Research Paper

Future of Food: Journal on Food, Agriculture and Society
6 (1) Summer 2018

Assessment of genetic diversity in *Agropyron desertorum* accessions using ISSR molecular marker

Mohsen Farshadfar [*1], Raheleh Nowrozi[2], Hooshmand Safari[3], Hooman Shirvani[1], Mostafa Amjadian[1]

[1] Department of Agriculture, Payame Noor University, Iran
[2] Islamic Azad University,Kermanshah, Iran
[3] Center of Agricultural Research and Natural Resources of Kermanshah, Agricultural Research, Education and Extension Organization (AREEO), Kermanshah, Iran

* Corresponding author: *farshadfarmohsen@yahoo.com* | +989183313221

Data of the article

First received : 17 December 2017 | Last revision received : 29 August 2018
Accepted: 30 August 2018 | Published online : 09 October 2018
URN: nbn:de:hebis: 34-2017120753925

Keywords

Genetic variability, ISSR, *Agropyron desertorum*

Abstract

Molecular markers, such as Inter-Simple Sequence Repeats (ISSR), are relatively easy to use and are highly effective for genetic diversity studies. This research was carried out to evaluate polymorphism and variation among Agropyron accessions. Genetic variability was studied for 13 accessions of *Agropyron desertorum* using molecular markers. Genetic variations for 13 accessions were screened using 15 ISSR primers and 12 primers were scored. The ISSR primers produced 61 bands, of which the polymorphism was observed in 60 bands. The primers IS5, IS11 and IS13 revealed the highest number of bands (7 bands) and IS3 showed the lowest number of bands (3 bands). The highest Polymorphism Information Content (PIC) value (0.44) belonged to IS3, which determined better genetic distance than other primers based on the PIC index. The IS9 with the lowest PIC value (0.27) did not separate the accessions. The average Resolving Power (RP) index was 4.19, of which primers IS5, IS11, IS12 and IS13 had the greatest value of RP. Primers IS3, IS7, and IS14 had the lowest values of RP. However, IS5, IS11 and IS13 had the greatest values, and IS3 and IS15 had the lowest amount of Marker Index (MI) and Effective Multiplex Ratio (EMR). In addition, IS5, IS11 and IS13 could be introduced as desirable primers for the determination of genetic variation based on all indices. The accession of G11 had the highest similarity (0.79) with G10, while the accessions of G4, G5 had the lowest similarity (0.39) with G8. The Agropyron accessions were classified by cluster analysis method based on Dice's coefficient. All of the 13 accessions were grouped into four clusters.

Introduction

Agropyron is one of the most important forage grasses that can be grown in weak and shallow soils, and is cultivated mainly to aid with pasture establishment and reclamation in different climates (Sanderson et al. 2002). The gene pool of Agropyron includes about 19 species in Iran and 150 species worldwide (Bor, 1970). Plant genetic resources are the basis of global food security. They comprise diversity of genetic material contained in traditional varieties, modern cultivars, crop wild relatives, and other wild species in order to improve the rangeland and increase forage production (Arghavani et al., 2010, Farshadfar & Farshadfar, 2008). Since, there is high variation within and among different species of Agropyron, the selection response for improving important traits is high (Arghavani et al., 2010). On the other hand, understanding genetic diversity of certain species is not only useful in addressing questions about evolutionary process and the development of conservation strategies, but also a prerequisite for efficient use of genetic resources in breeding programs. Interest in the genetic structure of natural populations of grass species has increased in the last few years because of the necessity of broadening the knowledge of genetic variations in cultivated species (Che & Li, 2007). Molecular markers provide a

Citation (APA):
Farshadfar,M., Nowrozi, R., Safari, H., Shirvani, H., Amjadian, M. (2018). Assessment of genetic diversity in *Agropyron desertorum* accessions using ISSR molecular marker. *Future of Food: Journal on Food, Agriculture and Society*, 6 (1), 20-29.

