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IRAP and REMAP-Based Genetic Diversity among Iranian, Turkish, and International Durum Wheat (*Triticum turgidum* L.) Cultivars

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ABSTRACT

Retrotransposons (RTNs) are a major source of genomic changes in plant genomes and, therefore, are extensively used as ideal molecular markers for genetic variability, DNA fingerprinting, and genetic mapping studies in plant species. In the present study, two RTN-based marker systems, inter-retrotransposon amplified polymorphisms (IRAPs), and the retrotransposon-microsatellite amplified polymorphisms (REMAPs) were used to assess genetic variability and structure in a collection of 94 durum wheat genotypes. In general, 63 and 141 loci were amplified using 6 IRAP and 15 REMAP primers, respectively. Percentage of polymorphic loci (PPL) in the studied collection for IRAP and REMAP markers were 47.15% and 47.81%, respectively. The average of expected heterozygosity (He), number of effective alleles (Ne), and Shannon's information index (I), separately estimated based on IRAP and REMAP data, were not considerably different. A model-based Bayesian method and cluster analysis using Neighbor joining (NJ) algorithm depicted five clusters. A moderate level of inter-group genetic variability was detected among the clusters (11%) obtained from STRUCTUR software (PhiPT =0.111; *P*=0.001) with the vast majority of variation (89%) still uncaptured within groups. Most of the accessions and landraces from Iran aggregated together in clusters I and III with the cultivars from Turkey. Also, Iranian and foreign durum wheat landraces were assigned to different clusters or subpopulations in both clustering methods. In conclusion, the results showed that the genetic diversity of Iranian durum wheat is low and it is necessary to extend the genetic base of durum wheat germplasm in Iran.

Keywords: Genetic diversity, Model-based cluster, LTR Retrotransposon, *Triticum turgidum* L.

INTRODUCTION

Durum wheat (*Triticum turgidum* L.) is a tetraploid species with an estimated 1C genome size of about 13,000 Mbp. It consists of A and B genomes (AABB) (Peng *et al*., 2011), evolved from domesticated wild emmer wheat (*T. dicoccoides* L.) and derived from a spontaneous cross between *T. urartu* L. (AA genome, 2n=14) and a B-genome diploid related to *Aegilops speltoides* (2n=14, donor of the B genome). Durum wheat originated in the Eastern Mediterranean and has been cultivated in

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this region for the past 12 thousand years (Key, 2005). Durum wheat is an important small-grain cereal with a yellow endosperm and high protein content, and it is used predominantly for production of pasta, couscous, bourghul, and other semolina foods (Mardi *et al*., 2011). Recently, more research attention has been dedicated to durum wheat because of its valuable production and better adaptation to low-rainfall semi-arid environments than common wheat, and its unique end products (Martos *et al*., 2005). Iranian durum wheat traditional germplasm includes a large number of unexploited landraces, which represent a potential treasure trove of genetic diversity. This high variation is important for improvement of various traits in durum wheat (Mardi *et al*., 2011). However, the wonders of breeding targeted at traits improvement has undeniably resulted in the erosion of genetic diversity of the elite germplasm, hindering further developments (Zaim *et al*., 2017).

The accurate estimation of genetic variation in a germplasm is crucial for survival, evolution, effective conservation, and more-efficient utilization of genetic resources in crop improvement programs (Laurentin, 2009; Kabbaj *et al*., 2017). Therefore, assessment of genetic diversity in durum wheat germplasm provides fundamental and useful information to broaden the genetic variation in future breeding programs. Traditionally, genetic variation analyses relied on morphological and phenotypic markers, but these markers have been restricted to a few phenotypic traits, strongly affected by environmental conditions and exhibiting little variation, especially for highly heritable traits (Rao, 2004). Currently, DNA-based molecular markers have become the most effective tool and feasible method for assessment of genetic diversity and structures in a plant collection, because they can overcome many of the limitations associated with phenotypic-based diversity analysis, are plentiful, and allow cultivar identification at early stages of plant development (Abouzied *et al*., 2013).

The ubiquitous nature of retrotransposons (RTNs), and their abundance in plant genomes makes them an ideal source of variation to be tagged via molecular markers (Kalendar *et al*., 1999; Nasri *et al*., 2013; Gholamzadeh Khoei *et al*., 2015). RTN insertional sites that are shared between germplasm accessions have a high probability of being present in their last common ancestor. Therefore, RTN insertional polymorphisms and dynamic feature can be used to determine pedigrees, phylogenies, genome evolution and speciation, and can serve as biodiversity indicators (Schulman *et al*., 2004; Bento *et al*., 2008). In recent years, various RTN-based molecular markers such as inter retrotransposon amplified polymorphism (IRAP) and retrotransposon microsatellite amplified polymorphism (REMAP) have been developed, which principally rely on tagging the unique joints formed between the RTN and the genomic DNA during the integration process (Kalendar *et al*., 1999). The IRAP method displays insertional polymorphisms by amplifying the DNA segments between two nearby RTNs using outward-facing primers. In REMAP, amplification between retrotransposons proximal to simple sequence repeats (SSRs) produces the marker bands (Kalendar and Schulman, 2006). IRAP and REMAP markers have been used for assessing the genetic diversity and genetic mapping of several plant genera and species, including wild almond (Sorkheh *et al*., 2017), *Linum usitatissimum* (Smykal *et al*., 2011; Abbasi Holasou *et al*., 2016), *Medicago sativa* L. (Abdollahi Mandoulakani *et al*., 2012; Abdollahi Mandoulakani *et al*., 2015b), Wheat (Abdollahi Mandoulakani *et al*., 2014; Nasri *et al*., 2013) and melon (Gholamzadeh Khoei *et al*., 2015; Abdollahi Mandoulakani *et al*., 2015c). Direct comparisons of IRAP and REMAP markers with other molecular markers in various crop plants have demonstrated the informativity, high polymorphism,

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reproducibility and their lower cost. However, similar to any other marker systems, IRAP and REMAP markers have own properties and some disadvantages, including the lack of information on the tagged genome sequence, which in turn hinders the possibility of deriving more targeted primers. The amplifications are dependent on the number and distance of RTN copies in the genome, and they are mostly dominant in nature (Ferreira Santana *et al*., 2012).

