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Molecular Similarity Relationships Among Iranian Bread Wheat Cultivars and Breeding Lines Using ISSR Markers

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Abstract

Inter simple sequence repeat (ISSR) markers were used to characterize and assess genetic diversity of Iranian bread wheat (*Triticum aestivum* L.) using 101 cultivars and breeding lines. Twenty-three ISSR primers amplified a total of 267 loci, of which 224 (83.9%) were polymorphic among the genotypes. The percentage of polymorphic loci (PPL) for primers ranged from 54% (UBC808) to 100% (441, A12 and UBC820). The mean of expected heterozygosity (He) for the primers varied from 0.26 (UBC808, UBC827 and A14) to 0.48 (A12, 441). The PPL, He, Shannon's information index (I), and number of effective alleles (Ne) for breeding lines were slightly higher than those of cultivars. The Dice similarity coefficient for the germplasm ranged from 0.76 (between two breeding lines) to 0.91 (between two breeding lines). The dendrogram grouped samples in four main clusters; most cultivars were placed into the same cluster close to each other with regard to their geographic origin. The genotypes in different groups might be used as potential parents in bread wheat breeding programs. Also, a high level of genetic similarity detected in this collection may demonstrate the narrow genetic base of Iranian wheat germplasm.

Keywords: expected heterozygosity, genetic variability, Shannon's information index, *Triticum aestivum* L.

Introduction

Bread wheat (*Triticum aestivum* L.), the most important cereal crop in the world, evolved from wild grasses, probably somewhere in the Near East (Briggle and Curtis, 1987). In the cradle of agriculture, Iran is considered as the centre of origin of wheat and as a primary centre of its genetic diversity, but Iranian wheat germplasm has experienced an overall reduction in its genetic basis as a result of high selection pressure, recurrent use of the adapted elite germplasm, and the adoption of particular breeding schemes.

Genetic diversity patterns can provide insights into evolutionary and demographic history of a taxon. Moreover, exploitation, evaluation, and strategies for the conservation *in situ* and *ex situ* of genetic diversity present in natural populations or local cultivars are imperative to guarantee sustainable development (Smolik *et al*., 2011). Dreisigacker *et al*. (2005) reported that the genetic variation of bread wheat landraces and cultivars conserved in seed banks is usually unknown. Thus, in many countries, strategies have been devoted to characterize and evaluate the genetic diversity of their natural populations and landraces, providing considerable opportunities for genetic research, breeding, and construction of core collections (Hou *et al*., 2005).

Genetic diversity among individuals or populations can be determined using morphological and molecular markers. Phenotypic characters have limitations since they are influenced by environmental factors and the developmental stage of the plant (Archak *et al*., 2003). Different molecular marker systems have been used to assess genetic diversity in wheat, including random amplified polymorphic DNA (RAPD) (Abdollahi Mandoulakani *et al*., 2010), amplified fragment length polymorphism (AFLP) (Khalighi *et al*., 2008), simple sequence repeats (SSRs) (Gupta *et al*., 2002), sequence-tagged-sites (STS) (Talbert *et al*., 1994), and retrotransposon-based markers (Carvalho *et al*., 2010). Inter simple sequence repeat (ISSR) markers is a PCR-based technique, amplify regions between adjacent and inversely oriented microsatellites using di-, tri-, tetra-, and penta-nucleotide SSR primers, with the advantage that knowledge of the DNA sequence of the target region is not required. ISSR amplification depends on the variation, motif, and frequency of SSRs that change among species (Zietkiewicz *et al*., 1994). ISSR is an ideal method for fingerprinting and a useful alternative to single-locus or hybridization-based methods. It is a powerful technique to determine intra- and inter-specific genetic diversity (Bornet and Branchard, 2001; Myśków *et al*., 2011; Pradeep Reddy *et al*., 2002; Smolik *et al*., 2011; Sofalian *et al*., 2009). The ISSR markers are increasingly applied in the plant sciences and have detected a sufficient degree of polymorphism in faba bean (Terzopoulos and Bebeli, 2008), safflower (Golkar *et al*., 2011), rice (Blair *et al*., 1999) and barley (Hou *et al*., 2005). ISSR mark-

ers have a better reproducibility than RAPDs, are easy to detect and can be produced at lower costs than AFLPs. They are simpler to use than the SSR technique and less restrictive than restriction fragment length polymorphism (RFLP), and may offer considerable variation among species (Archak *et al*., 2003)

