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IRAP AND REMAP-BASED ASSESSMENT OF GENETIC DIVERSITY IN CHICKPEA COLLECTION FROM IRAN

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Retrotransposons (RTN) make a significant contribution to the size, organization and genetic diversity of their host genomes. Several RTN families have been identified in chickpea (*Cicer arietinum* L.) and other closely related species. In the current research, integration activity and insertional polymorphism of the RTNs CARE1, Tms1Ret1, TPS and LORE were studied in 64 chickpea accessions collected in Iran using inter retrotransposon amplified polymorphism (IRAP) and retrotransposon-microsatellite amplified polymorphism (REMAP) techniques. Results indicated that all RTNs studied, are transpositionally active in chickpea genome and amplified scorable and polymorphic banding pattern. Among the RTN families used, the highest percentage of polymorphic loci (PPL) was produced by TPS family (81.82%). Totally, 129 loci were amplified using 18 IRAP and REMAP primers which 83 (64.34%) were polymorphic. The Dice genetic similarity coefficients among accessions ranged from 0.84 (accessions Tj48 and Ba4) to 0.98 (accessions Ka30 and Urm61), averaging 0.93. The parameters of expected heterozygosity (He), Shannon's information index (I) and number of effective alleles (Ne) were the highest for Urmia accessions. Cluster analysis based on UPGMA algorithm and Dice similarity coefficient categorized the 64 accessions in 7 main groups. The mean Fst values of all groups except for groups IV and VII, were lower than 0.20, demonstrating no clear differentiation among the groups, no genetic structure of the studied chickpea collection and probably occurrences of gene flow among the origins. In conclusion, although RTN-based markers were able to differentiate the chickpea

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accessions but the measured relative genetic similarity among accessions were not correlated with geographical distances between places of their origins.

Key words: Bayesian analysis, CARE1 retrotransposon, *Cicer arietinum* L., Genetic diversity

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a cool season grain legume with high nutritive value and serves as an important cheap source of protein in developing countries diet in addition to improving land fertility. Chickpea is a diploid plant (2n=2x=16) with an estimated haploid genome size of about 931 Mb (CORAM *et al*., 2007). It is a self-pollinated crop with natural cross-pollination of less than one percent. Chickpea has been cultivated for at least 7000 years and probably originating from the region of southeastern Turkey and adjoining area of Iran (VAN DER MAESEN, 1987). However, chickpea productivity is not enough to fulfill the requirement of an increasing human population. One major reason for the low productivity of cultivated chickpea is its narrow genetic base and its sexual incompatibility with other *Cicer* wild types in natural inter-specific crosses. Therefore, many chickpea breeding programs are focused on improving the genetic potential both to increase yield and to provide protection against abiotic and biotic stresses. In order to enhance the genetic potential, there must be a comprehensive understanding of the amount and pattern of the genetic variation that exists within and between the available cultivated accessions. World germplasm collections of the cultivated chickpea are lacking in diversity that many traits need to be included for effective improvement of the crop (ROBERTSON *et al*., 1997). However, this may be overcome by looking to the new accessions and wild relatives to widen the genetic bases of breeding programs through interspecific hybridization (SINGH and OCAMPO, 1997).