Tabel 1: List of 13 accessions of *Agropyron desertorum*

accessions	Code	Accessions	Code
G1	4051 – 2066	G8	Alborz 2077(Mix)
G2	3965 – 2059	G9	M – 4036
G3	8848	G10	3477 – 2058
G4	287 – 10	G11	747
G5	plc 1	G12	400
G6	plc 2	G13	3014
G7	341 – 2053		

Tabel 2: Compounds of optimized ISSR reaction

Total volume 20 µl	Compounds of each sample
12.6 µl	ddH2O
2 µl	PCR Buffer (X10)
1.5 µl	MgCl2 (50 mMol)
0.4 µl	Nucleotides mixture (10 mMol)
1.2 µl	Primer (10 µMol)
0.3 µl	Tag polymerase
2 µl	DNA (10 ng)

robust estimate of genetic similarity that was likely not obtained using morphological data alone (Surendhar et al., 2009). Often, the initial objective of DNA profiling of populations is to determine diversity among populations in order to develop genetically distinct subsets of populations in a breeding program or to check for duplicates in a gene bank. In these cases, it may be possible to determine diversity among populations by profiling bulked DNA of the individuals (Rouf Mian et al., 2002). Genetic variations based on DNA markers between and within different species of Agropyron were reported by many researchers (Arghavani et al., 2010; Sun et al., 1999; Sun et al., 2002; Refoufi et al., 2009; Szczepaniak et al., 2009; Dizkirici et al., 2010; Che et al., 2011; Yang et al., 2011). Many PCR-based DNA markers have been developed, including Randomly Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeat (SSR), Expressed Sequence Tag (EST), and Inter-Simple Se¬quence Repeats (ISSR). Among these, RAPD and ISSR are relatively simple to use and are highly effective in plant fingerprinting and phylogenetic studies, which require no prior knowledge of sequence information (Xu et al., 2012; Yousefi-azar-Khanian et al., 2016). The first report of inter-simple

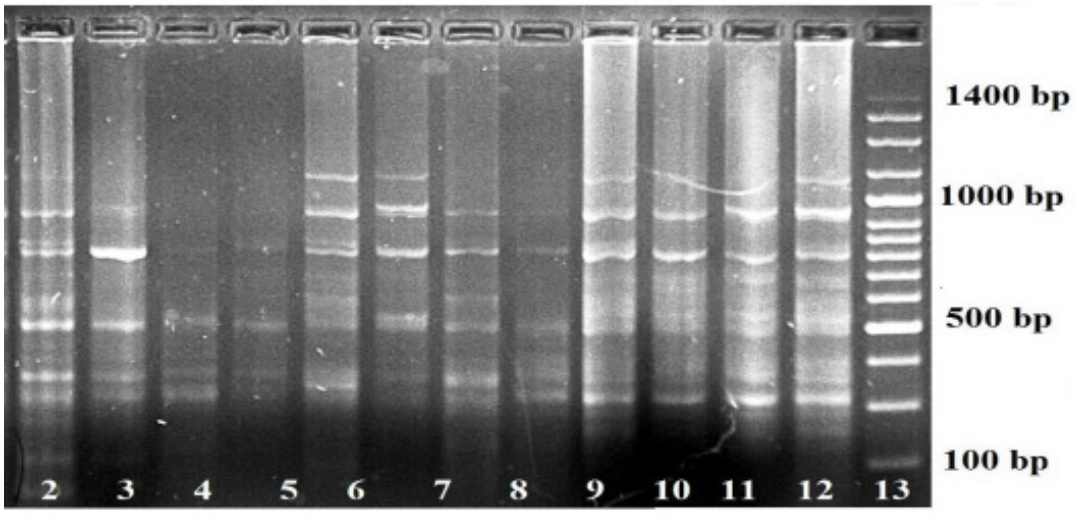

Figure 1: The band pattern for accessions using IS13 primer

sequence repeats (ISSRs) was published in 1994 (Zietkiewicz et al., 1994), which provides genomic information for a range of applications and it is widely used in population genetic studies (Behura, 2006). In other word, ISSR markers have great potential for studying natural populations (Wolfe et al., 1998). Sicard et al. (2005) mentioned that ISSR and RAPD markers were the most widely used compared to the other molecular markers for genetic variation analysis, however, Souframanien and Gopalkrishna (2004) reported that ISSR markers were more effective than RAPD to determine genetic diversity. The objectives of the present research include: (1) determination of the potential of ISSR to generate polymorphic markers in *Agropyron desertorum*; and, (2) identification of the relationship between different Agropyron accessions using ISSR molecular markers.

Materials and methods

Plant Materials: In order to evaluate the genetic variation, 13 accessions of *Agropyron desertorum* were provided from the Natural Resources Gene Bank of Iran at the Research Institute of Forest and Rangeland. (Table 1). The experiment was conducted at the Agriculture and Natural Resources Research center of Kermanshah in Iran.

