PLANT BREEDING AND SEED SCIENCE
Volume 71 2015

DE GRUYTER OPEN

DOI: 10.1515/plass-2015-0024

Hoda Moradkhani[1], Ali Ashraf Mehrabi[2], Alireza Etminan[1], Alireza Pour-Aboughadareh[3*]

[1]Department of Plant breeding, Kermanshah branch, Islamic Azad University, Kermanshah, Iran; [2]Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Ilam, Ilam, Iran; [3]Department of Plant Breeding and Production, Imam Khomeini International University, Qazvin, Iran; Corresponding author's e-mail: a.poraboghadareh@edu.ikiu.ac.ir

MOLECULAR DIVERSITY AND PHYLOGENY OF *TRITICUM-AEGILOPS* SPECIES POSSESSING D GENOME REVEALED BY SSR AND ISSR MARKERS

ABSTRACT

The aim of this study is investigation the applicability of SSR and ISSR markers in evaluating the genetic relationships in twenty accessions of *Aegilops* and *Triticum* species with D genome in different ploidy levels. Totally, 119 bands and 46 alleles were detected using ten primers for ISSR and SSR markers, respectively. Polymorphism Information Content values for all primers ranged from 0.345 to 0.375 with an average of 0.367 for SSR, and varied from 0.29 to 0.44 with the average 0.37 for ISSR marker. Analysis of molecular variance (AMOVA) revealed that 81% (ISSR) and 84% (SSR) of variability was partitioned among individuals within populations. Comparing the genetic diversity of *Aegilops* and *Triticum* accessions, based on genetic parameters, shows that genetic variation of *Ae. crassa* and *Ae. tauschii* species are higher than other species, especially in terms of Nei's gene diversity. Cluster analysis, based on both markers, separated total accessions in three groups. However, classification based on SSR marker data was not conformed to classification according to ISSR marker data. Principal co-ordinate analysis (PCoA) for SSR and ISSR data showed that, the first two components clarified 53.48% and 49.91% of the total variation, respectively. This analysis (PCoA), also, indicated consistent patterns of genetic relationships for ISSR data sets, however, the grouping of accessions was not completely accorded to their own geographical origins. Consequently, a high level of genetic diversity was revealed from the accessions sampled from different eco-geographical regions of Iran.

Key word: *Aegilops*, genetic diversity, ISSR, molecular phylogeny, *Triticum*, SSR.

INTRODUCTION

Genetic drift of germplasm in cereal especially of cultivated wheat provides a good stimulant for assessing about genetic diversity in its wild relatives. The

Communicated by Grzegorz Żurek

crop wild relatives relating to the genera *Aegilops* L. and *Triticum* L. are important genetic resources, because they are evolutionarily belonged to the major agricultural crop *T. aestivum* L. There exist 22 *Aegilops* and five *Triticum* species in three ploidy levels consist of diploid (2n=2x=14), tetraploid (2n=4x=28) and hexaploid (2n=6x=42) cytotype (Van Slageren 1994). Although, Iran has been known as the main center of distribution of wheat's ancestors (Kimber and Feldman 1987) and the associated compositions of *Triticum* and *Aegilops* as the richest wheat gene pool have been detected in this region, however, little information is available about the genetic diversity in wild wheat from Iran. In general, the origins of wild wheat in a range from west to northwest of Iran (East of the Fertile Crescent) are potentially ideal regions to explore for appropriate genes to further transfer into cultivated wheat (Van Slageren 1994). Thus, study on the crop wild relatives' genetic variation in these areas provides information about the closest relatives of crops such as bread wheat (Saeidi *et al.* 2006). As the D genome was involved in the formation of bread wheat (Powell *et al.* 1996; Dvorak *et al.* 1998), the genetic variability present within *Aegilops* species bearing the D genome are of remarkable interest. These species are potential sources of diversity and useful alleles for breeding purposes of *T. aestivum* and also can serve as a secondary gene pool to improve wheat's species (Kilian *et al.* 2011). The D genome is divided to three ploidy levels: diploid [2n=2x=14, *Ae. tauschii* with D genome]; tetraploid [2n=4x=28, *Ae. cylindrica* Host. with CD genome; *Ae. ventricosa* Tausch. with DN genome and the tetraploid cytotype of *Ae. crassa* with XD genome]; and hexaploid [2n=6x=42; *Ae. vavilovii* (Zhuk.) Chennav with XDS genome; *Ae. juvenalis* Thell. Eig. with XDU genome and the hexaploid cytotype of *Ae. crassa* with XDD genome] (Van Slageren 1994). The *Ae. tauschii* and the other species with D genome have been improved for disease and insect resistance (Cox *et al.* 1992), endosperm protein quality (Lagudah and Halloran 1988) and morpho-physiological traits (Le *et al.* 1986), and also some genes for resistance to biotic and abiotic stress have been discovered in these wild relatives (Limin and Fowler 1981; Schachtman *et al.* 1992). Therefore, these species have a good potential for wheat improvement.

