in relation to the produced corresponding gene sets, genotype determination and screening the genetic resources for useful genes. In this way, the first step is the sustainable using of Iranian wheat germ plasms (1). Searching for new gene resources, therefore, breeders maintain in successful applicability of native varieties and wild relatives of wheat as potentially useful and valuable resources of genetic diversity (7). One of the early needs for wheat improvement is estimating the genetic diversity among relatives of wheat for identification and improvement purposes. As genome A donors to hexaploid, tetraploid and diploid crop wheat plants (*Triticum monococum*) are considered as the valuable genetic resources in improving crop wheats (4). The moderate mountainous areas in the hillsides of Zagros and Alborz mountains in the west and northwest provinces of Iran are regarded as the main areas of genesis, distribution and diversification for different species of Einkorn diploid wheat. Knowing the precise status of the wheat wild populations in Iran has been of high priority among the interested researchers. As another important objective, thus, the present study investigates the status and structure of genetic diversity within and between populations

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of Einkorn diploid wheat in two species of Triticum boeoticum and Triticum urartu.

The genome anatomy of Einkorn wheat Triticum urartu (AA) as genome donor to bread and triticum durum has been well preserved in their crop varieties. One of the applied objectives of this study was identification and introduction of polymorphic microsatellite markers in this diploid species; the intended markers must be capable of passing to the improved populations of hexaploid bread wheat. Microsatellite markers are of the most effective molecular markers. They characterize by distribution in the genome, easy application, multi allelic nature and co-dominancy (6). Using 279 microsatellite gene locations in 1998, Roder et al plotted the first genetic map of wheat genome Based on the microsatellite markers (9). In 2002, Ahmad used 43 microsatellite markers to estimate the genetic diversity across 13 wheat genotypes from different origins (5). To date, many studies have been reported on the study of genetic diversity of wheat using the microsatellite markers (8,11,12).

2. Materials and Methods

The plant materials used in this research was 25 populations of wild Einkorn wheat collected from Zagros hillsides. The studied populations were from two species of Triticum boeoticum and Triticum urartu (Table 1).

In order to molecular analysis, the seeds of 25 genotypes were cultured in pots and the germinator. Through CTAB method, genomic DNA was extracted from the intact young leaves of two weeks single seedlings. Microsatellite locations were multiplied using 12 primers. The multiplied fragments were dissociated by the polyacrylamide gel and the gel was stained using the silver nitrate approach. Band scorings were carried out as 0 (no bands) and 1 (bands existed). Polymorphism information content was calculated based on the Microsatellite locations. The dissimilarity matrix and cluster analysis were performed according to UPGMA algorithm and Dice and Jaccard methods. The softwares used in data analysis included DARwin, XLstat Popgene and Excel.

Table 1. he studied populations of Einkorn Wheat used in the research

Number	Genus	Species	Collecting location
1	Triticum	urartu	Sarnaveh Village, Nourabad- Khorramabad road
2	Triticum	boeoticum	Ravansar Police station, Ravansar-Paveh road, Kermanshah
3	Triticum	boeoticum	Hurand-Ahar road- East Azerbaijan
4	Triticum	boeoticum	Kermanshah-Sonqor road- Kermanshah
5	Triticum	boeoticum	Sefid dasht (Gol zard)- Lorestan
6	Triticum	boeoticum	Dorud- Khorramabad road- 35Km to Khorramabad- Lorestan
7	Triticum	boeoticum	Bistoun, Hosseinabad, Kermanshah
8	Triticum	boeoticum	Nourabad, Delfan, Lorestan
9	Triticum	boeoticum	Javanroud crossroad, Kermanshah
10	Triticum	boeoticum	Saqqez countryside, Kordestan
11	Triticum	boeoticum	Ravansar-Kamyaran road, Kermanshah
12	Triticum	urartu	1 Km passed Khosroabad -, Eslamabad-Kerend road, Kermanshah
13	Triticum	boeoticum	2 Km to Hassanabad, Eslamabad, Kermanshah
14	Triticum	boeoticum	15Km passed Dehgolan-Qorveh-Sanandaj road, Kordestan
15	Triticum	boeoticum	20Km to Marivan, Chenareh village, Kordestan
16	Triticum	boeoticum	On the 10th Km of Ahar-Tabriz road, East Azerbaijan
17	Triticum	urartu	Zolfas village, Asadabad, Sonqor, Kermanshah
18	Triticum	boeoticum	Sepid dasht, 30 Km to Khorramabad, Lorestan
19	Triticum	boeoticum	Oshnavieh, Silvana, West Azerbaijan
20	Triticum	boeoticum	30 Km on Khorramabad-Dorud road, Lorestan
21	Triticum	boeoticum	30 Km on ahar-kaleibar road, East Azerbaijan
22	Triticum	urartu	Naqadeh countryside, West Azerbaijan
23	Triticum	boeoticum	Sonqor road, Kermanshah
24	Triticum	boeoticum	Qarehtapeh village, Asadabad, Sonqor, Kermanshah
25	Triticum	urartu	Bistoun, Hosseinabad, Kermanshah