DNA Extraction and ISSR Method: The total genomic DNA was extracted from young leaves of greenhouse-grown plants using a modified CTAB (Murry & Tompson, 1980) with modification described by De la Rosa et al. (2002). The quality and quantity of extracted DNA were examined using 0.8% agarose gel. The PCR mixtures were carried out according to Table 2. Template DNA was initially denatured at 92ºC for 5 minutes, followed by 35 cycles of PCR amplification under the following parameters: denaturation for 30 seconds at 95º C, primer annealing for 30 seconds at the temperature based on primer temperature (50, 55 and 60º C), and final extension for 1 minute at 72º C. A final incubation was performed for 5 minutes at 72º C to ensure that the primer extension reaction proceeded to completion. The PCR amplified products were separated by electrophoresis with 1.5% agarose gels and a TBE buffer. The gels were placed in the ethidium bromide for 30-45 minutes and visualized using gel documentation. ISSR bands were treated as binary characters and coded accordingly (presence =1, absence = 0).

Statistical Analysis: The Number of Scored Bands (NSB), the Number of Polymorphic Bands (NPB), the Percentage of Polymorphism Bands (PPB), and the Polymorphism Information Content ($PIC = 1 - \sum_{i=1}^{n} PI^2$)were calculated for each primer (Anderson et al., 1993), in addition to the Marker Index (MI= PIC × E) (Powell et al., 1996), Effective Multiplex Ratio (EMR= NPB × β) (Kumar et al., 2009), and Resolving Power (RP=∑IB) (Altıntas et al., 2008). Matrix similarity was computed based on Dice's coefficient NTSYSpc 2.02e (Rohlf & Taxonomy, 1998), and the cluster analysis was performed for grouping accessions based on Dice's coefficient according to the UPGMA method. Principal coordinate analysis was performed to better interpret the genetic variation between accessions DARwin 6 (Perrier & Jacquemoud-Collet, 2006), and finally, the analysis of molecular variance GenAlEx 6.2 (Peakall & Smouse, 2006) was performed for the three groups using cluster analysis.

ISSN-Internet 2197-411x OLCL 862804632

Tabel 3: Polymorphism percentage, total bands, PIC, MI, EMR, RP of ISSR markers

Primer code		No. of polymorphism induced (proliferated)	No. of polymorphism place	Polymorphism Percentage	PIC	MI	EMR	RP
IS3	5' GAGAGAGAGAGAGAGAYC 3'	3	3	100	0.44	1.33	3.00	2.15
IS10	5' GAGAGAGAGAGAGAGARC 3'	5	5	100	0.34	1.68	5.00	5.38
IS6	5'-CACACACACACACACAG -3'	5	5	100	0.41	2.04	5.00	3.08
IS7	5'DBDACACACACACACACA3'	4	4	100	0.43	1.70	4.00	2.77
IS13	5' AGAGAGAGAGAGAGAGYT 3'	7	6	85.71	0.32	2.39	5.14	6.46
IS5	5' AGAGAGAGAGAGAGAGC 3'	7	7	100	0.43	3.01	7.00	6.77
IS11	5' ACACACACACACACACC 3'	7	7	100	0.37	2.60	7.00	7.38
IS12	5' TGTGTGTGTGTGTGTGG 3'	5	5	100	0.37	1.87	5.00	6.92
IS9	5' CTCTCTCTCTCTCTCTG 3'	5	5	100	0.27	1.35	5.00	6.15
IS14	5' GACAGACAGACAGACA 3'	4	4	100	0.40	1.59	4.00	2.31
IS15	5' GGATGGATGGATGGAT 3'	4	4	100	0.33	1.30	4.00	5.38
IS16	5'DBDACACACACACACACA3'	5	5	100	0.41	2.06	5.00	4.15
		5.08	5.00	96.73	0.38	1.91	4.93	4.91

Results

ISSR Polymorphism: Primers, sequences, code, number of bands scored, number of polymorphic bands, percent of PPB and Polymorphism Information Content (PIC), Marker Index (MI), Effective Multiplex Ratio (EMR), Resolving power (RP) were showed for 12 ISSR primers in Table 3. For all primers, the numbers of 61 bands were scored but only 60 polymorphisms were observed. The IS5, IS11, IS13 primers had the highest number of bands with 7 bands, and primer IS3 with 3 bands had the lowest number of bands. Band pattern of accessions for IS13 was showed in Figure 1. The lowest percentage of polymorphism belonged to IS13 (85.71%) and the highest percentage of polymorphism was 100% for other primers. The average value of polymorphism percentage was 96.73. The mean value of PIC for all primers was 0.38, and the highest value of PIC belonged to IS3 (0.44), while the lowest value (0.33) belonged to IS15 primers. The mean amount of MI was 1.91, and the largest MI was IS5 with 3.01, and the lowest value was IS15 with 1.30. The average EMR was 4.93, and the IS5 and IS11 were 7. The average of RP was 4.91, and the largest value was IS11 with 7.38, though, the lowest was IS3 with 2.15.