Molecular markers provide valuable information in crop breeding, especially in studies on genetic diversity and genetic relationships between different accessions of many crop species. In particular, the polymerase chain reaction (PCR) is used to analyze the amplification fragment length polymorphism (AFLP), random amplified polymorphic DNA (RADP), simple sequence repeats (SSR), and inter-simple sequence repeats (ISSR). PCR-based methods are used to characterize mostly neutral, unique, and moderately repetitive sequences of the genome (Lagercrantz *et al.* 1993). SSR and ISSR, among all marker systems, are the most popular markers based on polymerase chain reaction. These markers (SSR and ISSR) have been used widely to analyze of the genetic diversity among different species of plants (Masoumi *et al.* 2012). Simple sequence repeats (SSRs) are tandem repeats of 1 to 6 nucleotides in both coding and non-

coding regions. SSRs have become a marker of choice in genotyping because of their high abundance, high level of allelic variation, as well as their co-dominant inheritance and analytical simplicity. Besides, these markers can be efficiently applied in phylogenetically studies (Lagercrantz *et al.* 1993; Fahima *et al.* 1998). Also, the SSR is successfully used to identify genetic diversity (Fahima *et al.* 1998), produce wheat genome map, as well as, estimate genetic relationship among accessions (Roder *et al.* 1998). On the other hand, the ISSR markers are detected by means of repeaters anchored primers that are amplified between SSRs. The ISSR markers are also suitable for detecting genetic polymorphisms among accessions of different crops (Nagaoka and Ogihara 1997). ISSR markers, furthermore, in most of plants, are more in demand because they are known to be repeatable, highly polymorphic, as well as very reproducible and highly informative (Bornet and Branchard 2001). In the present study, 20 accessions of *Aegilops* and *Triticum* were used for evaluating genetic diversity by using of SSR and ISSR markers and comparison of these markers, as well as investigating the relationships between *Triticum* and *Aegilops* accessions sampled from different geographical origins from Iran.

MATERIALS AND METHODS

Plant materials and DNA extraction

Plant materials consisted of forty accessions of *Aegilops* and *Triticum* with different ploidy levels provided by the Gene Bank of the Agricultural College of Ilam University (Ilam province is located in western Iran). The accessions used in this research shown in Table 1. From each accession, after the seed germination and growth, several individuals were randomly selected and about 5 gr young and clean leaves per plant were sampled. The total genomic DNA was isolated from the leaves of greenhouse-grown plants according to the CTAB protocol (Doyle and Doyle 1987). DNA quality was also analyzed by 1% agarose.

ISSR amplification

ISSR primers were selected from the Biotechnology Laboratory, University of British Columbia, Canada. PCR amplifications were performed in the reaction mixture 20 µl containing 2 µl of isolated DNA from each sample, 10 µl of each primer, 0.4 µl dNTPs Mix, 1.5 µl $Mgcl_2$, 0.3 µl Taq polymerase, 12.6 µl ddH_2O, and 2 µl PCR buffer. Amplification was run at 95ºC for 4 min, followed by 35 cycles of denaturation at 95ºC for 30 seconds, primer annealing at 50-60ºC (varied for each primer) for 30 seconds and primer elongation at 72ºC for 1 min. The final extension was 7 min at 72ºC. The amplification reaction prod-

ucts were separated on 1.5% denaturing agarose gels. The electrophoresis was run at 110 V, then staining was carried out by Ethidium Bromide.

Table 1

Geographical origins from where accessions of Aegilops and Triticum collected

Number	Accession	Ploidy level	Genome	Origin	Gene Bank Code
1	*T. aestivum*	6x	ABD	Iran, Esfahan	IUGB 00282
2	*T. aestivum*	6x	ABD	Iran, Esfahan	IUGB 00397
3	*T. aestivum*	6x	ABD	Iran, Esfahan	IUGB 00085
4	*T. aestivum*	6x	ABD	Iran, Kermanshah	IUGB 00425
5	*T. aestivum*	6x	ABD	Iran, Esfahan	IUGB 00266
6	*Ae. tauschii*	2x	D	Iran, Lahijan	IUGB 00374
7	*Ae. tauschii*	2x	D	Armenia	IUGB 00296
8	*Ae. tauschii*	2x	D	Iran, Gorgan	IUGB 00233
9	*Ae. tauschii*	2x	D	Turkmenistan	IUGB 00290
10	*Ae. tauschii*	2x	D	Iran, Lahijan	IUGB 00300
11	*Ae. crassa*	4x	MD	Iran, Kermanshah	IUGB 00379
12	*Ae. crassa*	4x	MD	Iran, Kermanshah	IUGB 00284
13	*Ae. crassa*	4x	MD	Iran, East Azerbaijan	IUGB 00280
14	*Ae. crassa*	4x	MD	Iran, Shahr-e-Kord	IUGB 00319
15	*Ae. cylindrica*	4x	DC	Iran, East Azerbaijan	IUGB 00201
16	*Ae. cylindrica*	4x	DC	Iran, Lurestan	IUGB 00210
17	*Ae. cylindrica*	4x	DC	Iran, Zanjan	IUGB 00239
18	*Ae. cylindrica*	4x	DC	Iran, Ardabil	IUGB 00258
19	*Ae. cylindrica*	4x	DC	Iran, Gorgan	IUGB 00236
20	*Ae. speltoides*	2x	S	Iran, Kermanshah	IUGB 00025

SSR amplification

SSR primers were selected based on the study of Roder *et al.* (1998). Similar to ISSR marker, PCR amplifications for SSR were carried out in 20 µl mixture containing 2 µl of isolated DNA from each sample, 10 µl of each primer, 0.4 µl dNTPs Mix, 1.5 µl $Mgcl_2$, 0.3 µl Taq polymerase, 12.6 µl ddH_2O, 2 µl PCR buffer. Amplification was run at 95ºC for 5 min, followed by 35 cycles of denaturation at 95ºC for 30 seconds, primer annealing at 50, 55 and 60ºC (varied for each primer) for 30 seconds and primer elongation at 72ºC for 1 min. The final extension was 5 min at 72ºC. The amplification reaction products were run on 3% agarose gel and visualized by means of Ethidium Bromide staining. Also, the electrophoresis was performed for 2/5-3 h at 110 V. The bands were finally

scored based on presence or absence of each single fragment as 1 and 0, respectively.