The objective of this study was to develop IRAP and REMAP markers in durum wheat to assess genetic structure and variability among accessions, cultivars, landraces and inbred lines of durum wheat with the aim of using them in breeding programs as well as for conservation management of this germplasm.

MATERIALS AND METHODS

Plant Materials and DNA Extraction

Ninety-four durum wheat genotypes collected from different locations in Iran, as well as from other countries including Turkey, Italy, and Syria, were analyzed (Table 1). Plant materials were kindly provided by the Dry Land Agricultural Research Institute (DARI, Maragheh, Iran) and National Plant Gene Bank of Iran (NPGBI, Karaj, Iran). Genomic DNA was extracted from young leaves of 20-day seedling (Ausubel *et al*., 1995). The quality and concentration of the DNA were measured using a spectrophotometer and electrophoresis in a 0.8% (w/v) agarose gel.

IRAP and REMAP Reactions

Ten single and 15 IRAP primer combinations (Tables 2 and 3) were used to analyze genetic diversity and RTN integration events in 94 durum wheat genotypes. The primers were tested on six durum wheat genotypes to choose those providing scorable and clear banding patterns. PCR amplifications of IRAP reactions were carried out in a Bio-Rad thermo cycler (Bio-Rad Laboratories, Hercules, CA, USA) in a total volume of 20 µL containing 40 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 of each dNTP, 1 U Taq DNA polymerase (SinaClon, Iran), and 10 pmol of each primer. The cycling program was an initial denaturation of 4 min at 94 °C, 36 cycles of 45 s at 94 °C, 40 s at 55-58 °C (Table 3), 2 min at 72 °C followed by a final extension step of 10 min at 72 °C. The PCR products were resolved by electrophoresis (Bio-Rad) using 1.8% Resolute^{TM} line Biozyme agarose gel in 0.5×TBE buffer with constant voltage of 70 V for 3-4 h. Gels were stained by ethidium bromide, then visualized under UV light and photographed using a gel documentation system.

Thirty-five REMAP primer combinations, derived from five single IRAP primers with seven ISSR primers, (Tables 2 and 3) were applied. PCR amplification reactions and temperature profile, electrophoresis and visualization of REMAP markers were as stated for IRAPs, but annealing temperature of REMAP primer combinations varied from 55 °C to 58 °C (Table 3).

Data Analysis

IRAP and REMAP amplified fragments were scored independently as 1 or 0 for their presence or absence at each position, and the binary data obtained were subjected to analysis. Genetic similarity matrices between individual pairs of genotype were calculated for IRAP, REMAP, and IRAP+REMAP data using the number of differences coefficients. These matrices were used to construct dendrograms using Neighbor joining (NJ) algorithm in MEGA 4.0 (Tamura *et al*., 2007). The cophenetic correlation coefficient was calculated to evaluate the adjustment between similarity matrices and respective dendrogram-derived

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Table 1. List of 94 durum wheat breeding lines, accessions, cultivars and landraces, the cluster and subpopulation they fall in based on Neighbor joining and Structure analysis, respectively. [a]

Name	Origin	Source	Cluster#	Subpop#
3615	NPGBI	Accession	3	3
1292	NPGBI	Accession	3	3
3608	NPGBI	Accession	3	3
3699	NPGBI	Accession	3	5
3611	NPGBI	Accession	3	5
3605	NPGBI	Accession	3	5
3652	NPGBI	Accession	3	3
1295	NPGBI	Accession	3	3
3715	NPGBI	Accession	3	3
3811	NPGBI	Accession	3	2
4012	NPGBI	Accession	1	1
3818	NPGBI	Accession	1	1
3822	NPGBI	Accession	1	1
3810	NPGBI	Accession	3	3
908	NPGBI	Accession	4	2
4025	NPGBI	Accession	4	5
3711	NPGBI	Accession	4	5
3791	NPGBI	Accession	3	3
3827	NPGBI	Accession	3	3
Kc-17307	NPGBI	Accession	1	1
Kc-173333	NPGBI	Accession	3	3
Kc-17395	NPGBI	Accession	5	2
Kc-9257	NPGBI	Accession	5	3
Kc-17278	NPGBI	Accession	1	2
Kc-17493	NPGBI	Accession	1	4
Kc-17399	NPGBI	Accession	3	3
Kc-17462	NPGBI	Accession	5	3
Kc-17369	NPGBI	Accession	2	3
Kc-9955	NPGBI	Accession	4	5
Kc-17366	NPGBI	Accession	3	4
Kc-17495	NPGBI	Accession	1	4
Kc-17430	NPGBI	Accession	1	4
Kc-17149	NPGBI	Accession	1	4
Kc-17362	NPGBI	Accession	1	1
Kc-1740	NPGBI	Accession	1	4
Kc-1744	NPGBI	Accession	1	2
Kc-1752	NPGBI	Accession	1	4
Kc-1732	NPGBI	Accession	1	4
Kc-17342	NPGBI	Accession	2	3
Haran-95	Turkey	Cultivar	1	2
Turabi	Turkey	Cultivar	1	2
Yelken 2000	Turkey	Cultivar	4	5
Tunca-179	Turkey	Cultivar	3	5
Sahinbey	Turkey	Cultivar	3	3
Selcuklu-97	Turkey	Cultivar	2	2
Gap	Turkey	Cultivar	2	2