Similarity Matrix: Similarity matrix based on Dice's coefficient for accessions varied between 0.39 and 0.79 (Table 4). The greatest value of similarity was observed between accessions G11 and G10 with 0.79, while the least value was G5, G4 with G8 (0.39).

The frequency distribution histogram showed that the number of accessions with about 0.41 distance was the greatest amount and accessions with about 0.65 distance were the least amount of frequency indicate that the number of accessions with moderate distance were higher than the others (Figure 2).

Cluster Analysis: Based on Dice's coefficient (Figure 3), the UPGMA hierarchical clustering for grouping accessions were identified for the four distinctive groups. The first group consisted of accessions G3, G4, and G5 with mean similarity 0.61. The second cluster included G1, G2, G6, and G7 with a 0.71 value of similarity. The third group

Tabel 4: Similarity matrix between Agropyron accessions based on ISSR primers

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13
G1	1.00												
G2	0.76	1.00											
G3	0.68	0.73	1.00										
G4	0.48	0.56	0.59	1.00									
G5	0.46	0.50	0.69	0.56	1.00								
G6	0.67	0.72	0.56	0.66	0.53	1.00							
G7	0.68	0.74	0.62	0.51	0.48	0.75	1.00						
G8	0.63	0.55	0.52	0.39	0.39	0.48	0.57	1.00					
G9	0.56	0.63	0.63	0.48	0.61	0.59	0.58	0.55	1.00				
G10	0.69	0.61	0.61	0.57	0.54	0.57	0.63	0.52	0.58	1.00			
G11	0.63	0.52	0.61	0.50	0.55	0.58	0.60	0.56	0.48	0.79	1.00		
G12	0.55	0.53	0.62	0.62	0.56	0.58	0.55	0.60	0.59	0.67	0.73	1.00	
G13	0.66	0.61	0.64	0.42	0.50	0.54	0.63	0.59	0.64	0.62	0.59	0.67	1.00

consisted of G10, G11, and G12 with 0.72 similarities. The forth cluster included G8, G9, and G13 with a 0.59 similarity coefficient. Similarity between clusters was showed in Table 4. Maximum similarity was between cluster 1 and cluster 4, while the least similarity was between cluster 2 and cluster 3.

Principal Coordinate Analysis (PCo): Scatter plot for accessions based on first (26.01) and the second (20.20) axis from principal coordinate analysis (Figure 4) showed that genetic variation did not match with the geographical distribution of accessions. These results, confirmed by cluster analysis and similarity matrix, grouped the accessions into four clusters.

Molecular Variance Analysis: Analysis of Molecular Variance (AMOVA) was performed for ISSR bands to determine significant differences between groups of accessions based on cluster analysis (Table 6). The results showed a statistically significant difference ($p<0.01$) between groups and the portion of variance percentage for the variables between group and within group were 23% and 77%, respectively. Results indicated that the portion of variance within groups with 77% was much greater than among groups with 23%.

Discussion

This research revealed that the genetic pattern of Agropyron accession can be determined using ISSR in the short-term. The results proved that ISSR markers are suitable for detecting the genetic variation in Agropyron accessions. The average of PPB in the present investigation indicated a high polymorphism among Agropyron accessions based on ISSR primers. Ma et al. (2008) reported the percentage of (PPB)=77.20% for accessions of Elymous sibiricus and mentioned that, according to ISSR markers, there was a high level of variation between accessions. Moreover, all primers used in the study were helpful for determination of genetic diversity based on PPB in Agropyron populations, however measurements, such as PIC, and the primers IS3, IS6, IS7, IS5, IS14, and IS16 were useful for polymorphism study and can be used in further analysis of genome for other Agropyron accessions in future research. Efficiency of ISSR primers were reported by other researchers to determine the