Large eukaryotic genomes are comprised mainly of transposable elements, the bulk of which are the Class I elements or retrotransposons (RTNs). The ubiquity, abundance, dispersion, and dynamism of the LTR (Long terminal repeat) RTNs in plant genomes have made them excellent sources of molecular markers. The methods generally rely on PCR amplification between a conserved RTN feature, most often the LTR, and another abundant, dispersed and conserved feature in the genome. The second site is a restriction site adapter in sequence specific amplified polymorphism (SSAP; WAUGH *et al*., 1997), a microsatellite in retrotransposon-microsatellite amplified polymorphism (REMAP; KALENDAR *et al*., 1999), another RTN in inter retrotransposon amplified polymorphism (IRAP; KALENDAR *et al*., 1999) and a random sequence in RAPD-retrotransposon amplified polymorphism (R-RAP; AALAMI *et al*., 2012). The degree of current activity, epoch of past activity, and speed of clearing from the genome all affect the phylogenetic resolution and genetic diversity obtained from RTN-based markers. The RTN-based markers have been applied successfully for the analysis of genetic diversity and phylogenetic evolution in genera and species as diverse as alfalfa (ABDOLLAHI MANDOULAKANI *et al*., 2012), wheat (NASRI *et al*., 2013), flax (SMYKAL *et al*., 2011), sunflower (VUKICH *et al*., 2009) and grapevine (ONOFRIO *et al*., 2010). Moreover, these molecular markers are used in the construction of genetic maps and the identification of genes, providing insights into plant evolution (MANNINEN *et al*., 2000). Direct comparisons of RTN methods with other marker systems have indicated that the RTN markers are more informative and polymorphic in a variety of crops (TAM *et al*., 2005). A major disadvantage of RTN-based molecular markers is the need for RTN sequence information to design the family-specific primers. However, related species have similar RTN sequences, meaning that primers

designed based on LTR sequences of an RTN can be readily used among closely related genera, and even sometimes between plant families (KALENDAR *et al*., 2011).

A variety of common molecular techniques have been used for measuring genetic variability in chickpea including amplified fragment length polymorphism (AFLP) (NGUYEN *et al*., 2003), random amplified polymorphic DNA (RAPD) (AHMAD *et al*., 2010) and simple sequence repeats (SSR) (SAEED *et al*., 2011). Given the activity of RTNs in driving genome diversification, RTN-based marker methods appear attractive to be used in chickpea. The sequence of the CARE1, a *TY3-gypsy* like LTR-RTN, has been identified in *C. arietinum* (RAJPUT and UPADHYAYA, 2009). Also, several RTN families in legumes closely related to chickpea have been sequenced including TPS family in *Pisum sativum* (PEARCE *et al*., 2000), LORE1 and LORE2 in *Lotus japonicas* (MADSEN *et al*., 2005; FUKAI *et al*., 2008) and Tms1Ret1 in *Medicago sativa* (PORCEDDU *et al*., 2002). To our knowledge, no IRAP and REMAP-based study has been conducted in chickpea. Therefore, the aim of the present study was to develop IRAP and REMAP markers for detecting the integration events and activity of RTN families CARE1, Tms1Ret1, TPS and LORE in *C. arietinum* accessions and to assess the extent of RTN-related variability in a chickpea collection from Iran in order to evaluate the potential application of IRAP and REMAP techniques in chickpea breeding programs.

MATERIALS AND METHODS

Plant materials and DNA extraction

Plant materials consisted of 64 chickpea accessions (Table 1) kindly provided by the University of Tehran (Prof M.R. Bihamta), Iran. Seeds were planted in small pots with 10 cm diameter containing perlite and vermiculite in the greenhouse with an ambient temperature 25°C. The leaves of 10 individual plants of each accession bulked, and then genomic DNA was extracted from freshly young leaves of 10-day seedlings using CTAB method (AUSUBEL *et al*., 1995) with minor modifications. The concentration and quality of the DNA were measured using a spectrophotometer and electrophoresis in a 0.8% (w/v) agarose gel.

IRAP and REMAP amplifications

Six single and 10 IRAP primer combinations were used to study the extent of RTN insertional polymorphism in 64 chickpea accessions (Table 2 and 3). The primers of the RTN families Tms1Ret1, LORE1, LORE2, TPS12a and TPS19 were extracted from the previously published articles (PORCEDDU *et al.,* 2002; ABDOLLAHI MANDOULAKANI *et al*., 2012). The CARE1-LTR2 primer was designed based on the LTR region of CARE1 RTN. The complete sequence of CARE1 (Accession number: DQ239702.2) was downloaded from the NCBI (http://www.ncbi.nlm.nih.gov/genebank/). BLAST search was performed to fined sequences highly similar to CARE1. The LTR region of these RTNs identified, then sequence alignments of the CARE1 LTRs and highly similar sequences was carried out to identify the conserved region within LTRs. CARE1-LTR2 primer was designed based of the conserved region of the LTR.