[a]*NPGBI* National Plant Gene Bank of Iran, *DARI* Dry Land Agricultural Research Institute, *ICARDA* International Center for Agricultural Research in the Dry Areas.

Table 1 continued ….

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Continued of Table 1. List of 94 durum wheat breeding lines, accessions, cultivars and landraces, the cluster and subpopulation they fall in based on Neighbor joining and Structure analysis, respectively. [a]

Name	Origin	Source	Cluster#	Subpop#
Berekmen-469	Turkey	Cultivar	4	5
Sarayollo	Turkey	Cultivar	5	2
Kizeltan-91	Turkey	Cultivar	5	3
Durbil	Turkey	Cultivar	5	3
Zenit	Turkey	Cultivar	4	5
Gokgol-79	Turkey	Cultivar	3	3
Eminbey	Turkey	Cultivar	1	1
Mirzabey-200	Turkey	Cultivar	2	2
Kunduru-1149	Turkey	Cultivar	3	3
Meram-2002	Turkey	Cultivar	3	3
Pinar-2001	Turkey	Cultivar	4	5
Chakmak-79	Turkey	Cultivar	3	3
Solen-2002	Turkey	Cultivar	5	2
Kunduru-414	Turkey	Cultivar	1	4
Lmren	Turkey	Cultivar	2	1
Geromtel-1	Turkey	Cultivar	2	2
Svevo	Turkey	Cultivar	4	5
Ankara-98	Turkey	Cultivar	5	5
Aydin-93	Turkey	Cultivar	5	3
Chesit-1252	Turkey	Cultivar	1	1
Saji	DARI	Cultivar	2	3
Sardari	DARI	Cultivar	1	1
Gredish	DARI	Cultivar	2	1
Chehel daneh	DARI	Cultivar	1	2
Zardak	DARI	Cultivar	3	3
Haurani	Syria	Cultivar	3	3
Wc-378	Ardabil (Moghan)	Landrace	1	4
Tn-12726	Ardabil	Landrace	3	3
Tn-12722	Ardabil (Moghan)	Landrace	4	5
Kc-1477	Lorestan (Shoshtar)	Landrace	1	4
Kc-950	Lorestan (Khoramabad)	Landrace	3	3
Kc-963	Lorestan (Khoramabad)	Landrace	3	3
Kc-678	Lorestan (Khoramabad)	Landrace	3	4
Tn-12715	Khuzestan (Dezful)	Landrace	5	5
Tn-12668	Khuzestan (Izeh)	Landrace	3	4
Kc-3654	Kermanshah	Landrace	3	3
Kc-3638	Kermanshah	Landrace	4	5
Tn-12501	Kermanshah	Landrace	4	5
Kc-3653	Kermanshah	Landrace	3	3
Tn-12595	Kermanshah	Landrace	1	4
P.s.no8	Italy	Landrace	4	5
P.s.no5	Italy	Landrace	4	5
Kunduru	Turkey	Line	1	1
Bcr/grol//mgn1/1	-	Line	3	3
Amar	DARI	Line	4	3
Pgs	ICARDA	Line	4	3
Ter/1//nrf1/st/	ICARDA	Line	3	3
61-30	ICARDA	Line	4	5

[a]*NPGBI* National Plant Gene Bank of Iran, *DARI* Dry Land Agricultural Research Institute, *ICARDA* International Center for Agricultural Research in the Dry Areas.

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Table 2. Name and sequences of the primers used. [a]

Primer	Sequence (5'-3')	Primer	Sequence (5'-3')
Bare1 (RTN)	ggaattcatagcatggataataaacgattatc	UBC811 (ISSR)	gagagagagagagagaac
Sukkula (RTN)	gatagaatcgcatcttgggcgtgac	UBC857 (ISSR)	acacacacacacacacYg
LTR725 (RTN)	gatagaatcgcatcttgggcgtgac	UBC840 (ISSR)	gagagagagagagagaYt
LTR2105 (RTN)	Actccatagatggatcttggtga	UBC8848 (ISSR)	cacacacacacacacaRg
LTR455 (RTN)	Ttgaatttctgctacgttcccc	UBC820 (ISSR)	Gtgtgtgtgtgtgtgtc
A12 (ISSR)	Gagagagagagacc	UBC855 (ISSR)	acacacacacacacacYt

[a] *Y* pyrimidine (C/T), *R* purine (A/G)

Table 3. Characteristics of the used IRAP and REMAP primers in 94 durum wheat genotypes. [a]