In order to extend the genetic base of Iranian wheat collection, new breeding lines have been currently introduced to this germplasm. To the best of our knowledge, little information is available on the molecular diversity of Iranian wheat cultivars and breeding lines using ISSR markers. Therefore, the objective of our study were to characterize 101 Iranian bread wheat cultivars and breeding lines using cultivar-specific ISSR markers and to assess the genetic diversity and relationships among the cultivars and breeding lines for further planning of germplasm conservation and breeding strategies.

Materials and methods

Plant materials and DNA isolation

Fifty Iranian wheat cultivars and 51 breeding lines provided by the Seed and Plant Improvement Institute, Karaj, Iran, were used (Tab. 1). Seeds were planted in small pots with a 10 cm diameter, containing a mixture of garden soil and vermiculite, in the greenhouse with 20°C of ambient temperature. Genomic DNA was extracted from young leaves of 20 day seedlings of five plants from each genotype using the method described by Doyle and Doyle (1990) with minor modifications. The concentration of genomic DNA samples was determined by a spectrophotometer and by 0.8% agarose gel electrophoresis.

Tab. 1. 101 bread wheat cultivars and breeding lines used in the current study

Cultivar	Cultivar	Breeding line	Breeding line	Breeding line
'Adl'	'Karaj3'	2	140	1720
'Alamut'	'Kaveh'	5	145	
'Akbari'	'Kavir'	8	146	
'Arta'	'Mahdavi'	12	147	
'Aria'	'Marvdasht'	13	149	
'Atrak'	'Moghan1'	15	151	
'Azadi'	'Moghan3'	16	152	
'Bam'	'Morvarid'	18	156	
'Bahar'	'M17'	19	158	
'Behrang'	'Navid'	20	159	
'Bayat'	'Niknezhad'	22	164	
'Chenab'	'Pishgam'	25	167	
'Darab2'	'Rasul'	28	168	
'Darya'	'Roshan'	30	169	
'Dena'	'Sardari'	32	170	
'Dez'	'Sepahan'	38	175	
'Falat'	'Shahpasnd'	110	177	
'Gaskozhen'	'Shahriar'	114	178	
'Gaspard'	'Shiraz'	115	179	
'Ghods'	'Sholeh'	121	183	
'Golestan'	'Sorkhtokhm'	122	608	
'Hamun'	'Sistan'	125	1546	
'Hirmand'	'Vee/nak'	132	1549	
'Inia'	'Zagros'	133	1661	
'Karaj2'	'Zarin'	137	1717	

ISSR analysis

Fifty-seven ISSR primers were tested on three cultivars and three breeding lines to choose the primers yielding a clear, scorable, and discernible banding pattern (Tab. 2). Gradient PCR was used to optimize the annealing temperature of each primer. PCRs were carried out in a Bio-Rad thermocycler (Bio-Rad Laboratories Inc., Hercules, CA, USA). Each 20 µl of the reaction contained 30 ng of genomic DNA, 1× PCR buffer (10 mM Tris-HCL, 50 mM KCL, PH=8.3), 1.5 mM $MgCl_2$, 0.2 µM dNTP, 0.5 U Taq DNA polymerase and 10 picomol primer. The thermal profile for ISSR-PCR was as follows: 94°C initial denaturation for 4 min, then 35 cycles of 94°C for 40 s, 53 to 61°C (Tab. 2) for 40 s, and 72°C for 2 min, then 72°C for 5 min. ISSR bands were separated by electrophoresis in 1.8% agarose gels (Trade Mark) and visualized with ethidium bromide (1.0 μgml^{-1}). The electrophoretic patterns of the PCR products were photographed under UV light using a Gel-Doc image analysis system (Gel Logic 212 PRO).