The IRAP PCR reactions were performed consistent with the protocol described by ABDOLLAHI MANDOULAKANI *et al.,* (2012). Thermal cycling was performed in a Bio-Rad thermal cycler (Bio-Rad Laboratories Inc., Hercules, CA, USA) with the following profile: 94°C for 4 min, followed by 35 cycles of: 94°C for 40 s, 48°C to 55°C for 40 s, 72°C for 2 min followed by 72°C for 5 min. The PCR products were separated by electrophoresis (Bio-Rad) using 1.8% agarose gel in 0.5× TBE buffer with constant voltage of 65 V for 3 to 4 h. Gels were stained by ethidium

bromide. DNA fragments were then visualized under UV light and photographed using a gel documentation system.

Table 1. Origin and abbreviation of the analyzed chickpea accessions.

Accession no.	Origin	Abbreviation	Accession no.	Origin	Abbreviation
534	Ardabil	Ar1	999	Korosh	Ko33
490	Ardabil	Ar2	606	Mahan	Ma34
559	Ardabil	Ar3	109	Mamaghan	Ma35
642	Bam	Ba4	552	Mianeh	Mi36
473	Darghaz	Dg5	56	Shiraz	Sh37
474	Darghaz	Dg6	345	Torbat-e Jam	TJ38
629	Esfahan	Es7	323	Torbat-e Jam	TJ39
466	Esfahan	Es8	335	Torbat-e Jam	TJ40
478	Esfahan	Es9	328	Torbat-e Jam	TJ41
36	Esfahan	Es10	325	Torbat-e Jam	TJ42
29	Esfahan	Es11	370	Torbat-e Jam	TJ43
492	FAO	Fa12	403	Torbat-e Jam	TJ44
23	Qazvin	Qa13	356	Torbat-e Jam	TJ45
38	Quchan	Qu14	396	Torbat-e Jam	TJ46
998	Jam	Ja15	394	Torbat-e Jam	TJ47
308	Jiroft	Ji16	375	Torbat-e Jam	TJ48
318	Jiroft	Ji17	357	Torbat-e Jam	TJ49
317	Jiroft	Ji18	139	Torbat-e Jam	TJ50
307	Jiroft	Ji19	555	Urmia	Ur51
269	Jiroft	Ji20	525	Urmia	Ur52
30	Jiroft	Ji21	512	Urmia	Ur53
259	Jiroft	Ji22	511	Urmia	Ur54
289	Jiroft	Ji23	508	Urmia	Ur55
284	Jiroft	Ji24	239	Urmia	Ur56
245	Jiroft	Ji25	236	Urmia	Ur57
12	Karaj	Ka26	198	Urmia	Ur58
2	Karaj	Ka27	235	Urmia	Ur59
22	Karaj	Ka28	187	Urmia	Ur60
16	Karaj	Ka29	233	Urmia	Ur61
154	Karaj	Ka30	216	Urmia	Ur62
120	Karaj	Ka31	912	Urmia	Ur63
563	Khoy	Kh32	128	Urmia	Ur64

Table 2. Sequences and source of the primers used in the current study.

Primer	Sequence (5′→3′)	Source	Primer	Sequence (5′→3′)	Source
CARE1-LTR2	agtggactttacgaatgcttgag	*Cicer arietinum*	TPS12a	gggctttgactaatggacctc	*Pisum sativum*
TPS19	ggagtagaagtagagagcc	*Pisum sativum*	LORE1	gagtcctgagtaaccaactaac	*Lotus japonicus*
Tms1Ret1	cggttttgtggggttgtgttaggccca	*Medicago sativa*	LORE2	cagcttgaggacaagctgagtc	*Lotus japonicus*
A12	gagagagagagacc		A7	agagagagagagagagagagt	
443	acacacacacacacact		430	gtggtggtggtggtggtggtga	
UBC825	acacacacacacact		UBC815	ctctctctctctctt	
UBC857	acacacacacacacYg		UBC840	gagagagagagagaYt	
UBC867	ggcggcggcggcggcggcggcgc		UBC862	agcagcagcagcagcagc	

Y: pyrimidine (C/T)

Table 3. Genetic parameters calculated for the used IRAP and REMAP primers used in 64 chickpea accessions.