Primer name	Tm	TL	PL	PPL	He	Ne	I	BS (bp)
IRAP								
Sukkula	55	10	4	40	0.16	1.28	0.24	400-1500
LTR2105	55	12	7	58.33	0.26	1.40	0.37	400-1500
LTR725	55	10	5	50	0.21	1.37	0.30	300-1400
Bare1-Sukkula	56	10	5	50	0.23	1.42	0.32	300-2000
LTR455-Sukkula	58	11	6	54.54	0.22	1.38	0.32	300-2000
LTR725-Sukkula	55	10	3	30	0.13	1.25	0.19	200-1000
Total		63	30					
Average		10.5	5	47.15	0.20	1.35	0.29	
REMAP								
LTR725-A12	55	4	0	0	0	1	0	300-500
Sukkula-UBC811	55	12	4	33.34	0.15	1.12	0.22	300-1000
LTR725-UBC811	58	8	4	50	0.20	1.36	0.30	300-2000
LTR455-UBC811	58	9	5	55.56	0.23	1.40	0.34	300-700
LTR725-UBC857	56	10	5	50	0.21	1.37	0.31	300-2000
Bare1-UBC840	58	8	5	62.5	0.29	1.54	0.40	500-2000
LTR725-UBC848	58	10	6	60	0.25	1.43	0.30	300-2000
LTR2105-UBC840	55	11	7	63.63	0.27	1.48	0.39	500-1500
LTR2105-UBC848	55	11	6	54.54	0.23	1.39	0.33	100-2000
LTR2105-UBC857	55	11	6	54.54	0.23	1.30	0.28	200-2000
Bare1-UBC811	57	14	9	64.28	0.23	1.06	0.32	100-800
LTR725-UBC820	58	7	2	28.57	0.11	1.19	0.17	200-2000
LTR725-UBC855	55	9	4	44.44	0.20	1.37	0.29	200-1500
LTR2105-A12	56	8	5	62.5	0.29	1.53	0.41	500-2000
LTR2105-UBC855	55	9	3	33.33	0.15	1.27	0.21	300-1700
Total		141	71					
Average		9.4	4.7	47.81	0.20	1.32	0.28	

[a] *TM* Annealing temperature, *TL* Total loci, *PL* Polymorphic loci, *PPL* Percentage of polymorphic loci, *He* Mean of expected heterozygosity, *Ne* Number of effective alleles, *I* Shannon's information index, *BS* Band size.

matrices (cophenetic matrix). To estimate the degree of association among the three cophenetic matrices obtained from IRAP, REMAP and combined data, Mantel test was implemented in NTSYSpc (Rohlf, 2000).

Population structure was analyzed using a Bayesian clustering approach implemented in Structure 2.3.4 (Pritchard *et al*., 2000). Ten independent replicates were performed setting the number of subpopulations (k) from 1 to 20, burn in period and MCMC iterations, both to 100,000 and an admixture model and correlated allele frequencies. The optimal K value (as true cluster number) was

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estimated by the posterior probability [ln P(D)] and an ad hoc statistic ΔK based on the rate of change in [ln P(D)] between successive K (Evanno *et al*., 2005) using the software Structure Harvester (Figure 1). Inferred ancestry estimates of genotypes (Q-matrix) were derived for the selected subpopulation (Pritchard *et al*., 2000). The genetic differentiation among STRUCTURE inferred groups was also estimated using analysis of molecular variance (AMOVA) as implemented in GenAlEx 6.4 (Peakall and Smouse, 2006). The number of loci, percentage of polymorphic loci (PPL), mean of expect heterozygosity (He), number of effective alleles (Ne), Shannon's information index (I) and standard error of mean heterozygosity were calculated using the same software for the subpopulations derived from STRUCTURE analysis.

RESULTS

IRAP Analysis

Among the 25 IRAP primers tested, three single (Sukkula, LTR725 and LTR2105) and three IRAP primer combinations (Bare1-Sukkula, LTR455-Sukkula and LTR725-Sukkula) (Table 3) generated scorable and polymorphic banding profiles among the 94 durum wheat genotypes. Six IRAP primers generated a total of 63 loci, of which 30 were polymorphic (47.15%). Length of the amplified fragments ranged from 200 to