Data analysis

To characterize genetic variation, some of parameters, namely Shannon's information index (I), Nei's gene diversity (He) and the observed number of alleles (Ne) were calculated for species. All of the above calculations were carried out using PopGen 1.31 software (Yeh *et al.* 1999). The analysis of molecular variance (AMOVA) was conducted by the GenAlEx software (Peakall and Smouse 2006). Cluster analysis based on Jaccard similarity coefficients matrix among individuals was performed using the Ward method by DARwin 5.0.146 software (Darwin 2009). Principal co-ordinated analysis (PCoA) was also carried out to show accessions' multiple distribution in a scatter-plot by GenAlEx 6.41 software (Peakall and Smouse 2006).

RESULTS AND DISCUSSION

Diversity analysis using SSR marker

Ten SSR primers were firstly screened for their ability to produce polymorphic patterns across the twenty *Aegilops-Triticum* accessions (Fig. 1). All primers which were repeatable and produced high resolution bands for all the accessions were selected for evaluation of genetic diversity in the accessions (Table 2). As a whole, 46 alleles were recognized. The number of alleles per microsatellite varied from 2 to 7 (for Xgwm33 and Xgwm469, respectively) with an average of 4.2 alleles per locus. This result was not the same as what Naghavi *et al.* (2007) and Saeidi *et al.* (2006) got. That is, the former was a range from 6 to 15 with an average of 9.21 alleles per locus; the latter was a range from 4 to 12 with an average of 7.3 alleles per locus by SSR marker. These different results might be due to use accessions from different geographical areas, as well as, different number of each accessions and their ploidy levels. The major frequency alleles ranged from 0.5 (for Xgwm539) to 0.66 (for Xgwm16) with an average of 0.56 alleles. The observed heterozygosity for primers varied from zero to 0.93 with a mean value of 0.61, so that primer Xgwm271 had the highest value. Furthermore, the polymorphism information content (PIC) and Nie's gene diversity values were various and primers Xgwm539 and Xgmw16 had the highest and lowest values in the terms of these parameters (Table 2). Previously, the studies conducted by Naghavi *et al.* (2007), Saeidi *et al.* (2006) and Tahernezhad *et al.* (2010) showed different value of PIC and Nei's gene diversity by means of SSR marker in wild population of wheat.

Table 2

The SSR primers used for genetic diversity of wild ancestors of bread wheat, their major frequency allele, Nei's gene diversity, Observed heterozygosity and Polymorphism information content (PIC).

Primers	Chromosome location	Major frequency allele	Nei's gene diversity	Observed heterozygosity	PIC
Xgwm30	2D	0.60	0.48	0.66	0.36
Xgwm44	7D	0.63	0.46	0.00	0.35
Xgwm121	7D	0.53	0.49	0.81	0.37
Xgwm469	6D	0.57	0.48	0.65	0.36
Xgwm608	4D	0.58	0.48	0.58	0.36
Xgwm16	5D	0.66	0.44	0.33	0.34
Xgwm33	1D	0.53	0.49	0.92	0.37
Xgwm341	3D	0.53	0.49	0.40	0.37
Xgwm539	2D	0.50	0.50	0.84	0.37
Xgwm271	5D	0.53	0.49	0.93	0.37
Mean		0.56	0.49	0.61	0.36

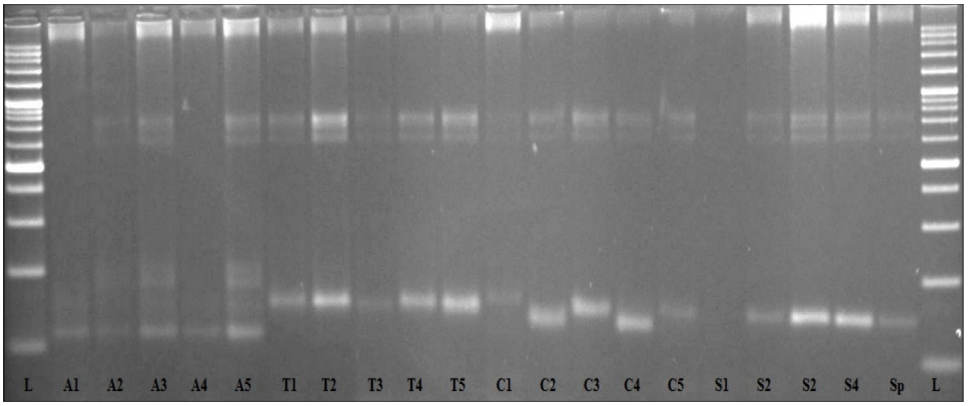

Fig. 1 The banding pattern of SSR marker in Aegilops and Triticum species possessing D genome in different ploidy levels. L, A, T, C, S and Sp indicate: leader, T. aestivum, Ae. tauschii, Ae. crassa, Ae. cylindrica and Ae. speltoides species, respectively.

The AMOVA analysis showed that the percentages of variance attributable to the differences between and within groups were 16% and 84% for SSR marker (Table 3). Also, a summary of genetic diversity parameters (Table 4) showed that the observed heterozygosity (*Ho*) ranged from 0.53 (*T. aestivum*) to 0.70 (*Ae. crassa*) with a mean of 0.61. The highest and lowest value of the mean of Nei's gene diversity index (*He*) belonged to *Ae. cylindrica* (0.46) and *T. aesti-*

vum (0.38) species, respectively. According to the mean of Shannon's information index (*I*), *Ae. tauschii* and *Ae. crassa* species had the highest values (0.58), and *T. aestivum* species had the lowest one (0.53). The *Ae. crassa*, generally, comparing to the other studied species, had the highest value of *Ho*, *He* and *Ne*. In the study conducted by Naghavi *et al.* (2009) by means of SSR markers, a high level of genetic diversity was reported in *Aegilops* accessions which were sampled from some parts of Iran.