ISSN-Internet 2197-411x OLCL 862804632

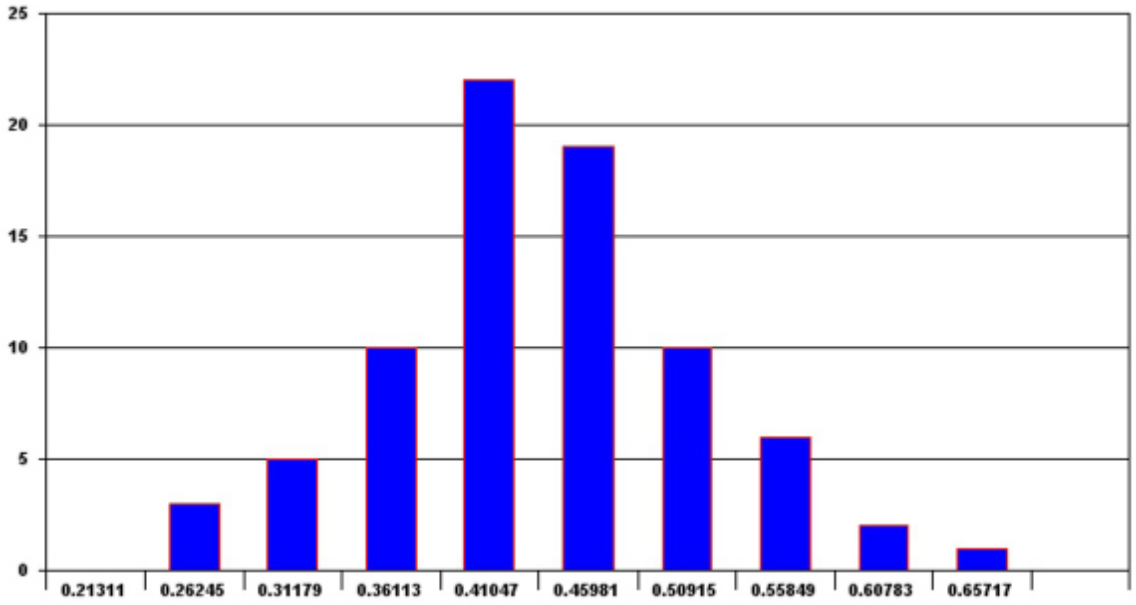

Figure 2: Amount of distance estimated between *Agropyron accessions*

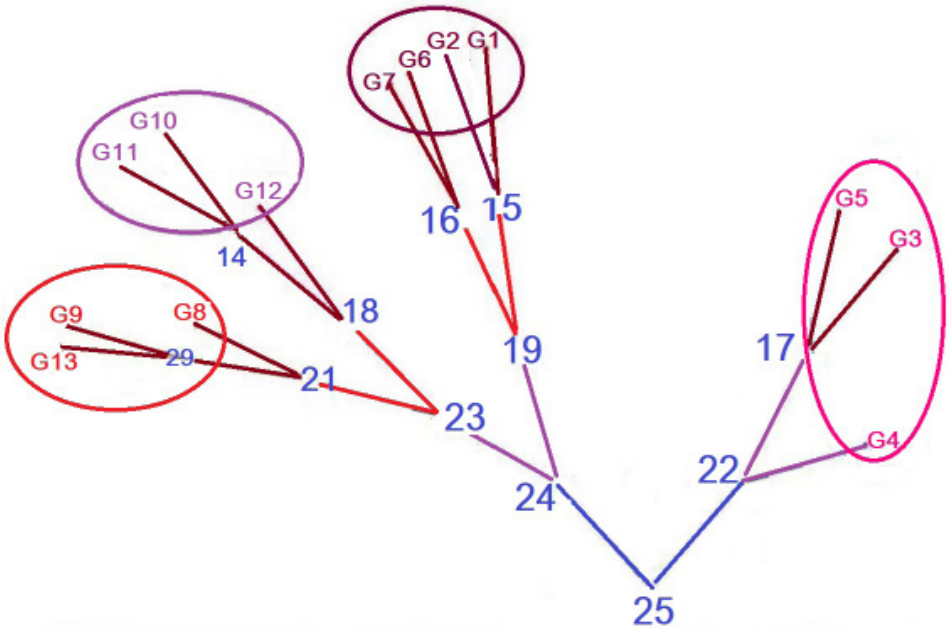

Figure 3: Dendrogram of *Agropyron* accession based on ISSR primers

genetic diversity between and within different plant species (Pivoriene & Pasakinskiene, 2008; Hu et al., 2011; Fasih et al., 2013; Shirvani et al., 2013; Shirvani et al., 2014). Based on Dice's coefficient, the similarity between accessions was low, therefore it can be stated that there was high genetic variation among accessions. In general, according to all indices, the primers IS5, IS11, and IS13 were the best ones to identify genetic variation among Agropyron. Esnault and Refoufi (2008), by using RAPD and isozymes, measured the genetic variation among Agropyron species with different ploidy levels. Grouping of accessions, based on cluster analysis and principal coordinate analysis, indicated that genetic variations are not in accordance with the geographical distribution of accessions. There are several possible explanations for these results, such that some of them are connected through nature, and also that the structure of different molecular markers are designed from various regions of genome, among other reasons. An additional problem was the possibility of overestimating genetic similarity