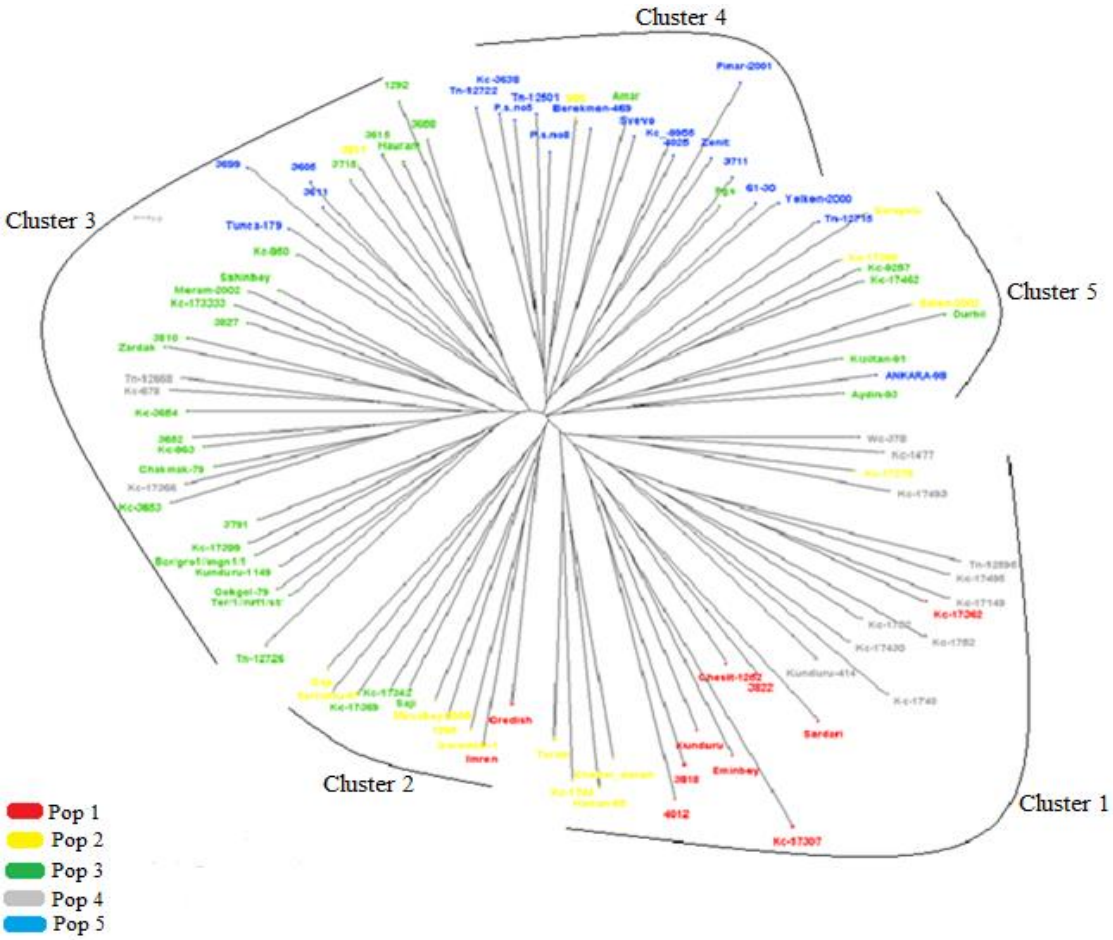

Figure. 1 Neighbor joining dendrogram of 94 durum wheat genotypes based on IRAP+REMAP markers, colored individuals (red, yellow, green, black, blue) corresponds to the subpopulations derived from Bayesian-based clustering using Structure software.

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2,000 bp (Table 3). Primer LTR2105, generated the maximum number of amplified and polymorphic loci, and showed the highest PPL, He, and I. The lowest values of these parameters were achieved for the primer combination LTR725-Sukkula. The average of polymorphic loci was five per primer.

REMAP Analysis

Fifteen primers, out of 35 REMAP primers tested, produced 141 distinguishable and scorable loci, of which 71 (47%) were polymorphic. All single retroelement-based primers generated clear and polymorphic banding patterns in combination with ISSR primers. The size of amplified loci varied from 100 to 2000 bp (Table 3). Primer combination LTR725-A12 produced monomorphic banding pattern. Primer combination Bare1-UBC811 amplified the highest number of loci (14) and the maximum amount of PPL (Table 3). The average of REMAP polymorphic loci was 4.7 per primer.

Combined Data Analysis

To compare the efficiency of IRAP and REMAP markers, parameters Ne, I, He and PPL were separately calculated for IRAP, REMAP, and IRAP+REMAP markers. No remarkable differences were detected between the two marker classes, although the calculated parameters for REMAP markers were slightly more than those of IRAPs (Table 5). Across all analyzed datasets of durum wheat (accession, landraces and cultivars), the mean values of the genetic variation (He), number of effective alleles (Ne), Shannon's information index (I) and percentage of polymorphic loci (PPL) demonstrated no considerable genetic variation within each dataset (Table 6). Furthermore, little differences among accessions, cultivars, and landraces were observed in terms of these parameters (Table 6). The landraces had the mean He of 0.199, while the accessions and cultivars had the mean He of 0.211 and 0.213, respectively (Table 6).

Cophenetic matrices of IRAP and REMAP markers were significantly correlated with the IRAP+REMAP data, but Mantel test between IRAP and REMAP cophenetic matrices evidenced no significant correlation (r=0.059). Hence, IRAP+REMAP markers were used to construct a NJ dendrogram, which resulted in the identification of five major groups among the tested germplasm (Figure 2). In each cluster, genotypes with different geographical origin were observed. Clusters I and III were dominated by the accessions

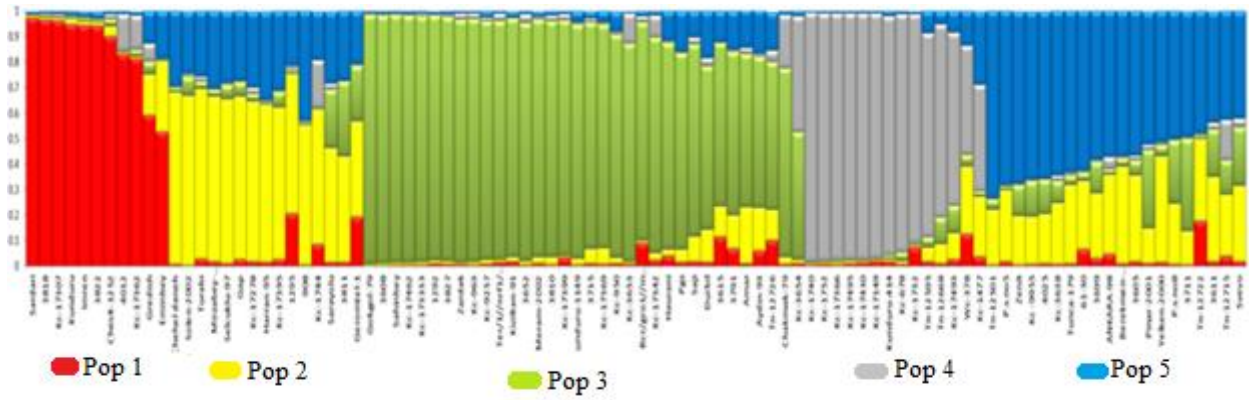

Figure. 2 A Bayesian model-based clustering of the 94 durum wheat genotypes. Bar plots show the membership coefficient estimate (Q) for each genotype for the inferred clusters with maximum log-likelihood probability. Bar colors and lengths represent inferred clusters and Q, respectively, identified by Structure for K=5.