Table 3

AMOVA analysis based on SSR and ISSR markers for Triticum and Aegilops species

Source of variance	DF	SS		MS		Est v.		Vp	
		SSR	ISSR	SSR	ISSR	SSR	ISSR	SSR	ISSR
Between	3	27.54	123.96	9.181	41.32	0.90	4.55	16	19
Within	15	73.30	296.25	4.887	19.75	4.88	19.75	84	81
Total	18	100.84	420.21			5.78	24.30	100	100

DF, SS, MS, Est.v and Vp indicate; degree of freedom, Sum of square, Mean of square, Estimated variance, Variance percentage

Table 4

Genetic diversity parameters for Triticum and Aegilops species based on SSR and ISSR

Species	Number of accessions in each species	I (±SD)	Ho (±SD)	He (±SD)	Ne (±SD)
SSR marker					
T. aestivum	5	0.53 ±0.28	0.53 ±0.34	0.38 ±0.20	1.74 ±0.39
Ae. tauschii	5	0.58 ±0.21	0.61 ±0.38	0.41 ±0.15	1.79 ±0.32
Ae. crassa	4	0.58 ±0.22	0.70 ±0.43	0.41 ±0.17	1.81 ±0.36
Ae. cylindrical	5	0.57 ±0.21	0.61 ±0.39	0.46 ±0.15	1.76 ±0.32
ISSR marker					
T. aestivum	5	0.26 ±0.02	0.17 ±0.01	1.29 ±0.03	1.05 ±0.09
Ae. tauschii	5	0.40 ±0.02	0.26 ±0.01	1.45 ± 0.03	1.60 ±0.06
Ae. crassa	4	0.38 ±0.02	0.24 ±0.01	1.40 ±0.03	1.57 ±0.07
Ae. cylindrical	5	0.32 ±0.02	0.22 ±0.01	1.39 ±0.03	1.23 ±0.08

Diversity analysis using ISSR marker

All of the ten ISSR primers were polymorphic when considered over all accessions. Totally 119 band recognized. The number of alleles per primer ranged from 8 (for primer UBC820) to 15 (for primers UBC826 and UBC878) with an average of 11.9 alleles per locus. In general, 119 DNA fragments were scored with an average of 11.9 fragments per primer and 119 of them (100%) were polymorphic (Fig. 2). It seems that the potential of ISSR markers to produce genetic information by means of polymorphic fragments depends on the microsatellite frequency and their distribution in the species' genome wide scale. Polymorphism information content (PIC) ranged from 0.29 (UBC818) to 0.44 (UBC826) with an average 0.37 (Table 5). Saidi *et al.* (2013) in a study on genetic diversity of accessions of *Lamiaceae* family by ISSR marker reported that the PIC values ranged from 0.28 to 0.45 with an average of 0.41. Actually, PIC is not fixed and it depends on the number of allele per locus, GT content and type of motif (Saeidi *et al.* 2006). However, the PIC value has been widely used in many genetic diversity studies (Tahernezhad *et al.* 2010). The results of AMOVA analysis showed that there is 19% and 81% of variance among between and within groups (Table 3). The genetic diversity data for various species based on ISSR revealed that the effective number of alleles (*Ne*) ranged from 1.05 (*T. aestivum*) to 1.60 (*Ae. tauschii*) with a mean of 1.4 alleles. The mean of Nei's gene diversity index (*He*) was the highest in the *Ae. tauschii* species (1.45) while, the lowest value was estimated for *T. aestivum* species (1.29). Also, the mean of Shannon's information index (*I*) was highest in the *Ae. tauschii* species (0.40) however, the lowest mean was estimated for *T. aestivum* species (0.26). The *Ae. tauschii*, generally, comparing to the other studied species, had the highest value of *I*, *Ho*, *He* and *Ne* (Table 4). The result of our present study revealed that the number of effective alleles in *T. aestivum* is less than *Ae. tauschii* and other accessions, indicating that there is a great potential in discovery and use of *Ae. tauschii* for wheat breeding programs. Thus, these results show that there can be a level of genetic diversity among the *Aegilops* and *Triticum* accessions, indicating the high efficiency of the ISSR marker technique to reveal genetic diversity in the case of accessions of wheat.

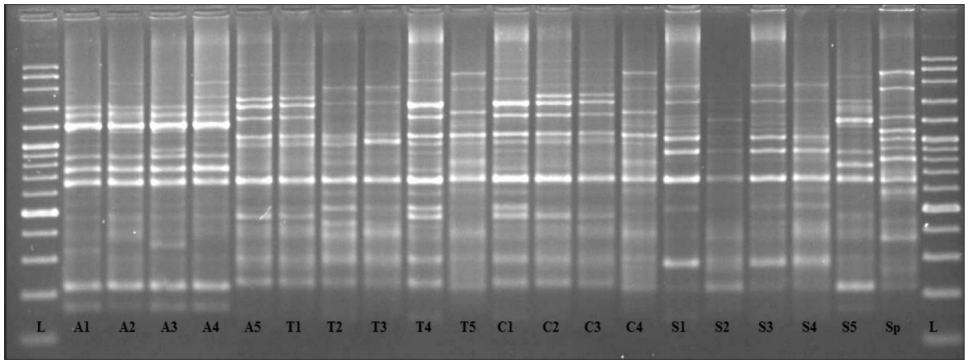

Fig. 2 The banding pattern of ISSR marker in *Aegilops* and *Triticum* species possessing D genome in different ploidy levels. L, A, T, C, S and Sp indicate: leader, T. aestivum, *Ae. tauschii, Ae. crassa, Ae. cylindrica* and *Ae. speltoides* species, respectively

Table 5

The ISSR primers used for genetic diversity of wild ancestors of bread wheat, their sequence, polymorphic fragment, number of polymorphic loci, number of amplification loci and polymorphic information content (PIC).