Table 5: Similarity coefficient between cluster

Clusters	Cluster1	Cluster2	Cluster3	Cluster4
Cluster1	1.000			
Cluster2	0.790	1.000		
Cluster3	0.820	0.768	1.000	
Cluster4	0.837	0.811	0.791	1.000

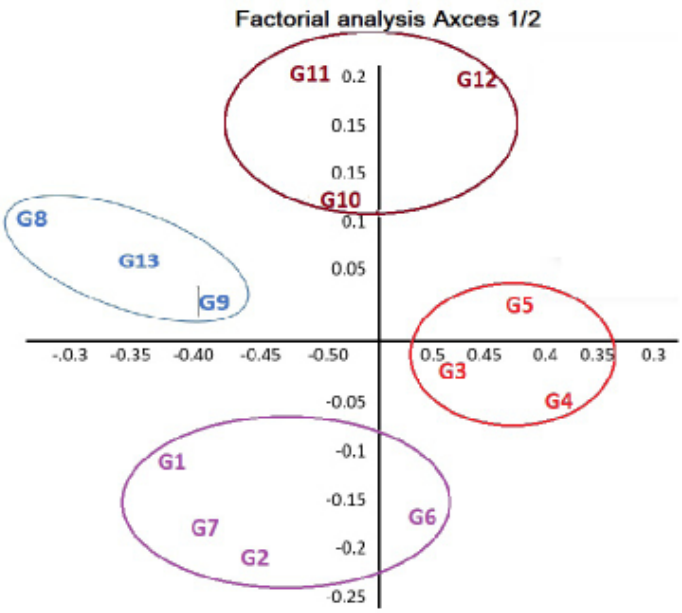

Figure 5: Distribution diagram of accessions in regard to the first and second PCo

Table 6: Analysis of molecular of variance based on ISSR markers

S.O.V	Df	SS	MS	Est. Var.	Var%	PhiPT
Between group	3	59.423	19.808	3.053	23%	0.235*
Within group	9	89.500	9.944	9.944	77%	
Total	12	148.923		12.997	100%	

as the fragments with the same size could have different origins (Suvendu et al., 2009; Poczai et al., 2013; Etminan et al., 2016). The results from the cluster analysis were confirmed by AMOVA. On the other hand, the results of estimated variance indicating the genetic variation the within group variable was more than the between group variable, and can be explained by the high genetic variation between accessions (Etminan et al., 2018; Moradkhani et al., 2015). Finally, based on ISSR markers, the accessions of the first group (G3, G4, G5) had higher

ISSN-Internet 2197-411x OLCL 862804632

genetic distance compared to the accessions of the forth group (G8, G9, G13). As depicted by the high diversity between these two groups, they would be appropriate partners in crossing programs to obtain high yield and heterosis.

Conclusions

The assessment of genetic diversity is important, not only for crop improvement, but also for efficient management and conservation of germplasm resources. The current study confirmed the importance of molecular studies data in detecting genetic variation among genotypes to select diverse parents in order to carry out a new crossing program successfully. We believe that there is a need for molecular marker studies as a complementary study to the morphological traits in the field. The primers IS3, IS6, IS7, IS5, IS14, and IS16 were useful for polymorphism study and can be used for the analysis of genome and other Agropyron accessions in future research. The grouping of accessions based on cluster analysis and principal coordinate analysis indicated that genetic variations are not in accordance with the geographical distribution of accessions. The greatest value of similarity was observed between accessions G11 and G10 with 0.79, while the least value was G5, G4 and G8 with 0.39. Due to the high diversity between these two groups, they would be considered appropriate partners in crossing programs to obtain high yield and heterosis.

Acknowledgements

This research has been sponsored by Payam Noor University, thanks and appreciation of the relevant authorities.

Conflict of Interests

The authors hereby declare that there is no conflict of interests.

References

Altıntas, S., Toklu, F., Kafkas, S., Kilian, B., Brandolini, A., & Zkan, H. O. (2008). Estimating genetic diversity in durum and bread wheat cultivars from Turkey using AFLP and SAMPL markers. *Plant Breeding*, 127, 9-14

Anderson, J. A., Church, J. E., Autrique, S. D., Thanksley, S., Sorrells, M. E. (1993). Optimizing parental selection for genetic linkage map. *Genome*, 36, 181-188.

Arghavani, A., Asghari, A., Shokrpour, M., Mohammaddost, C. (2010). Genetic diversity in ecotypes of two agropyron species using RAPD markers. *Research Journal of Environmental Sciences*, 4, 50-56.