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originating from NPGBI, and consisted of the maximum number of genotypes. In addition, two lines, namely, Ter/1//nrf1/st/ and Bcr/grol//mgn1/L were classified together in clusters III. Other lines (except Kunduru) were classified in cluster IV. Genotypes KC-17430, KC-1740, KC-1732, KC-1752, KC-17495 and KC-17149, with the same geographical origin (NPGBI) grouped together in cluster I. Cluster II consisted of seven cultivars from Turkey and DARI and two accessions from NPGBI (Figure 2).

A model-based Bayesian approach (Figure 3) approximately confirmed the results obtained by NJ cluster analysis, although there were some discrepancies between the two classification methods. Sub-populations II and V had the maximum numbers of admixture samples. Subpopulation I contained five accessions, five cultivars, and one line. Subpopulation II consisted of nine cultivars, mostly from Turkey, and six accessions from NPGBI. Genotypes with different geographical origin (NPGBI, Turkey, Iran and ICARDA) constituted Subpopulation III. Also, most lines were classified together in this subpopulation. Eight accessions from NPGBI and five landraces from Iran, along with Kunduru-414 (cultivar) from Turkey were in subpopulation IV. Subpopulation V contained six accessions from NPGBI, seven cultivars from Turkey, one line from ICARDA, and 6 landraces from Iran and Italy. Subpopulation III exhibited the highest level of variability (He=0.439, I=0.626), whereas subpopulation I exhibited the lowest level (He=0.322, I=0.482). AMOVA indicated that grouping into five sub-populations captured 11% of the total genetic variation (PhiPT =0.111; P=0.001), while the largest portion (89%) remained uncaptured within groups, supporting the existence of potentially more sub-populations within the studied collection.

DISCUSSION

RTN Insertional Polymorphism in Durum Wheat Genome

Due to their ability to spread into a genome by self-duplication, transposable elements may affect the adaptation and evolutionary potential of their hosts through events such as insertion mutation, gene interruption, increment of gene expression, and chromosomal rearrangements (Huan-Van *et al*., 2005). Therefore, transposable element-based marker systems could

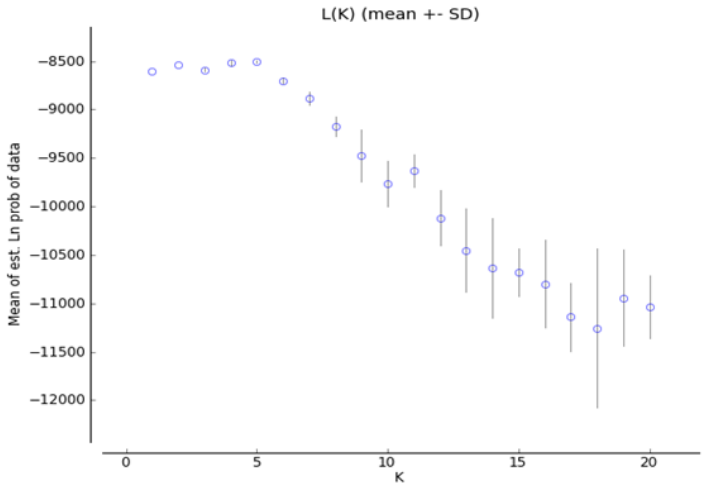

Figure. 3 Estimation of the most probable number of clusters (K), based on five independent runs and K ranging from 1 to 20.

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provide authentic and reliable information regarding genotype identification and performances. In this study, 6 IRAP and 15 REMAP primers were shown to amplify polymorphic and discernible banding patterns and were used to study genetic diversity among 94 durum wheat accessions, cultivars, landraces and breeding lines. Single IRAP primers LTR2105, LTR725, and Sukkula generated scorable banding patterns, indicating the presence and insertional activity of these elements in durum wheat genome (Table 4). The multiplicity of the bands of IRAP primer LTR2105 supports the idea that the LTR families tend to form local clusters in the genome of durum wheat (Vicient *et al*., 1999). Primers Bare1 and LTR455 amplified no bands as single IRAP primers, but they amplified bands in IRAP reaction when combined with Sukkula primer. This might reflect the insertion of Bare1 and LTR455 in or near Sukkula elements in durum wheat genome. The insertion of Bare1 near the Nikita, Sukkula and other RTNs has been previously observed in the genome of Portuguese durum and breed wheat cultivars (Carvalho *et al*., 2010; Carvalho *et al*., 2012). Therefore, the wide activity of RTN families across the grasses may be a general phenomenon. The applicability of the barley RTNs for genome analysis in the genera *Aegilops*, *Hordeum*, and *Triticum* has been previously demonstrated (Kalendar *et al*., 1999;; Nasri *et al*., 2013; Sen *et al*., 2017). Vicient *et al*. (1999) indicated that grasses share transcriptionally, translationally, and insertionally active RTN families. Our study showed that Bare1 and Sukkula, have relatives in durum wheat genome and are transpositionally active, as evidenced earlier (Carvalho *et al*., 2010). Sukkula was present in all IRAP primer combinations generating discernible banding pattern, indicating its high frequency and activity as well as a possible role of this RTN family in the construction, organization, and evolution of the durum wheat genome. This result indicates that active retrotransposon families (including Sukkula) have the potential to be major contributors to variability in genome size. Primer LTR455, did not produce bands as a single primer in IRAP reactions, but this primer amplified bands in REMAP reactions, suggesting their presence in the durum wheat genome as solo LTRs and their preferential integration near microsatellite motifs. Most of the RTNs used here generated bands in REMAP reactions, showing their insertion near or in SSR motifs. In the present study, both techniques produced enough polymorphic bands for genetic diversity analysis; however, REMAP markers, particularly primer combinations Bare1-UBC811, produced a high number of fragments (Table 3).