Primer code	Primer Sequences	Polymorphic fragment [%]	Number of polymorphic loci	Number of amplification loci	PIC
UBC818	5'CACACACACACACACAG3'	100	13	13	0.29
UBC820	5'GTGTGTGTGTGTGTGTC3'	100	8	8	0.37
UBC826	5'ACACACACACACACACC3'	100	15	15	0.44
UBC878	5'GGATGGATGGATGGAT3'	100	15	15	0.37
UBC843	5'CTCTCTCTCTCTCTCTRA3'	100	13	13	0.31
UBC889	5'DBDACACACACACACAC3'	100	12	12	0.42
UBC859	5'TGTGTGTGTGTGTGTGGRC3'	100	10	10	0.43
UBC827	5'ACACACACACACACACACG3'	100	11	11	0.34
UBC808	5'AGAGAGAGAGAGAGAGC3'	100	10	10	0.37
UBC873	5'GACAGACAGACAGACA3'	100	12	12	0.42

I, *Ho*, *He*, *Ne* and SD indicate; Shannon's information index, observed heterozygosity, Nei's gene diversity, effective number of alleles and Standard deviation, respectively

Cluster analysis

In order to study genetic relationships among accessions cluster analysis was calculated based on Jaccard similarity coefficients and Ward algorithm. The

obtained dendrograms were constructed to express the results of the cluster analysis based on the SSR (Fig. 3) and ISSR (Fig. 4), respectively. Cluster analysis distinguished 20 accessions into three major groups based on both marker systems. According to SSR marker, the first cluster included two accessions of *T. aestivum*, two accessions of *Ae. tauschii*, two accessions of *Ae. crassa* and two accessions of *Ae. cylindrica*; and the second cluster comprised two accessions of *Ae. tauschii*, one accessions of *Ae. crassa* and one accession of *Ae. cylindrica* together with *Ae. speltoides*. Finally, the third cluster included three accession of *T. aestivum*, two accessions of *Ae. cylindrica* as well as one accession of *Ae. tauschii*, and one accession of *Ae. crassa*. The remarkable point is that the accessions sampled from geographically distant regions in this study, displayed a high degree of genetic diversity. For example, in the second cluster, an accession of *Ae. cylindrica* and *Ae. speltoides* which are from two different geographical regions, exhibited a high degree of genetic diversity. Therefore, it should be noted that geographically close regions could be ecologically quite different, and conversely, regions which are geographically distant from each other, can be very similar in their environmental conditions (Moghaddam *et al.* 2000). On the other hand, based on ISSR marker, accessions showed a different pattern of variation. According to this phenogram, cluster 1 included accessions no.1, 2, 3 and 5 (*T. aestivum*). Accession no.12, 13, 14 and 4 (three accessions of *Ae. crassa* and one accessions of *T. aestivum*) classified in the second cluster and the other accessions placed into the third cluster (comprising *Ae. speltoides*, all of *Ae. tauschii* and *Ae. cylandirica* accessions). In general, the Ward cluster plots showed distinct levels of divergence depending on the marker employed. The dendrograms obtained for SSR and ISSR markers showed a dissimilar pattern for accessions' distribution in different clusters; this, as it seems, may refer to the difference in genome and primers sequences. Furthermore, as revealed by AMOVA, there is a higher level of genetic variation within species than among them, indicating a remarkable genetic diversity. Such a pattern of genetic diversity has been confirmed in many other crop wild relatives (Wolfe and Liston 1998; Deshpande *et al.* 2001; Naghavi *et al.* 2010; Saidi *et al.* 2013). Therefore, the results of AMOVA supported genetic diversity within species which was obtained by cluster analysis. In general, with regard to considerable genetic diversity of *Aegilops* accessions, especially of *Ae. tauschii* and *Ae. crassa*, it is recommended to evaluate the germplasm of these accessions. The previous studies reported high levels of genetic diversity in *Aegilops* germplasm, especially in *Ae. tauschii* collected from different geographical origins of Iran by means of allozyms system (Dudnikov and Kawahara 2006), AFLP marker (Naghavi *et al.* 2007), morphological characters (Aghaei *et al.* 2008) and microsatellite marker (Naghavi *et al.* 2010; Tahernezhad *et al.* 2010).