Results and Discussion

Most of the multiplied loci showed optimal polymorphisms. As the result of multiplying 12 microsatellite locations, 87 alleles were identificated with 2 to 14 alleles in/for each location. The mean number of alleles was 7.25 in all the loci (Table 2). This was consistent with the previous studies on microsatellite locations in wheat such as the research by Stodart et al (2005) reporting 3 to 29 alleles with the mean value of 10 and the study by Maccaferri et al (2003) with reported number of 2 to 12 and the mean value of 5.6 (11,13). Polymorphism information content ranged from 0.37 to 0.92 with the mean value of 0.75. The values were consistent with the results of Agrama et al (2003) ranging from 0.23 to 0.81 (with the mean value of 0.62), and the study by Roder et

al (1995) reporting the values of 0.23 to 0.79 and the mean value of 0.7 (3, 9). Based on Dice and Jaccard method, the Dissimilarity coefficient of the genotypes was calculated using the software DARwin and UPGMA algorithm was applied in cluster analysis.

In order to compute the dissociation power (D) of microsatellite markers the software popgene was used. Thus, populations of the species urartu and boeoticum were studied as the two groups in terms of genomic profiles resulted from 87 scored bands among the test materials. Accordingly, the dissociation power of the microsatellite marker and PI were determined as 0.95 and 0.051 respectively, indicating high effectiveness of the markers in varieties identification.

In order to assess the information obtained from the studied microsatellite loci, the information resulted from 12 scored bands of these 12 microsatellite markers was complementary investigated by the principal component analysis. Based on the analysis results (table 3), the first and second axes contained %18.29 and %16.7 of information, respectively. In total, the 6 axes implied two thirds of information. The results indicated the proper distribution of the selected microsatellite locations across the studied genomes. This was already considered in selecting primers by using the ones related to the markers from 7 chromosomes in the genome A of common wheat. Biplot drawing with the first and second axes containing %34.99 of information in the scored bands showed good differentiation across the genotypes. The two species of urartu and boeoticum were located in biplot plate in a differentiated manner (Figure 3).

The studied populations of Einkorn Wheat plants exhibited higher diversities in the west provinces (Lorestan, Kermanshah and Kordestan) compared to the northwest ones (East Azerbaijan and West Azerbajan). Triticum boeoticum showed the highest diversity in Lorestan province so that the collected samples existed in all three groups including 20 genotypes of the species (Figures 1 and 2). In addition, Triticum urartu had the highest diversity in Kermanshah province.

The results of the present study indicated high effectiveness of the microsatellite markers in studying the genetic diversity of Einkorn wheat species. The low number of the microsatellite markers makes good differentiation across the mentioned species, indicating intraspecies differences. It's partly because of optimal distribution of the microsatellite in A genome. Another possible reason is the wild nature of the studied species and high diversity across them. In general, it seems essential to pay special attention to Einkorn wheat germ plasm in the west provinces given the significant diversity of its species in these regions. In order to exploit of this genetic diversity, improvement programs need to be planned based on the precise identification of these populations for optimal genes. Since Triticum urartu is considered as the donor species of A genome for Triticum aestivvm and Triticum durum, special investigations on the species germ plasm in these regions particularly Kermanshah province will be of high importance (2).

Table 2. Properties of the Primers Used in studying and identifacion of gene locations and polymorphism content in 25 populations of two Einkorn wheat species

Locus name	Number of Alleles	Primer annealing temperature	polymorphism content
Xgwm130-7A	7	60	0.83
Xgwm156-5A	11	60	0.90
Xgwm160-4A	2	60	0.37
Xgwm334-6A	6	50	0.81
Xgwm357-1A	3	55	0.59
Xgwm369-3A	14	60	0.92
Xgwm372-2A	9	60	0.87
Xgwm5-3A	2	50	0.37
Xgwm99-1A	7	60	0.83
Xgwm164-1A	5	55	0.76
Xgwm570-6A	14	60	0.92
Xgwm410-5A	7	55	0.83