Genetic Relationship among Durum Genotypes

The measured correlations between the three generated cophenetic matrices from IRAP, REMAP, and IRAP+REMAP dendrogram evidenced a relatively high and significant congruence of IRAP and REMAP with IRAP+REMAP. However, the matrices estimated by the techniques individually depicted a low and non-significant correlation (r=0.059). These results are similar to findings in barley (Kalendar *et al*., 1999), rice (Branco *et al*., 2007), alfalfa (Abdollahi Mandoulakani *et al*., 2012) and wheat (Nasri *et al*., 2013). Since REMAP primers amplified DNA regions that could not be covered by IRAP, IRAP+REMAP data were used to reveal the phylogeny between the studied germplasm. Using 204 amplified IRAP+REMAP loci and cluster analysis based on NJ algorithm, five groups were identified among 94 genotypes (Figure 2). The highest cophenetic correlation coefficient (r=0.73) supports that this dendrogram is a good representation of our IRAP+REMAP data. The population structure and relationship among individual genotypes were analyzed using Bayesian model-based clustering as well (Figure 3). The results showed the highest peak at k=5 (Figure 1), suggesting

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that the analyzed durum germplasm can be divided into 5 genetically distinct groups and confirming the results obtained with NJ clustering method. Most of the accessions and landraces from Iran aggregated together in clusters I and III with the cultivars from Turkey. Genotypes located in subpopulations I, III and V were less scattered in NJ dendrogram than the accessions located in subpopulations II and IV. Iranian and foreign durum wheat landraces were assigned to different clusters or subpopulations in both clustering methods. In addition, most of the lines were located in subpopulation III.

In the present study, we compared the genetic diversity of durum wheat accessions, landraces, cultivars, and breeding lines originating from Iran and some other countries around the world. The low level of genetic variation detected (I=0.31, He=0.21, Ne=1.39) might be ascribed to the self-pollinating nature of durum wheat and low insertional activity of the used RTNs, or the creation of diversity bottleneck through breeding selection and evaluation. Mardi et al. (2011) stated that genetic diversity of Iranian genotypes was higher compared to the foreign entries. They indicated that Iranian landraces had the mean He of 0.28 and an average of 7.2 alleles per locus, while the Iranian traditional and modern improved cultivars had the mean He of 0.27 and 0.26 and 0.26 and an average of 7.1 and 6.7 alleles per locus, respectively. Additionally, these authors explained that Iranian durum wheat landraces might be a good source of genetic variability, to be explored in crosses

Table 4. Polymorphism comparison of retrotransposon families in durum wheat germplasm. [a]

Retrotransposon family	TL	PL	PPL	He	Ne	I
Sukkula	53	22	42%	0.18	1.29	0.26
LTR2105	62	34	55%	0.24	1.40	0.33
LTR725	68	29	43	0.16	1.29	0.23
Bare1	32	19	59	0.25	1.34	0.34
LTR455	20	11	55	0.22	1.39	0.33

[a] *TL* Total loci, *PL* Polymorphic loci, *PPL* Percentage of polymorphic loci, *He* Mean of expected heterozygosity, *Ne* Number of effective alleles, *I* Shannon's information index

Table 5. Comparison of genetic diversity indices derived from IRAP, REMAP and IRAP+REMAP data in durum wheat germplasm. [a]

Molecular Markers	I (SE)	He (SE)	Ne (SE)	PPL (%)
IRAP	0.30 (0.04)	0.21 (0.028)	1.37 (0.051)	47.62
REMAP	0.32 (0.027)	0.22 (0.019)	1.39 (0.034)	50.35
IRAP+REMAP	0.31 (0.022)	0.21 (0.015)	1.39 (0.028)	49.51

[a] *I* Shannon's information index, *SE* Standard error, *He* Mean of expected heterozygosity, *Ne* Number of effective alleles, *PPL* Percentage of polymorphic Loci.

Table 6. Comparison of detected polymorphism among accessions, cultivars and landraces. [a]

Genotype	I (SE)	He (SE)	Ne (SE)	PPL (%)
Accession	0.30 (0.022)	0.211 (0.015)	1.37 (0.028)	49.51
Cultivar	0.31 (0.022)	0.213 (0.015)	1.38 (0.029)	49.51
Landrace	0.29 (0.022)	0.199 (0.015)	1.36 (0.028)	47.06
Total	0.31 (0.022)	0.215 (0.015)	1.39 (0.028)	49.51

[a] *I* Shannon's information index, *SE* Standard error, *He* Mean of expected heterozygosity, *Ne* Number of effective alleles, *PPL* Percentage of polymorphic Loci.