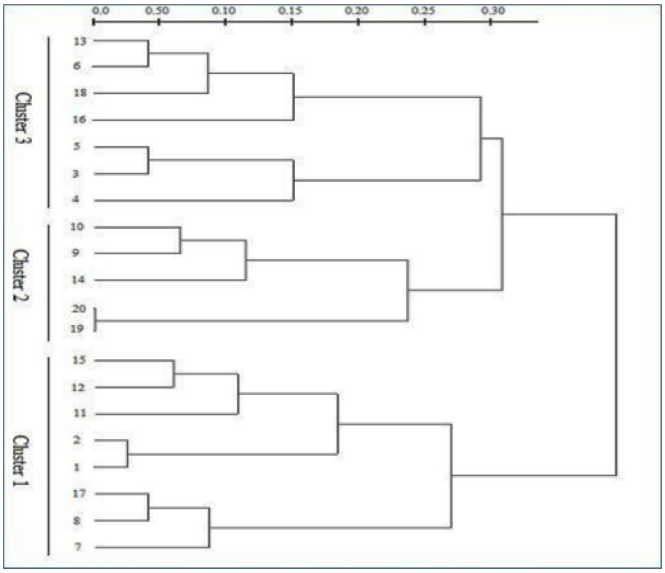

Fig. 3 Dendrogram showing the relationships among accessions of Triticum and Aegilops species based on an analysis of SSR data using the Jaccard similarity coefficients and the Ward clustering method. For accession number see Table 1.

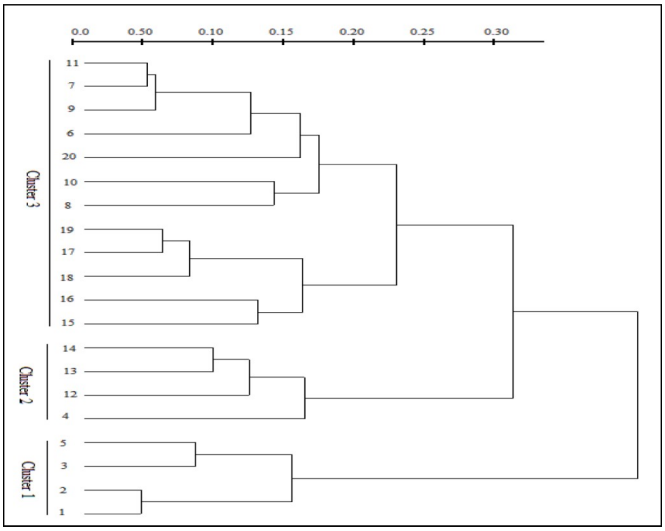

Fig. 4 Dendrogram showing the relationships among accessions of Triticum and Aegilops species based on an analysis of ISSR data using the Jaccard similarity coefficients and the Ward clustering method. For accession number see Table 1.

Principal coordinate analysis

Principal co-ordinates analysis (PCoA) is an ordination technique that is similar to principle components analysis (PCA). This technique has the advantage over PCA because it can investigate any ecological distance. The PCoA was done with SSR and ISSR data in order to study the relationships among accessions (Fig. 5 and 6). The results showed that the first two principal coordinates explain 53.48% and 49.91% of total variation (for SSR and ISSR, respectively). Albeit the general patterns of PCoAs for both marker data were almost similar, nonetheless, it was less clear for ISSR data. Thus, based on ISSR marker, separation of all the accessions was not based on their expected geographic distribution. Considering the patterns obtained of PCoA and cluster analysis shows that there is a little relationship between genetic divergence and geographical origins, so that the accessions from similar geographical regions are separated into different groups. On contrary, accessions from different geographical regions relatively tended to be clustered as one sub-group of the dendrogram. These differences in patterns between different accessions could be expected for wild species, indicating that these species represent a large gene pool with a significant level of diversity in these species.

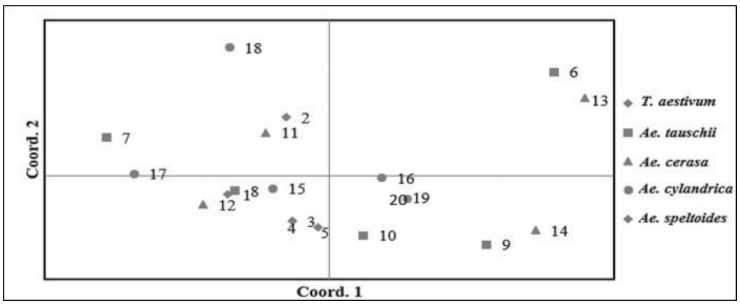

Fig. 5 Plot obtained from the first and second coordinate in 20 accessions of *Triticum* and *Aegilops* species according to SSR marker

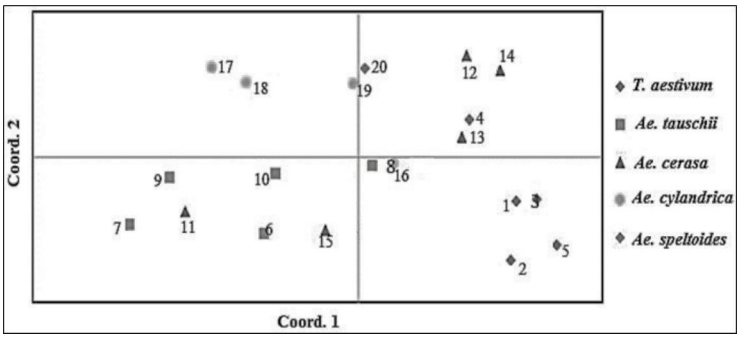

Fig. 6 Plot obtained from the first and second coordinate in 20 accessions of *Triticum* and *Aegilops* species according to ISSR marker