Data analysis

For subsequent statistical analysis, ISSR markers were binary-coded using 1 for presence and 0 for absence of band in each genotype. The genetic similarity matrix derived from ISSR data were calculated between 101 genotypes using the Dice similarity coefficient and subsequently used to construct the dendrogram using the complete linkage algorithm. To verify the adjustment between similarity matrix and respective dendrogram-derived matrix (cophenetic matrix), the cophenetic correlation coefficient was estimated. A similarity matrix were also used to establish the dcenter matrice and subsequently used to accomplish principal coordinate analysis (PCoA) to resolve the patterns of genetic relationships among 101 studied genotypes. All of these computations were implemented in NTSYSpc 2.1 (Rohlf, 2000). Number of loci, percentage of polymorphic loci (PPL), number of loci with a frequency higher or equal to 5%, number of private or specific loci, number of less common loci with frequency lower or equal to 25% and 50%, mean of expected heterozygosity (He), Shannon's information index (I) and number of effective alleles (Ne) (Lynch and Milligan, 1994) were also calculated for each primers as well as for cultivars, breeding lines and cultivars + breeding lines using GenAlEx 6.41 software (Peakall and Smouse, 2006). To partition of total genetic variation within and among cultivars and breeding lines, analysis of molecular variance (AMOVA) was carried out with this software as well.

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Tab. 2. The primer sequences and annealing temperatures used in the study

Primer	Sequence (5'-3)'	Annealing temperature	Primer	Sequence (5'-3)'	Annealing temperature
UBC818	$(CA)_8G$	54	UBC864	(ATG)6	53
441	$(TG)_9C$	61	UBC855	$(AC)_8YT$	56
UBC807	$(AG)_8T$	54	UBC816	$(CA)_8T$	53
UBC808	$(CA)_7G$	54	UBC 827	$(AC)_8G$	58
A13	$(GT)_6CC$	55	UBC 812	$(GA)_8A$	53
UBC840	$(GA)_8Y^*T$	54	UBC 811	$(GA)_8C$	54
UBC825	$(AC)_8T$	55	UBC 834	$(AG)_8YT$	55
UBC848	$(CA)_8R^*G$	54	A12	$(GA)_6CC$	53
A15	$(AG)_6/RG$	55	UBC 836	$(AG)_7YA$	55
A14	$(CA)_6/AC$	54	UBC 844	$(CT)_8RC$	56
UBC857	$(AC)_8YG$	57	UBC 820	$(GT)_8C$	55
UBC880	(G(GA)2)3	53			

*Y: C or T, R: A or T

Results

In the present study, out of 57 ISSR primers screened, 23 anchored dinucleotide primers amplified 267 clear and discernible loci which 224 were polymorphic (83.9%) (Tab. 2 and 3). No cultivar- or breeding line-specific marker was produced. The frequency of all amplified loci was more than 5%. No less common loci with frequency lower than 50% were amplified. The number of total loci per primer ranged from 7 (UBC844) to 16 (UBC811), with an average of 11.6. The number of polymorphic loci per primer ranged from 5 to 14 with a mean value of 9.78. The PPL, He, I, and Ne were calculated in order to characterize the capacity of each primer to detect polymorphism and to evaluate the discriminating ability of each primer in this collection. PPL detected by primers UBC840, UBC820 and A12 were 100%. UBC808 generated 54% polymorphism in the studied germplasm. The polymorphism detected by primers A13 and UBC840 are presented in Fig. 1 and 2, respectively. The size of the amplified fragments ranged from 75 to 2000 bp. He for primers varied from 0.26 (UBC808, A14, UBC827) to 0.48 (A12, 441) averaging 0.36 (Tab. 3).

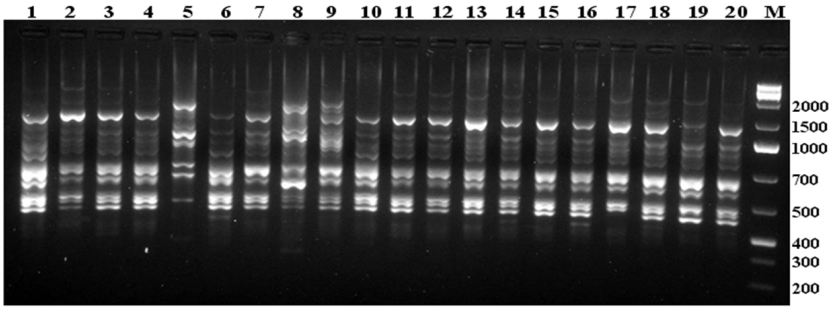

Fig. 1. The ISSR profiles obtained by primer A13, lanes 1 to 20: Pishgam, Darya, Moghan3, Dez, 121, 608, 1549, 149, 1720, Adl, Golestan, Shiraz, Rasol, 122, Shahpasand, Sorkh tokhm, Alamot, Akbari, 1661, 20, M: 1 kb DNA ladder (Fermentas, SM1303)