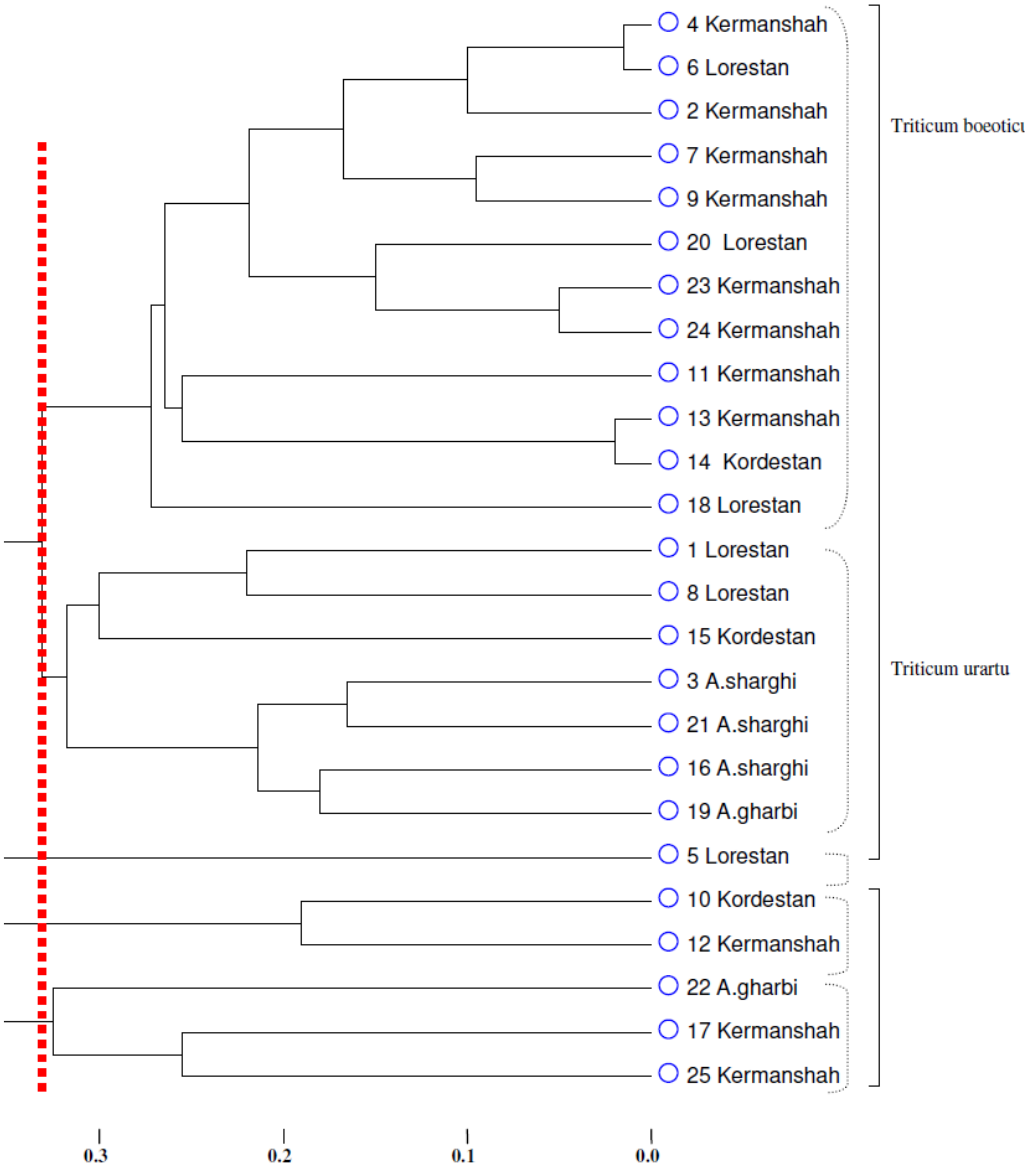

Figure 1. Grouping of 25 Einkorn wheat genotypes (the two species Triticum boeoticum and Triticum urartu) using a dendrogram prepared by 78 scored bands from 12 microsatellite locations (UPGMA algorithm on Dice dissimilarity matrix)