Comparison of ISSR and SSR markers analysis

Overall, molecular markers provide a good estimate of genetic diversity because of their independence of confounding effects made by environmental factors (Powell *et al.* 1996). In this study, two PCR-based systems (ISSR and SSRs) were employed to evaluate the genetic diversity among 20 accessions of *Aegilops* and *Triticum* species. According to the observed polymorphism by means of different SSR and ISSR markers, a high level of genetic diversity among studied *Aegilops-Triticum* accessions was indicated. Cross all the analyzed accessions, the average PIC values were different in two used marker systems. The average of PIC value for the ISSRs was higher than the SSRs, indicating the ability of ISSR than the SSRs to show the high efficiency of ISSR marker in revealing genetic diversity in accessions of wheat. Our results indicated that, for each species, the Shannon's information index followed by observed heterozygosity, and effective number of alleles were higher in SSR marker than ISSR marker. These differences might be due to the use of *Aegilops* and *Triticum* accessions which possess various ploidy levels, sampled from different geographical origins as well as the use of dissimilar primers sequences. Comparing the genetic diversity of *Aegilops* and *Triticum* accessions, based on genetic parameters, shows that genetic variation of *Ae. crassa* and *Ae. tauschii* species are higher than other species, especially in Nei's gene diversity parameter. Naghavi *et al.* (2008) also reported that the highest genetic diversity in *Ae. tauschii* followed by *Ae. crassa*. Therefore, based on these results, we can suggest that microsatellite markers have the potential to detect genetic variability in wild species. In addition, previous studies have reported a high level of genetic diversity in wheat germplasm. These studies showed that *Ae. tauschii* species, than modern wheat cultivars, has a greater variation in terms of the seed storage proteins and genome (Lubbers *et al.* 1991; Dvorak *et al.* 1998; Yan *et al.* 2003; Peterson *et al.* 2006; Saeidi *et al.* 2006; Naghavi *et al.* 2008). Cluster analysis for SSR and ISSR markers constructed three main groups, which were not conformed to each other. Also, principle co-ordinate analysis (PCoA) showed that the distribution of *Ae. tauschii*, *Ae. crassa* and *T. aestivum*, among all accessions, based on ISSR is more clear than their distribution based on SSR. Overall, the assessment of genetic diversity estimated by these different molecular marker systems can provide different levels of important information in the management of germplasm resources (Naghavi *et al.* 2009).

CONCLUSION

Studying the relationships among breeding materials and crop wild relatives (such as information about the degree and distribution of genetic diversity) has a meaningful effect on crop improvement; hence, it is so evident that molecular markers will increasingly have an important role in crop improvement pro-

grams. Our study indicated that the *Aegilops* and *Triticum* species, which are endemic to the major geographic regions of the South of the Caspian Sea and Fertile Crescent, have a high genetic diversity in the different eco-geographical regions of Iran. Therefore, detecting species which have high level of genetic variation leads us to conserve them for the breeding programs and also for the easy management of genetic resources. This study, consequently, shows that how certain parts of Iran can provide an available source of potentially useful variation for wheat improvement.

REFERENCES

Aghaei M.J., Mozafari J., Taleei A.R., Naghavi M.R., Omidi M. 2008. Distribution and diversity of *Aegilops tauschii* in Iran. Genetic Resources and Crop Evolution. 55: 341-349.

Bornet B., Branchard M. 2001. Nonanchored inter simple sequence repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting. Molecular Biology Reports. 19: 209-215.

Cox T.S., Raupp W.J., Wilson D.L., Gill B.S., Leath S., Bockus W.W., Browder L.E. 1992. Resistance to foliar diseases in a collection of *Triticum tauschii* germplasm. Plant Disease Journal. 76: 1061-1064.

Darwin. 2009. Dissimilarity Analysis and Representation for Windows. Available at http://darwin.cirad.fr/darwin.

Deshpande K., Apte G.S., Bahulikar R., Lagu M., Kulkarni B., Suresh H., Singh N., Rao M., Gupta V., Pant A., Ranjekar P. 2001. Genetic diversity across the natural populations of three montane plant species from the WG, India revealed by ISSR repeats. Molecular Ecology. 10: 2397-2408.

Doyle J.J., Doyle J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical. Bulletin. 19:11-15.

Dudnikov A.J., Kawahara T. 2006. *Aegilops tauschii*: genetic variation in Iran. Genetic Resources and Crop Evolution. 53:579-586.

Dvorak J., Luo M.C., Yang Z.L., Zhang H.B. 1998. The structure of the *Aegilops tauschii* gene pool and the evolution of hexaploid wheat. Theoretical and Applied Genetics. 97: 657-670.

Fahima T, Roder M., Grama A., Vnero E. 1998. Microsatellite DNA polymorphism divergence in *Triticum dicoccoides* accessions highly resistance to yellow rust. Theoretical and Applied Genetics. 96: 187-195.

Kilian B., Mammen K., Millet E., Sharma R., Graner A., Salamini F., Hammer K., Ozkan H. 2011. *Aegilops*. In: Kole C (ed) Wild crop relatives, genomic and breeding resources, cereals. Springer-Verlag, Berlin, Germany.

Kimber G., Feldman M. 1987. Wild Wheat. An Introduction. Special report 353, University of Missouri.

Lagercrantz U., Ellegren H., Andersson L. 1993. The abundance of various polymorphic microsatellite motifs differs between plants and vertebrates. Nucleic Acids Research. 21: 1111–1115.

Lagudah E.S., Halloran G.M. 1988. Phylogenetic relationships of *Triticum tauschii* the D genome donor to hexaploid wheat. 1: Variation in HMW subunits of glutenin and gliadins. Theoretical and Applied Genetics. 75: 592-598.

Le H.T., Reikosky D.A., Olien C.R., Cress C.E. 1986. Freezing hardiness of some accessions of *Triticum tauschii* and *Triticum turgidum* var. durum. Canadian Journal of Plant Science. 66: 893-899.

Limin A.E., Fowler D.B. 1981. Cold hardiness of some relatives of hexaploid wheat. Canadian Journal of Botany. 59: 572-573.