Primer	AT	TAL	PL	PPL	He	I	Ne	BS (bp)
CARE1-LTR2	55	9	3	33	0.12	0.17	1.05	600-2500
Tms1Ret1	48	4	1	25	0.07	0.12	1.10	550-1400
TPS12a	48	10	10	100	0.43	0.62	1.80	200-1750
CARE1-LTR2-430	53	9	4	44	0.14	0.22	1.23	100-1000
CARE1-LTR2-815	58.8	6	4	67	0.18	0.28	1.30	600-2500
CARE1-LTR2-862	54	11	6	54	0.15	0.23	1.24	100-1500
CARE1-LTR2-867	53	4	2	50	0.22	0.31	1.40	500-1000
LORE1-825	50	7	0	0	0.05	0.08	1.09	380-1500
LORE1-840	50	5	5	100	0.34	0.51	1.53	480-1500
LORE1-857	50	9	6	67	0.21	0.33	1.33	300-2000
Tms1Ret1-A7	48	7	5	71	0.26	0.39	1.42	75-850
Tms1Ret1-A12	48	8	7	87	0.36	0.53	1.65	100-750
Tms1Ret1-443	48	4	2	50	0.22	0.31	1.40	400-850
Tms1Ret1-825	50	6	6	100	0.33	0.48	1.58	500-1500
Tms1Ret1-840	50	7	4	57	0.10	0.16	1.14	300-1200
TPS12a-825	50	10	9	90	0.25	0.37	1.34	200-2500
TPS12a-862	50	6	4	67	0.17	0.27	1.22	75-750
TPS19-840	50	7	5	71	0.23	0.34	1.42	100-1500
Total	-	129	83		-	-	-	-
Mean		7	4.61	-	0.21	0.32	1.35	-

AT: annealing temperature, TAL: total amplified loci, PL: polymorphic loci, PPL: percentage of polymorphic loci, He: average of expected heterozygosity, Ne: number of effective alleles, I: Shannon's information index, BS: band size

Ninety REMAP primer combinations, derived from five single IRAP primers (designed based on RTN families CARE1, Tms1Ret1, LORE1, TPS12a and TPS19) with 42 ISSR primers were tested on 6 chickpea individuals to select the primer combinations producing scorable and discernible banding patterns (Table 2 and 3). PCR reactions and temperature profile, electrophoresis, and visualization of REMAP markers were the same as for IRAP, but annealing temperature of REMAP primer combinations varied from 48°C to 58.8°C (Table 3).

Data analysis

The amplified fragments were scored independently as 1 or 0 for their presence or absence at each position, and the obtained binary data was used for analysis. The genetic similarity matric for IRAP+REMAP data was established among accessions using Dice similarity coefficient, and then UPGMA dendrogram implemented in NTSYSpc 2.1 (ROHLF, 2000). To verify the adjustment between genetic similarity matric and respective dendrogram-derived matric (cophenetic matric), the cophenetic correlation coefficient (r) was estimated. To evaluate the genetic structure of the chickpea accessions and confirm the UPGMA clustering, Structure 2.3.1 (FALUSH *et al*., 2007) software was used with no admixture parameter, 50000 generations of burn-in period, 100000 MCMC iterations and different values of K. The optimal K value was determined according to EVANNO *et al.,* (2005). In order to assign the accessions reliably to a given cluster, the estimated membership coefficients of individuals were measured using this software as well. Genetic similarity matric was used to establish dcenter matric and subsequently used to accomplish principal coordinate analysis (PCoA) in NTSYSpc 2.1. To measure the discriminating ability of each primer, percentage of polymorphic loci (PPL), average of expected heterozygosity (He), Shannon's information index (I) and number of effective alleles (Ne) were calculated for each primer. In order to compare the RTN insertional polymorphism and genetic variability within and among the origins, accessions belong to the same origin were considered as a population (the origins with one accession was removed from the analysis) and parameters including number of loci, PPL, number of loci with a frequency higher or equal to 5%, number of private loci, number of less common loci with frequency lower or equal to 25% and 50%, He, Ne, I and standard errors were measured for each origin. In order to partition the total genetic variation among and within origins, analysis of molecular variance (AMOVA) was carried out based on IRAP+REMAP data. To compare the extent of the activity, variability and discriminating power of each RTN family, PPL, He, Ne and I were calculated for each RTN family, as well. All these computations implemented in GenAlEx 6.41 (PEAKALL and SMOUSE, 2006). Sequence alignments and primer design was performed using FastPCR (KALENDAR *et al*., 2011).