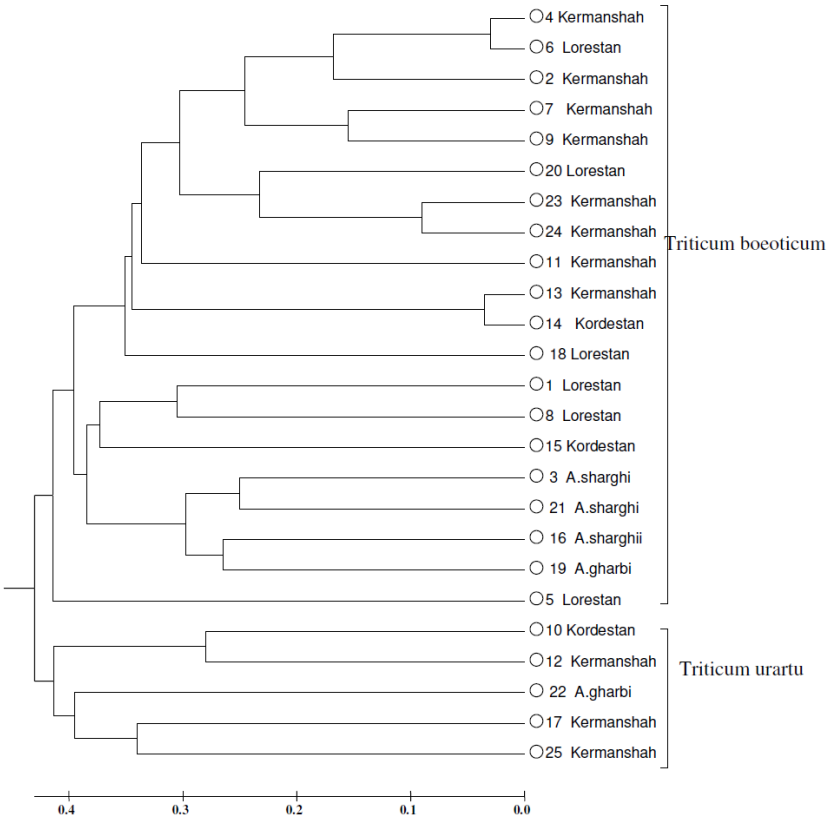

Figure 2. Grouping of 25 Einkorn wheat genotypes (the two species Triticum boeoticum and Triticum urartu) using a dendrogram prepared by 78 scored bands from 12 microsatellite locations (UPGMA algorithm on Jaccard dissimilarity matrix)

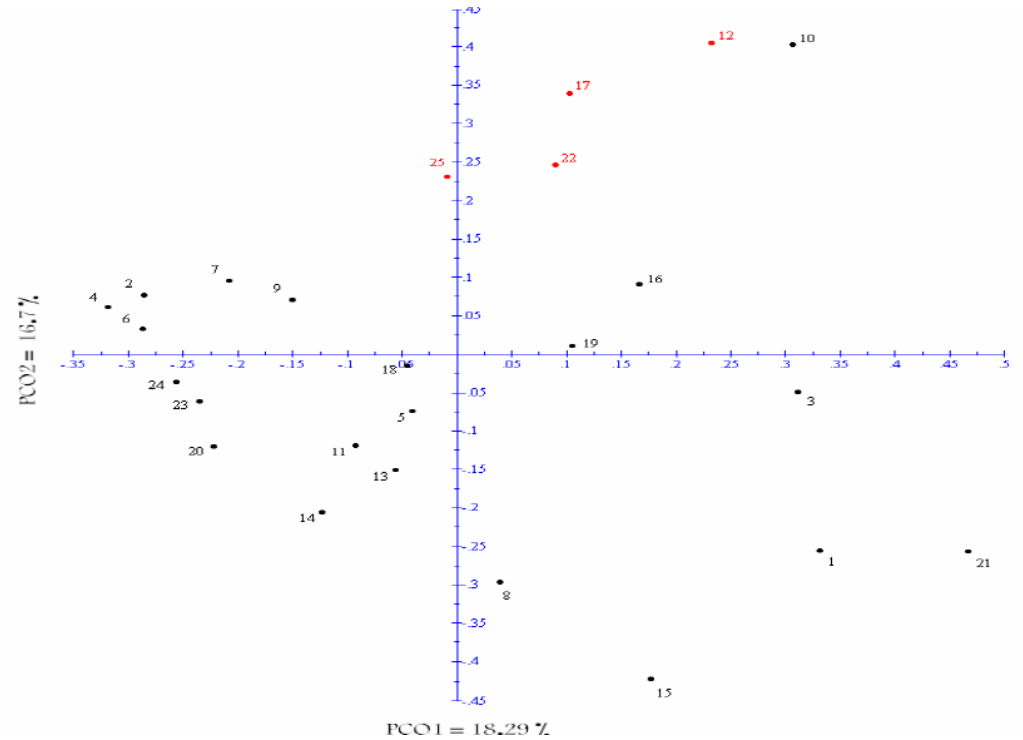

Figure 3. The principal component analysis and biplot deawing for the studied 25 genotypes using the information of the first and second coordinate axes

Table 3. Results of The principal component analysis (Information distribution across the axes indicates the proper distribution of 12 studied markers in genomes)

Coordinate axis	Eigen value	Variance contribution	Cumulative contribution
1	0.048	18.29	18.29
2	0.044	16.70	34.99
3	0.030	11.52	46.51
4	0.027	10.18	56.69
5	0.020	7.62	64.31
6	0.018	6.96	71.27
7	0.014	5.49	76.76
8	0.014	5.25	82.01
9	0.007	2.59	84.60
10	0.005	1.90	86.50

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