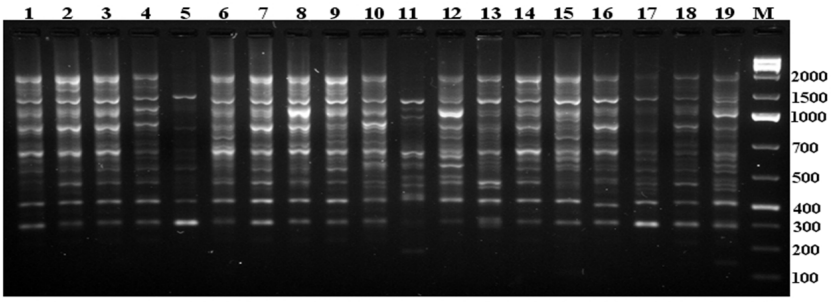

Fig. 2 The ISSR profiles obtained by primer UBC840, lanes 1 to 19: breeding lines 111, 158, 170, 152, 159, 110, 151, 156, 20, 28, 16, 175, 1661, 114, 2, 5, 122, 1720, 115, M: 1 kb DNA ladder (Fermentas, SM1303)

Tab. 3. Total loci, polymorphic loci, PPL, He, I and Ne of ISSR primers in 101 bread wheat genotypes

Primer name	Total loci	Poly-morphic loci	PPL*	He*	I*	Ne*	Band size (bp)
A13	10	9	90	0.31	0.48	1.50	200-1500
UBC840	12	12	100	0.34	0.49	1.36	400-2000
UBC825	12	11	91	0.35	0.51	1.61	400-1500
UBC848	11	6	54	0.34	0.50	1.60	200-1500
A15	11	10	90	0.42	0.60	1.81	400-2000
441	13	10	76	0.48	0.67	1.92	300-2000
UBC818	10	9	90	0.41	0.58	1.75	75-700
A14	11	10	90	0.26	0.37	1.48	300-2000
UBC807	10	9	90	0.41	0.58	1.78	400-1500
UBC857	10	6	60	0.27	0.42	1.42	500-2000
UBC880	12	10	83	0.37	0.53	1.68	200-1500
UBC864	14	12	85	0.39	0.55	1.73	200-1500
UBC855	13	11	84	0.41	0.58	1.80	300-1500
UBC816	14	12	85	0.27	0.39	1.49	75-1000
UBC827	11	7	63	0.26	0.38	1.46	200-1000
UBC812	12	8	66	0.43	0.60	1.82	300-2000
UBC811	10	9	90	0.31	0.44	1.58	300-1500
UBC834	16	14	87	0.44	0.62	1.86	200-2000
UBC808	13	12	92	0.24	0.36	1.49	200-2000
A12	10	10	100	0.48	0.67	1.91	300-2000
UBC836	15	12	80	0.33	0.48	1.61	200-1500
UBC844	7	5	71	0.30	0.43	1.55	400-1000
UBC820	10	10	100	0.41	0.60	1.74	200-1500
Total	267	224					
Mean	11.6	9.7	83	0.36	0.51	1.65	

*PPL: percentage of polymorphic loci, He: mean of expected heterozygocity, I: Shannon's information index, Ne: number of effective alleles

The Dice similarity coefficient values between 101 cultivars and breeding lines ranged from 0.76 to 0.91, averaging 0.86, 0.85 and 0.85 for cultivars, breeding lines, and cultivars + breeding lines. The PPL, Ne, He and I for breeding lines were slightly higher than those for cultivars. The highest similarity value was obtained between breeding lines 175 and 170, while lines 121 and 151 were found as genetically most dissimilar. Dice similarity coefficients