Lubbers E.L., Gill K.S., Cox T.S., Gill B.S. 1991. Variation of molecular markers among geographically diverse accessions of *Triticum tauschii*. Genome. 34: 354-361.

Masoumi S.M., Kahrizi D., Rostami-Ahmadvandi H., Soorni J., Kiani S., Mostafaie A., Yari K. 2012. Genetic diversity study of some medicinal plant accessions belong to Apiaceae family based on seed storage proteins patterns. Molecular Biology Reports. 39: 10361-10365.

Moghaddam M., Ehdaie B., Waines G. 2000. Genetic diversity in populations of wild diploid wheat (*Triticum urartu* Thum. ex. Gandil) revealed by Isozymes markers. Genetic Resources and Crop Evolution. 47: 323-334.

Morgante M., Olivieri A.M. 1993. PCR-amplified microsatellites as markers in plant genetics. The Plant Journal. 3: 175-182.

Nagaoka T., Ogihara Y. 1997. Applicability of intersimple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theoretical and Applied Genetics. 94: 597 -602.

Naghavi M.R., Mardi M., Pirseyedi S.M., Kazemi M., Potki P., Ghaffari M.R. 2007. Comparison of genetic variation among accessions of *Aegilops tauschii* using AFLP and SSR markers. Genetic Resources and Crop Evolution. 54: 237-240.

Naghavi M.R., Aghaei M.J., Taleei A.R., Omidi M., Hassani M.E. 2008. Genetic diversity of hexaploid wheat and three Aegilops species using microsatellite markers. In: Rudi A, Eastwood R, Lagudah E, Langridge P, Lynne MM (ed) The 11th International Wheat Genetics Symposium, Sydney University Press, Sydney.

Naghavi M.R., Aghaei M.J., Taleei A.R., Omidi M., Mozafari J., Hassani M.E. 2009. Genetic diversity of the D-genome in *T. aestivum* and *Aegilops* species using SSR markers. Genetic Resources and Crop Evolution. 56: 499-506.

Naghavi M.R., Hajikram M., Taleei A.R., Aghaei M.J. 2010. Microsatellite analysis of genetic diversity and population genetic structure of *Aegilops tauschii* Coss. in northern Iran. Genetic Resources and Crop Evolution. 57: 423-430.

Peakall R., Smouse P.E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology. 6: 288-295.

Pestsova E., Korzun V., Gncharov N.P., Hammer K., Ganal M.W., Roder M.S. 2000. Microsatellite analysis of *Aegilops tauschii* germplasm. Theoretical and Applied Genetics. 101: 100-106.

Peterson G., Seberg O., Yde M., Berthelsen K. 2006. Phylogenetic relation of *Triticum* and *Aegilops* evidence for the origin of the A, B and D genomes of common wheat (*Triticum aestivum*). Molecular Phylogenetics and Evolution. 39: 70-82.

Powell W., Morgante M., Andre C., Hanafey M., Vogel J., Tingey S., Rafalski A. 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding. 2: 225-238.

Roder M.S., Korzun V., Wendehake K., Plaschke J., Tixier M.H., Leroy P., Ganal M.W. 1998. A microsatellite map of wheat. Genetic. 149: 2007-2023.

Saeidi H, Rahiminejad M.R., Vallian S., Heslop-Harison J.S. 2006. Biodiversity of diploid D-genome *Aegilops tauschii* Coss. In Iran measured using microsatellites. Genetic Resources and Crop Evolution. 53: 1477-1484.

Saidi M., Movahedi K., Mehrabi M., Kahrizi D. 2013. Molecular genetic diversity of Satureja bachtiarica. Molecular Biology Reports. 40(11): 6501-6508.

Schachtman D.P., Lagudah E.S., Munns R. 1992. The expression of salt tolerance from *Triticum tauschii* in hexaploid wheat. Theoretical Applied Genetics. 84: 714-719.

Tahernezhad Z., Zamani M.J., Solouki M., Zahravi M., Imamjomeh A.A., Aghaei M.J., Bihamta M.R. 2010. Genetic diversity of Iranian *Aegilops tauschii* Coss. Using microsatellite molecular markers and morphological traits. Molecular Biology Reports 37: 3413-3420.

Tatikonda L., Wani S.P., Kannan S., Beerelli N., Sreedevi T.K., Hoisington D.A., Devi P., Varshney R.A. 2009. AFLP-based molecular characterization of an elite germplasm collection of *Jatropha curcas* L. a biofuel plant. Plant Science. 176: 505-513.

Van Slageren M.N. 1994. Wild wheat: a monograph of *Aegilops* L. and *Amblyopyrom* (Jaub & Spach) Eig. Wageningen, Netherlands.

Wolfe A.D., Liston A. 1998. Contributions of PCR-based methods to plant systematics and evolutionary biology. In: Soltis PS, Soltis DE, Doyle JJ (eds) Molecular systematics of plants: DNA sequencing. Kluwer, New York, USA.

Yan Y., Hsam S.L.K., Yu J.Z, Jiang Y., Ohtsuka I., Zeller F.J. 2003. HMW and LMW glutenin alleles among putative tetraploid and hexaploid European spelt wheat (*Triticum spelta* L.) progenitors. Theoretical Applied Genetics. 107: 1321-1330.

Yeh F.C., Yang R.C., Boyle T. 1999. Microsoft Window-based freeware for population genetic analysis (POPGENE), ver.1.31. http://ftp.microsoft.com/softlib/MSLFILES/HPGL.EXE.

Zietkiewicz E., Rafalski A., Labuda D. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genetics. 20: 176-183.