Behura, S. K. (2006). Molecular marker systems in insects: Current trends and future avenues. *Molecular Ecology*, 15, 3087–3113.

Bor, N. L. (1970). Flora Iranica. In K. H. Rechinger (Ed.) *Gramineae* (pp. 571-573). Graz, Austria: Akademische Druck- und Verlagsantalt.

Che, Y. H., & Li, L. H. (2007). Genetic diversity of prolamines in Agropyron mongolicum Keng indigenous to northern China. *Genetic Resources and Crop Evolution*, 54, 1145–1151. http://dx.doi.org/10.1007/s10722-006-9006-7

Che, Y. H., Yang, Y. P., Yang, X. M., Li, X. Q., & Li, L. H. (2011). Genetic diversity between ex situ and in situ samples of Agropyron cristatum (L.) Gaertn based on simple sequence repeat molecular markers. *Crop and Pasture Science* 62(8), 639-644. http://dx.doi.org/10.1071/CP11065

De la Rosa, R., James, C., & Tobutt, K. R. (2002). Isolation and characrerization of polymorphic microsatellite in olive (Olea europaea L.) and their transferability to other genera in Oleaceae. *Molecular Ecology Note*, 2, 265-267.

Dizkirici, A., Kaya, Z., Cabi, E., & Dogan, M. (2010). Phylogenetic relationships of Elymus L. and related genera (Poaceae) based on the nuclear ribosomal internal transcribed spacer sequences. *Turkish Journal of Botany*, 34, 467-478.

Etminan, A., Pour-Aboughadareh, A., & Mohammadi, R. (2016). Applicability of start codon targeted (SCoT) and inter-simple sequence repeat (ISSR) markers for genetic diversity analysis in durum wheat genotypes. *Biotechnol Biotechnol Equipment*, 30, 1075–1081.

Etminan, A., Pour-Aboughadareh, A., Noori, A., Ahmadi-Rad, A., Shooshtari, L., Mahdavian, Z., & Yousefiazar-Khanian, M. (2018). Genetic relationship and diversity among wild Saliva accessions revealed by ISSR and SCOT markers. Biotechnology & Biotechnological Equipment. *https://doi.org/10.1080/13102818.2018.1447397*

Farshadfar, M., & Farshadfar, E. (2008). Genetic variability among Lucerne cultivars based on biochemical (SDS-PAGE) and morphological markers. *Journal of Applied Sciences*, 8, 1867-1874.

Fasih, Z., Farshadfar, M., & Safari, H. (2013). Genetic diversity evaluation of within and between populations for Festuca arundinacea by ISSR markers. *International Journal of Agriculture and Crop Sciences*, 5(10), 1468-1472.

Hu, L., Huang, T., Liu, X. J., & Cai, Y. D. (2011). Predicting protein phenotypes based on protein-protein interaction network. *PLoS One*, 6(3), 68-76.

Kumar, M., Mishra, G. P., Singh, R., Kumar, J., Naik, P. K., & Singh Sh B. (2009). Correspondence of ISSR and RAPD markers for comparative analysis of genetic diversity among different apricot genotypes from cold arid deserts of Trans-Himalayas. *Physiology and Molecular Biology of Plants*, 15(3), 225-236.

Ma, X., Zhang, X. Q., Zhou, Y. H., Bai, S. Q., & Liu, W. (2008). Assessing genetic diversity of Elymus sibiricus (Poaceae: Triticeae) populations from Qinghai-Tibet plateau by ISSR markers. *Biochemical Systematics and Ecology*, 36, 514–522.

Moradkhani, H., Mehrabi, A. A., & Etminan, A. (2015). Molecular diversity and phylogeny of Triticum-Aegilops species possessing D genome revealed by SSR and ISSR markers. *Plant Breed Seed Science*, 71, 82–95.

Murry, M. G., & Tompson, W. F. (1980). Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research*, 8, 4321-4325.

Peakall, R. & Smouse, P. E. (2006). Genetic Analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, 6, 288-295.

Perrier, X., & Jacquemoud Collet, J. P. (2006). DARwin software [Computer Software]. Retrieved from *http://darwin.cirad.fr/*

Pivoriene, O., & Pasakinskiene, I. (2008). Genetic diversity assessment in perennial Ryegrass and Festulolium by ISSR finger printing. *Agriculture*, 95(2), 125-133.

Poczai, P., Varga, I., & Laos, M. (2013). Advances in plant gene-targeted and functional markers: a review. *Plant Methods*, 9, 6–37.

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.