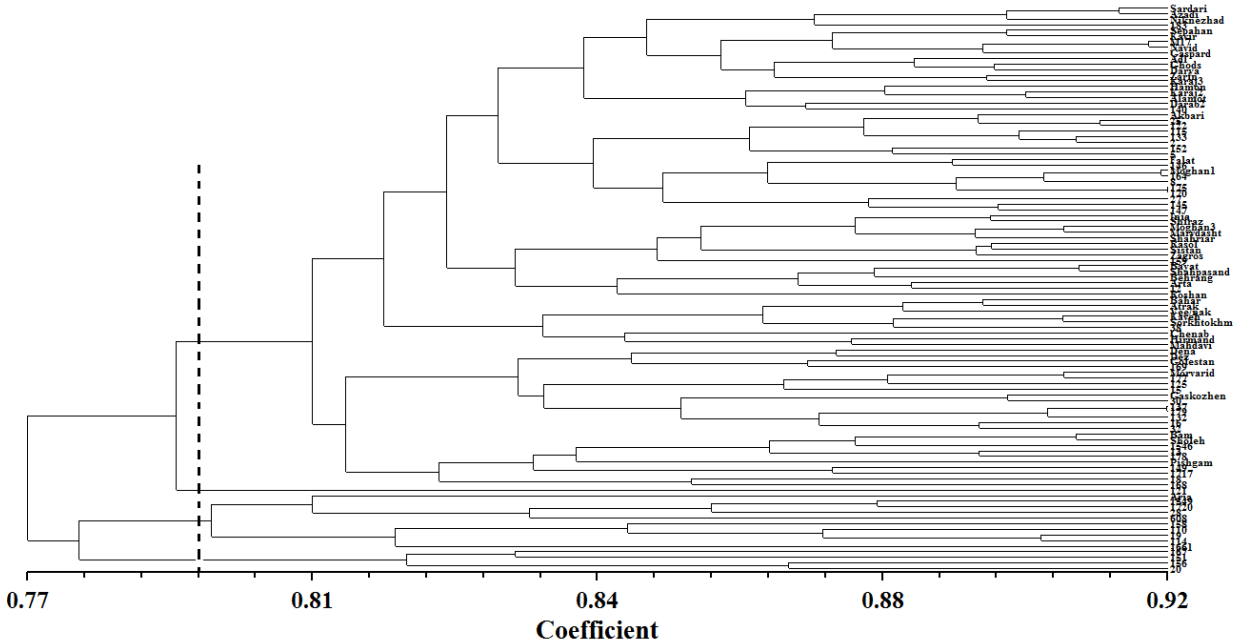

Fig. 3. Dendrogram of 101 bread wheat genotypes constructed with complete linkage method using Dice similarity coefficient based on 267 ISSR loci

Tab. 4. Four clusters of 101 bread wheat cultivars and breeding lines identified by ISSR data

Cluster I				Cluster II	Cluster III	Cluster IV
'Sardari'	2	'Behrang'	179	121	'Aria'	167
'Azadi'	152	'Arta'	132		1549	151
'Niknezhad'	5	12	16		1720	156
183	'Falat'	'Roshan'	32		28	20
'Sepahan'	146	'Bahar'	'Bam'		608	
'Kavir'	'Moghan1'	'Atrak'	'Sholeh'		158	
'M17'	164	'Vee/nak'	1546		110	
'Navid'	8	'Kaveh'	13		19	
'Gaspard'	175	'Shorkhtokhm'	178		114	
'Adl'	170	38	'Pishgam'		1661	
'Ghods'	27	'Chenab	149			
'Darya'	145	'Hirmand'	1717			
'Zarin'	147	'Mahdavi'	18			
'Karaj3'	'Inia'	'Dena'	168			
'Hamun'	'Shiraz'	'Dez'				
'Karaj2'	'Moghan3'	'Golestan'				
'Alamut'	'Marvdasht'	169				
'Darab2'	'Shahriar'	'Morvarid'				
140	'Rasul'	177				
'Akbari'	'Sistan'	125				
25	'Zagros'	15				
122	159	'Gaskozhen'				
115	'Bayat'	30				
133	'Shahpasand'	137				

with complete linkage clustering algorithm grouped the 101 wheat genotypes into four clusters. Most of the cultivars and breeding lines located in cluster I. Cluster II consisted of one sample, line 121. All cultivars were in the vicinity of each other in cluster I except for cultivar Aria which is in cluster III (Fig 3 and Tab. 4). PCoA was performed to visualize the association among lines and cultivars. The first two components of PCoA explained 7.6% of the total variation, probably suggesting the evenly distribution of amplified loci in wheat genome and no considerable correlation between them (Fig. 4).