RESULTS

RTN insertional polymorphism in chickpea genome

Three single IRAP primers and 15 REMAP primer combinations amplified discernible and scorable banding patterns and were used to analyze RTN-derived diversity in 64 chickpea accessions. Single IRAP primers CARE1-LTR2, Tms1Ret1 and TPS12a produced distinguishable and polymorphic banding patterns. No fragments were amplified by single IRAP primers TPS19, LORE1 and LORE2. Out of the 90 REMAP primer combinations tested, 15 combinations produced scorable banding pattern. Totally, 129 loci were produced using 18 IRAP and REMAP primers, which 83 (64.34%) were polymorphic (Figure 1). Primers TPS12a, LORE1-840, Tms1Ret1-825 were 100% polymorphic. The He values for primers ranged from 0.05 (LORE1-825) to 0.43 (TPS12a) with a mean value of 0.21 while Ne ranged from 1.05 (CARE1-LTR2) to

1.8 (TPS12a) and I ranged from 0.08 (LORE1-825) to 0.62 (TPS12a). Primer TPS12a generated the highest value of He, Ne and I. The size of the amplified loci ranged from 75 to 2500 bp (Table 3). Among the used RTN families, TPS revealed the highest values of the PPL, Ne, I and He in studied chickpea accessions (Table 4).

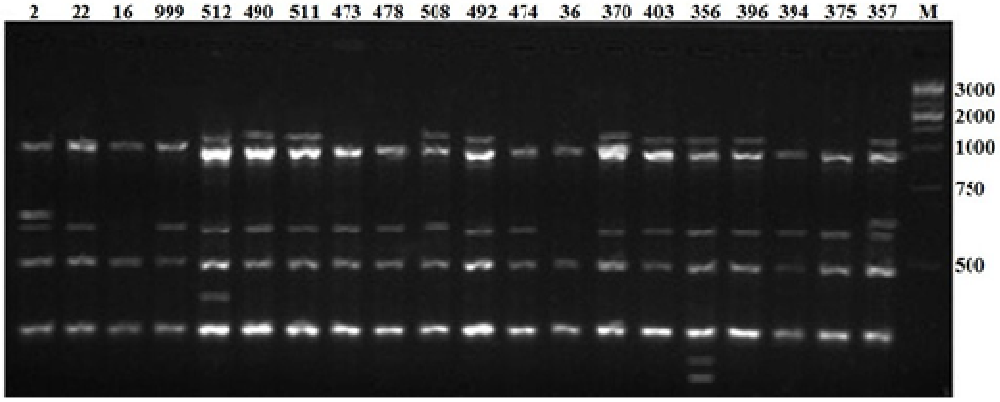

Figure 1. The DNA markers profile in chickpea accessions generated by the REMAP primer Tms1RET1-840. Numbers on top of the lanes are accession numbers.

The insertional polymorphism and genetic diversity was compared among origins. The highest He, I and Ne values observed for Urmia accessions. The highest value of PPL was found for Torbat-e Jam accessions (34.88), followed by Urmia accessions (34.11). Frequency of the all amplified loci was more than 5%. No less common loci with frequency less than 25 and 50% was detected in the origins. The private loci was produced for all origins except for origins Jiroft and Urmia (Table 5). Nei Genetic distance among origins ranged from 0.04 (Ardabil and Esfahan) to 0.08 (Darghaz and Jiroft), averaging 0.09.