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with elite durum wheat germplasm. Nasri et al. (2013), in a study of genetic diversity among 101 Iranian bread wheat cultivars and breeding lines, reported instead a low level of genetic variation (I=0.5, He=0.34, and Dice similarity coefficient=0.8). In our study, the mean of He, I, and Ne values estimated for different groups of studied durum wheat genotypes, decreased from cultivars to accessions and landraces (from 0.213 to 0.211 and 0.20; from 0.31 to 0.30 and 0.29; and from 1.38 to 1.37 and 1.36, respectively) (Table 6). Although genetic variability among populations (11%) represents only a portion of the total variability, it was concluded that the analyzed durum germplasm were genetically structured. Similar results were found by Ren et al. (2013) using SNP markers in accessions of durum wheat, whose analysis of molecular variance revealed that 99.5% of the variation was found within populations and only 0.5% between populations. Loveless and Hamrick (1984) reported that the dispersal of seeds determines the patterns of genes dispersion within and among populations. Low level of variability among population can be explained by the high value of gene flow via germplasm exchanges among different regions. In particular, Iran, Syria, and Turkey have relied on the efforts of ICARDA to derive superior varieties. This might be one of the driver of gene flow between cultivars. Another reason for the low level of variability could be due to the human practices such as seed trading and crossing among Iranian, Syrian, and Turkish cultivars. However, the strong selection pressure imposed through genetic improvement has eroded a large part of the genetic diversity available to breeders.

In conclusion, the results suggest that IRAP and REMAP markers provide powerful tools to study genetic relationships among durum wheat accessions, cultivars, landraces, and lines. Our results showed that the genetic diversity of Iranian durum wheat is low and it is necessary to extend the genetic base of durum wheat germplasm in Iran. Introduction of new useful traits from the primitive types, or foreign cultivars and landraces could increase the polymorphism of modern durum wheat varieties, and facilitate future genetic gain. Besides, the use of the strong and complementary statistical methods such as NJ cluster analysis and Baysian methods proved to be useful for determination of genetic relationships among durum wheat genotypes and for definition of the genetic structure of this collection. These data might be very useful in the future for planning wheat breeding programs and defining strategies for germplasm conservation. Knowledge of the population structure has great importance for studies focusing on association mapping as well, which can detect correlations between phenotypes and linked markers on the basis of linkage disequilibrium (Gupta *et al*., 2005).

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تنوع ژنتیکی مبتنی بر نشانگرهای IRAP و REMAP در ژنوتیپ‌های گندم دوروم ایرانی، ترکیه‌ای و بین المللی

ن. مرزنگ، ب. عبدالهی مندولکانی، س. شعف، م. قدیم زاده، ا. برنوسی، ح. عباسی هولاسو، و ب. صادق زاده

چکیده

رتروترنسپوزون‌ها یک منبع عمده‌ای از تغییرات ژنومی در ژنوم‌های گیاهی می‌باشند و بنابراین به عنوان نشانگرهای مولکولی ایده‌آل جهت مطالعه تنوع ژنتیکی، انگشت‌نگاری DNA و مکان‌یابی ژنتیکی در گونه‌های گیاهی بشمار می‌روند. در این مطالعه دو سیستم نشانگری مبتنی بر رتروترنسپوزون‌ها (IRAP و REMAP) برای مطالعه تنوع ژنتیکی و تعیین ساختار ژنتیکی ۹۴ ژنوتیپ گندم دوروم مورد استفاده قرار گرفت. در کل با استفاده از ۶ آغازگر IRAP و ۱۵ آغازگر REMAP به ترتیب ۶۳ و ۱۴۱ مکان تکثیر شد. درصد چندشکلی برای آغازگرهای IRAP و REMAP در ژرم پلاسم مطالعه شده به ترتیب ۴۷/۱۵ و ۴۷/۸۱ درصد بود. بین میانگین هتروزیگوسیتی مورد انتظار (He)، تعداد الل‌های مؤثر (Ne) و شاخص شانن (I) محاسبه شده بر اساس نشانگرهای IRAP و REMAP اختلاف قابل توجهی مشاهده نشد. گروهبندی مبتنی بر هر دو روش بیزین و تجزیه خوشه‌ای بر مبنای الگوریتم Neighbor joining (NJ)، ژنوتیپ‌های مورد مطالعه را در پنج گروه قرار داد. تنوع ژنتیکی قابل ملاحضه‌ای (۱۱ درصد) بین گروه‌های حاصل از تجزیه ساختار ژنتیکی با استفاده از نرم‌افزار STRUCTUR در مقایسه با تنوع درون گروهی (۸۹ درصد) حاصل شد (PhiPT =0.111; P=0.001). بسیاری از نمونه‌ها و ارقام بومی ایرانی و ترکیه‌ای در گروه‌های یک و سه در مجاورت یکدیگر قرار گرفتند. همچنین ارقام بومی ایرانی و خارجی گندم‌های دوروم مطالعه شده بر اساس هر دور روش تجزیه خوشه‌ای در گروه‌ها و زیرجمعیت‌های متفاوتی قرار گرفتند. بطور کلی نتایج این تحقیق نشان داد که تنوع ژنتیکی گندم‌های دوروم ایرانی پایین است و گسترش پایه ژنتیکی ژرم پلاسم گندم دوروم ایران ضروری می‌باشد.

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