Discussion

In the current investigation, 50 agro-economically important Iranian bread wheat cultivars and 51 breeding lines were examined via ISSR markers at a molecular level. A high level of polymorphism (82.77%) was revealed by 23 anchored dinuclotide ISSR primers, showing the high dis-

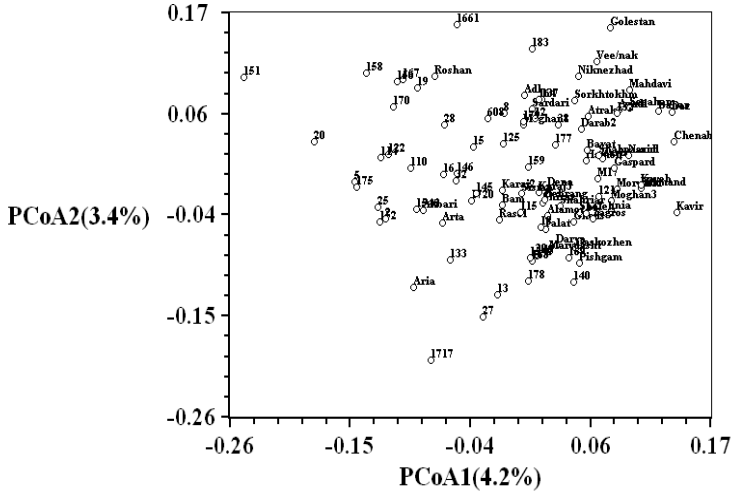

Fig. 4. Principle coordinate analysis plot of 101 bread wheat cultivars and breeding lines revealed by ISSR data

criminative and resolving power of the used ISSRs in the studied germplasm of bread wheat. The high percentage of polymorphism detected by ISSR markers have been reported in Portuguese bread wheat cultivars (98.5%) (Carvalho *et al*., 2010), in Chinese barley accessions (98.13%) (Hou *et al*., 2005), and in Mediterranean faba bean cultivars (98.9%) (Terzopoulos and Bebeli, 2008). Nagaoka and Ogihara (1997) stated that primers with dinucleotide motifs constituted more suitable primers for amplifying ISSRs in wheat, regardless of whether the genotype is diploid, tetraploid, or hexaploid. Blair *et al*. (1999) also considered dinucleotide motif primers to be more amenable to ISSR analysis in rice, but primers based on the more infrequent trinucleotide and tetranucleotide SSRs amplified few bands in rice (Blair *et al*., 1999). Carvalho *et al*. (2010) reported a high level of polymorphism by using the pentanucleotide primer UBC880 [$(GGAGA)_3$] in bread and durum wheat. In this study, a few tri- and teranucleotide ISSR primers amplified no clear and polymorphic banding patterns. A result similar to ours was reported by Najaphy *et al*. (2012). ISSR primers UBC820, UBC840, and A12 with the dinucleotide motifs GA and GT produced 100% polymorphism.

A narrow range of Dice genetic similarity (0.76 to 0.91) was found within the studied germplasm, indicating the limited amount of genetic diversity in Iranian bread wheat cultivars. This was confirmed by the low values of I and He obtained in our investigation. Mohammadi *et al*. (2009) reported the high values of SSR-based gene diversity and polymorphic information content (PIC) of 0.7 and 0.66 for 27 Iranian local commercial and adapted wheat cultivars. The differences between that investigation and our study may be derived from the different number of studied samples and used marker systems. Carvalho *et al*. (2010) detected considerable genetic diversity among the old Portuguese wheat cultivars using ISSR markers. Several authors reported a narrow genetic diversity in wheat when assessed with RAPD and DNA amplification fingerprinting (DAF) (Abdollahi Mandoulakani *et al*., 2010), AFLPs (Khalighi *et al*., 2008; Shoaib and Arabi, 2006), and selective amplified microsatellite polymorphic loci (SAMPL) (Altintas *et al*., 2008). Nevertheless, other researchers reported that ISSRs supplanted the polymorphism rates achieved by other markers in wheat and other plant species (Nagaoka and Ogihara, 1997). AMOVA was conducted to partition variance components among cultivars and breeding lines. Higher level of genetic variation detected within cultivars+breeding lines (97%) compared to among cultivars and breeding lines, indicating that overall genetic differentiation among lines and cultivars was low (p=0.01, PhiPT=0.028).