Table 4. Comparison of the used retrotransposon (RTN) families in view of polymorphism detection in analyzed chickpea collection.

RTN family	NP	TAL	NLP	PPL	Ne±SE	I±SE	He±SE
CARE1	5	39	7.8	48.72	1.25±0.05	0.23±0.04	0.15±0.03
Tms1Ret1	6	36	6	69.44	1.41±0.06	0.35±0.05	0.24±0.03
TPS	4	33	8.25	81.82	1.50±0.07	0.42±0.05	0.29±0.03
LORE1	3	21	7	57.14	1.30±0.06	0.29±0.06	0.19±0.04

NP: number of primers, TAL: total amplified loci, NLP: number of loci per primer, PPL: percentage of polymorphic loci, Ne: number of effective alleles, I: Shannon's information index, He: average of expected heterozygosity, SE: standard error

Seven main clusters were identified based on UPGMA algorithm and Dice similarity coefficient using 129 IRAP and REMAP loci (Figure 2). The membership coefficient estimates (Q) for each accession for the inferred clusters with maximum log-likelihood probability (Figure

3) did not unambiguously assign some accessions to one of the defined clusters using UPGMA method. The mean Fst values for most of the groups (except for groups IV and VII) were lower than 0.20 (Table 6), indicating no strong differentiation among the groups. The highest similarity (0.98) was found between accessions Ka30 and Ur61 while accessions Ba4 and TJ48 had the minimum value of similarity (0.84). The average of the similarity in the studied chickpea collection was 0.93. Principal coordinate analysis (PCoA) was used to identify and resolve patterns of the genetic relationships among studied accessions. The first, second and third axes explained 12, 11 and 7% of the cumulative variations, respectively, which were then plotted to identify the diversity of the genotypes (Figure 4). To assess and partition the total genetic variation among and within origins, AMOVA was carried out based on 7 origins using 129 IRAP and REMAP loci. Significant differences were detected within origins (PhiPT=0.086, P≤0.01). The level of genetic variation was higher within origins (91%) compared to among origins (9%).