Refoufi A, & Esnault, M. A. (2008). Population genetic diversity in the polyploid complex of wheatgrasses using isoenzyme and RAPD data. *Biologia Plantarum*, 52(3), 543-547. *http://dx.doi.org/10.1007/s10535-008-0106-4*

Rohlf, F. & Taxonomy, N. P. N. (1998). *Multivariate Analysis System, Version 2.02* [Computer Software]. New York: Exeter Software, Applied Biostatistics Inc.

Rouf Mian, M. A., Andrew, A. H., & John, C. Z. (2002). Determination of Genetic Diversity in Tall Fescue with AFLP Markers. *Crop Science*, 42, 944-950.

Shirvani, H., Etminan, A., & Safari, H. (2013). Evaluation of genetic diversity within and between populations for Agropyron Trichophorum by ISSR marker. International *Journal of Farming and Allied Sciences,* 2(21).

Shirvani, H., Etminan, A., & Safari, H. (2014). Genetic variation of agropyron trichophorum accessions using ISSR molecular marker. *Journal of Biodiversity and Environmental Sciences*, 5(4).

Sicard, D., Nanni, L., Porfiri, O., Bulfon, D., & Papa, R. (2005). Genetic diversity of phaseolus vulgaris L. and P. coccineus L. landraces in central Italy. *Plant Breeding,* 124(5), 464–472. *http://dx.doi.org/10.1111/j.1439-0523.2005.01137.x*

Souframanien, J., & Gopalkrishna, T. (2004). A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. *Theoretical and Applied Genetics*, 109, 1687-1693. *http://dx.doi.org/10.1007/s00122-004-1797-3*

Sun, G. L., Diaz, O., Salomon, B., & von Bothmer, R. (1999). Genetic diversity in Elymus caninus as revealed by isozyme, RAPD, and microsatellite markers. *Genome*, 42, 420-431.

Sun, G. L., Salomon, B., & von Bothmer, R. (2002). Microsatelllite polymorphism and genetic differentiation in three Norwegian populations of Elymus alaskanus (Poaceae). *Plant Systematics and Evolution*, 234, 101–110. *http://dx.doi.org/10.1007/s00606-002-0211-3*

Surendhar, R. Ch., Parsad, B. A., Mallikarjuna, S. B. P., Kaladhar, K., & Sarla, N. (2009). ISSR markers based on GA and AG repeats reveal genetic relationship among rice varieties tolerant to drought, flood, or salinity. *Journal of Zhejiang University*, 10(2), 133–141. *http://dx.doi.org/10.1631/jzus.B0820183*

ISSN-Internet 2197-411x OLCL 862804632

Suvendu Mondal, S., Sutar, R., & Badigannavar, A. M. (2009). Assessment of genetic diversity in cultivated groundnut (Arachis hypogaea L.) with differential responses to rust and late leaf spot using ISSR markers. *Indian Journal of Genetics*, 69(3), 219-224.

Szczepaniak, M., Bieniek, W., Boroń, P., Szklarczyk, M., & Mizianty, M. (2009). A contribution to characterisation of genetic variation in some natural Polish populations of Elymus repens (L.) Gould and Elymus hispidus (Opiz) Melderis (Poaceae) as revealed by RAPD markers. *Plant Biology*, 11(5), 766–773. *http://dx.doi.org/10.1111/j.1438-8677.2008.00171.x*

Wolfe, A. D., Xiang, Q. Y., & Kephart, S. R. (1998). Assessing hybridization in natural populations of Penstemon (Scrophulariaceae) using hyper variable inter simple sequence repeat markers. *Molecular Ecology*, 7, 1107–1125.

Xu, G. H., Su, W. Y., Shu, Y. J., Cong, W. W., Wu, L., & Guo, C. H. (2012). RAPD and ISSR-assisted identification and development of three new SCAR markers specific for the thinopyrum elongatum E (Poaceae) genome. *Genetics and Molecular Research*, 11(2), 1741-1751. *http://dx.doi.org/10.4238/2012.June.29.7*

Yang, R. W., Tsujimoto, H., Ding, C. B., Zhang, L., Wang, X. L., & Zhou, Y. H. (2011). Phylogenetic relationships among hystrix species and related species based on expressed sequence tag-polymerase chain reaction. *Journal of Systematics and Evolution*, 49(1), 65–71. *http://dx.doi.org/10.1111/j.1759-6831.2010.00107.x*

Yousefiazar-Khanian, M., Asghari, A., Ahmadi, J. (2016). Genetic diversity of salvia species assessed by ISSR and RAPD markers. *Not Bot Horti Agrobo*, 44, 431–436.

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