A cluster analysis using the Dice similarity coefficient and a complete linkage algorithm identified four clusters. The most of the cultivars and breeding lines were in the cluster I. No cultivars were located in cluster II, III, and IV except Aria in cluster III. The breeding lines were dispersed in four clusters, although most of them were in cluster I. These results confirmed the limited and narrow genetic diversity found in Iranian cultivated bread wheat germplasm. Different wheat breeding programs in recent decades have reduced the genetic variability of Iranian commercial wheat cultivars. This can be evidenced by epidemic extension of wheat yellow rust and other biotic and abiotic stresses in most regions of Iran under cultivation of bread wheat in the recent years. A decrease in allelic diversity after the 1960's was demonstrated in a study of 559 French bread wheat accessions from year 1800 to 2000 using 42 SSRs (Roussel *et al*., 2004) and in 480 European wheat cultivars from 1840 to 2000 using 39 SSRs (Roussel *et al*., 2005). The genetically most dissimilar breeding lines were found as 121 and 151. Knowledge about the genetic relationships of genotypes provides useful and helpful information for germplasm management and for planning intraspecific crosses between highly diverse cultivars for wheat breeding programs. Hence, the breeding lines with sufficient genetic distance could be introduced as potentially appropriate parents in wheat breeding programs to explore heterosis as well as to produce mapping populations, provided that factors such as combining ability and other agronomically important traits are considered.

Conclusions

Knowledge of the level of genetic variation among accessions is a prerequisite for germplasm conservation and breeding programs. The current study demonstrated that the genetic basis of Iranian bread wheat germplasm has been narrowed and more new accessions and landraces should be introduced to this germplasm to extend its diversity for further use in wheat breeding programs. The ISSR technique is an efficient tool for classifying wheat cultivars but more primers and different molecular marker systems need to be combined for an accurate and comprehensive assessment of genetic diversity and to develop cultivar-specific markers in Iranian bread wheat germplasm. However, the resulting groups classified by ISSRs already can be used as sources of genotypes appropriate for breeding programs in wheat.

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References

Abdollahi Mandoulakani B, Shahnejat-Bushehri AA, Sayed Tabatabaei BE, Torabi S, Mohammadi Hajiabad A (2010). Genetic diversity among wheat cultivars using molecular markers. J Crop Improv 24:299-309.

Altintas S, Toklu F, Kafkas S, Kilian B, Brandolini A, Ozkan H (2008). Estimating genetic diversity in durum and bread

wheat cultivars from Turkey using AFLP and SAMPL markers. Plant Breed 127:9-14.

Archak S, Gaikwad AB, Gautam D, Rao EV, Swamy KR, Karihaloo JL (2003). Comparative assessment of DNA fingerprinting techniques (RAPD, ISSR and AFLP) for genetic analysis of cashew (*Anacardium occidentale* L.) accessions of India. Genome 3:362-369.

Blair MW, Panaud O, McCouch SR (1999). Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). Theor Appl Genet 98:780-792.

Bornet B, Branchard M (2001). Nonanchored inter simple sequence repeat (ISSR) markers: reproducible genome fingerprinting. Plant Mol Biol Rep 19:209-215.

Briggle LW, Curtis BC (1987). Wheat Worldwide. In "Wheat and Wheat Improvement", EG Heyne, ed Ed. 2nd. American Society of Agronomy Inc. Publishers, Madison, Wisconsin, 31 p.

Carvalho A, Guedes-Pinto H, Martin-Lopes, Lima-Brito P (2010). Genetic variability of old Portuguese bread wheat cultivar assayed by IRAP and REMAP markers. Ann Appl Biol 156:337-345.