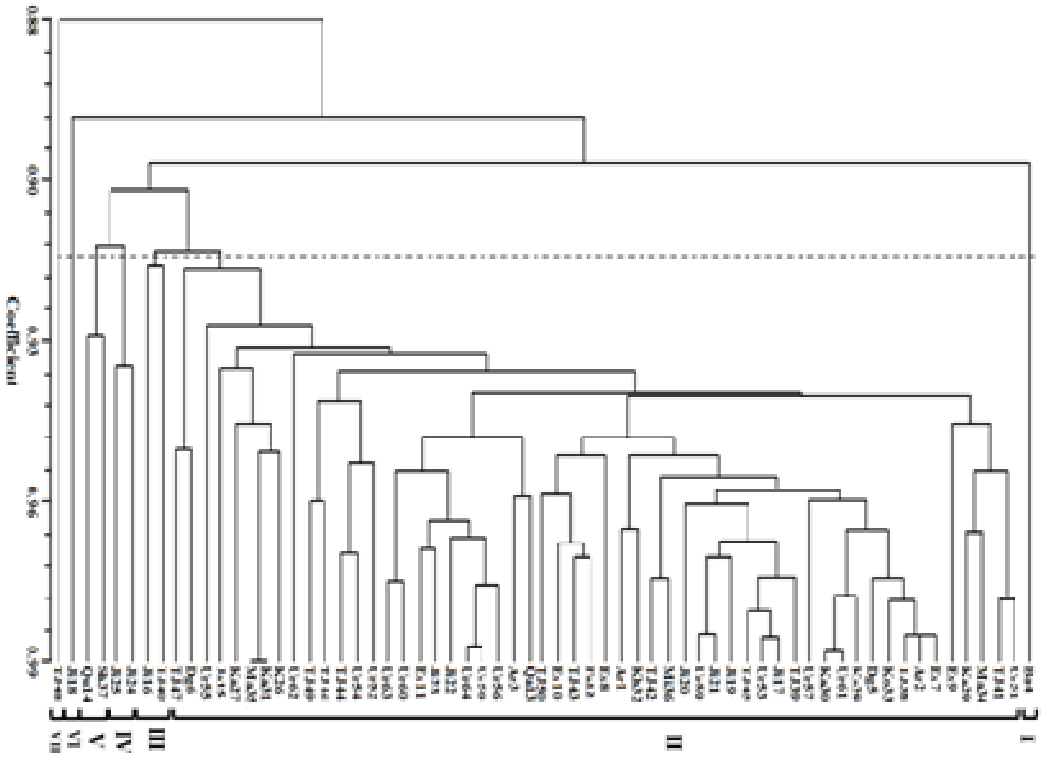

Figure 2. Associations among 64 chickpea accessions using Dice similarity coefficient and UPGMA algorithm based on 129 IRAP and REMAP loci.

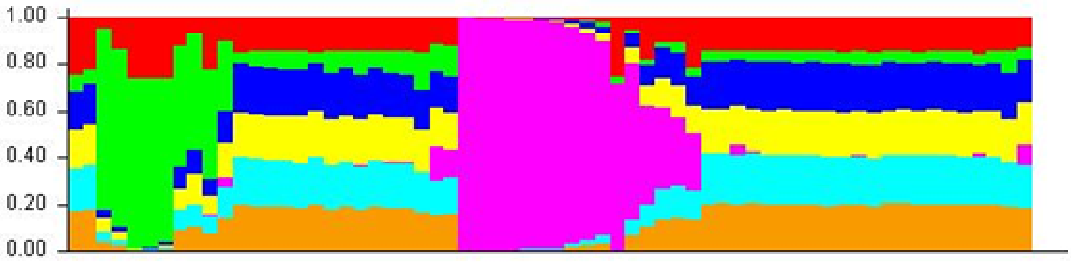

Figure 3. A Bayesian model-based clustering of the analyzed accessions. Bar plots show the membership coefficient estimate (Q) for each accession for the inferred clusters with maximum log-likelihood probability. Bar colors and lengths represent inferred clusters and Q, respectively, identified by Structure for K=7.

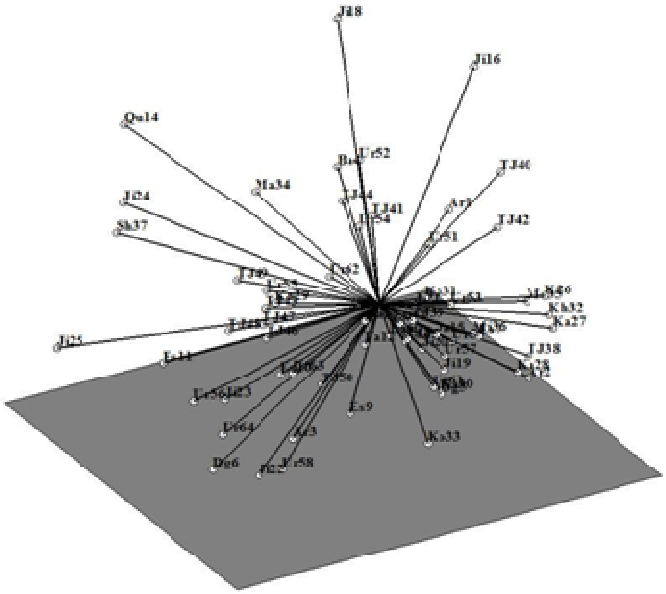

Figure 4**.** Three dimensional plots of the genetic relationship among 64 chickpea accessions as revealed by principal coordinate analysis (PCoA).