Doyle JJ, Doyle JL (1990). Isolation of plant DNA from fresh tissue. Focus 12:13-15.

Dreisigacker S, Zhang P, Warburton ML, Skovmand D, Hoisington D, Melchinger AE (2005). Genetic diversity among and within CIMMYT wheat landrace accessions investigated with SSRs and implications for plant genetic resources management. Crop Sci 45:653-661.

Golkar P, Arzani A, Rezaei AM (2011). Genetic variation in safflower (*Carthamus tinctorious* L.) for seed quality-related traits and inter-simple sequence repeat (ISSR) markers. Int J Mol Sci 12:2664-2677.

Gupta PK, Balyan HS, Edwards KJ, Isaac P, Korzun V, Röder M, Gautier MF, Joudrier P, Schlatter AR, Dubcovsky J, De la Pena RC, Khairallah M, Penner G, Hayden MJ, Sharp P, Keller B, Wang RCC, Hardouin JP, Jack P (2002). Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. Theor Appl Genet 105:413-422.

Hou YC, Yan ZH,Wei YM, Zheng YL (2005). Genetic diversity in barley from west China based on RAPD and ISSR analysis. Barley Gen Newsletter 35:9-22.

Khalighi M, Arzani A, Poursiahbidi MM (2008). Assessment of genetic diversity in *Triticum* spp. and *Aegilops* spp. using AFLP markers. Afr J Biotech 7:546-552.

Lynch M, Milligan BG (1994). Analysis of population genetic structure with RAPD markers. Mol Ecol 3:91-99.

Mohammadi SA, Khodarahmi M, Jamalirad S, Jalal Kamali MR (2009). Genetic diversity in a collection of old and new bread wheat cultivars from Iran as revealed by simple sequence repeat-based analysis. Ann Appl Biol 154:67-76.

Myśków B, Milczarski P, Masojć P (2011). Comparison of RAPD, ISSR and SSR markers in assessing genetic diversity among rye (*Secale cereale* L.) inbred lines. Plant Breed Seed Sci 62:107-115.

Nagaoka T, Ogihara Y (1997). Applicability of inter-simple sequence repeat markers in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor Appl Genet 94:597-602.

Najaphy A, Ashrafi Parchin R, Farshadfar E (2012). Comparison of phenotypic and molecular characterizations of some important wheat cultivars and advanced breeding lines. Aust J Crop Sci 2:326-332.

Peakall R, Smouse PE (2006). GenAlEx 6: Genetic analysis in excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288-295.

Pradeep Reddy M, Sarla N, Siddiq EA (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica 128:9-17.

Rohlf FJ (2000). NTSYS-pc: numerical taxonomy and multivariate analysis system, version 2.1. Exeter Software, New York.

Roussel V, Koenig J, Beckert M, Balfourier F (2004). Molecular diversity in French bread wheat accessions related to temporal trends and breeding programs. Theor Appl Genet 108:920-930.

Roussel V, Leisova L, Exbrayat F, Stehno Z, Balfourier F (2005). SSR allelic diversity changes in 480 European bread wheat varieties released from 1840 to 2000. Theor Appl Genet 111:162-170.

Shoaib A, Arabi MIE (2006). Genetic diversity among Syrian cultivated and landraces wheat revealed by AFLP markers. Genet Resour Crop Evol 53:901-906.

Smolik M, Ochmian I, Smolik B (2011). RAPD and ISSR Methods Used for Fingerprinting Selected, Closely Related Cultivars of *Aronia melanocarpa*. Not Bot Horti Agrobo 39(2):276-284.

Sofalian O, Chaparzadeh N, Dolati M (2009). Genetic diversity in spring wheat landraces from Northwest of Iran assessed by ISSR markers. Not Bot Horti Agrobo 37(2):252-256.

Talbert LE, Blake NK, Chee PW, Blake TK, Magyar GM (1994). Evaluation of sequence-tagged-site-facilitated PCR products as molecular markers in wheat. Theor Appl Genet 87:789-794.

Terzopoulos PJ, Bebeli PJ (2008). Genetic diversity analysis of Mediterranean faba bean (*Vicia faba* L.) with ISSR markers. Field Crops Res 108:39-44.

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