DISSCUSSION

RTN activity and polymorphism in chickpea accessions

To our knowledge, this is the first report of IRAP and REMAP-based assessment of RTN activity and genetic diversity in chickpea. Out of the primers tested, 3 IRAP and 15 REMAP amplified distinguishable and scorable banding pattern and applied to study the extent of the used RTN activity and diversity in 64 chickpea accessions. Several investigations have demonstrated that primers designed based on LTR sequences of RTN families can be readily used across species lines, among closely related genera and even sometimes between plant families (KALENDAR *et al*., 2011; NASRI *et al.*, 2013), hence RTN families from chickpea (CARE1) and other closely related species (Tms1Ret1, TPS and LORE1) were used in the current study. No discernible and scorable fragments were generated using IRAP primer combinations, probably suggesting that the used RTN elements do not insert into or near each other in chickpea genome. No fragments amplified by using primers LORE1 and TPS19 in IRAP reactions while they produced amplicons with a few number of SSRs in REMAP reactions. This might reflect the low copy number and preferential insertion of these elements near SSR motifs in chickpea genome. The preferential insertions of the RTN families in the vicinity of microsatellites have been previously reported in cultivated alfalfa (ABDOLLAHI MANDOULAKANI *et al*., 2012), barley (VICIENT *et al*., 2005) and wheat (CARVALHO *et al*., 2010).

The RTN family CARE1 yielded the most number of amplified loci, but the low level of polymorphism, indicating the high copy number but low activity of this RTN in chickpea genome. RTNs Tms1Ret1 and TPS12a produced polymorphic banding patterns in studied chickpea accessions in single IRAP and REMAP reactions. This most likely shows that these elements have relatives in *C. arietinum* and are transpositionaly active. PEARCE *et al*. (2000) stated that TPS elements have relatives in the more distantly related *Vicia* species and have been present throughout the evolution of the *Pisum* and *Vicia* genera from their common ancestor. They indicated that TPS-based markers generated a high level of insertional polymorphism in pea and have been active during *Pisum* species evolution. The presence and insertional polymorphism of the TPS elements in *Medicago sativa* has been also documented (ABDOLLAHI MANDOULAKANI *et al*., 2012). Our study shows that TPS elements have also relatives in *C. arietinum* and probably come from the common ancestors before the divergence of *Medicago, Pisum, Vicia* and *Cicer* in evolutionary process and might be widespread in legumes. Also, the highest insertional polymorphism of the TPS elements in our study demonstrated the more recent activation of these elements in chickpea genome. High polymorphism and no significant cluster of Tms1Ret1-based markers in *Medicago* genomes have been previously reported (PORCEDDU *et al*., 2002). In the current study, relatively high copy number and polymorphism was detected using the markers developed based on Tms1Ret1, suggesting its presence and transpositional activity in chickpea genome. LORE2, an aged *Ty3-gypsy* element (estimated as 600,000 years old), yet active in *L. japonicus* (FUKAI *et al*., 2008) amplified no bands in studied chickpea accessions, demonstrating its absence or much and fast divergence in *C. arietinum* genome.

Genetic relationship and structure of the chickpea accessions

The cophenetic matrices generated by the IRAP and REMAP techniques individually, evidenced a relatively low and non-significant correlation (0.13). This result suggest that similarly to what is found in barley (KALENDAR *et al*., 1999), rice (BRANCO *et al*., 2007), alfalfa (ABDOLLAHI MANDOULAKANI *et al*., 2012) and wheat (NASRI *et al*., 2013), REMAP primers amplified DNA regions that could not be covered by IRAP. Hence, to accumulate the power of both techniques

and achieve a confident classification of the chickpea accessions, combined data of IRAP and REMAP markers were applied to reveal the association between accessions. Using 129 amplified IRAP+REMAP loci and cluster analysis based on UPGMA algorithm, 7 groups were identified among 64 accessions (Figure 2). The highest cophenetic correlation coefficient (r=0.80) enabled us to propose that this dendrogram is a good representation of our IRAP+REMAP data. However, in order to determine whether this chickpea collection is differentiated in 7 groups, genetic structure analysis was performed with the software Structure 2.3.1. After performing several runs for different values of K (number of groups), K=7 was approximately considered as the suitable value to estimate the mean fixation index (Fst) for each group (Table 6). Except for groups IV and VII, all mean Fst values were lower than 0.20, indicating no strong differentiation among the groups, no genetic structure of the studied chickpea collection and relatively high gene flow among the origins of the accessions. This assumption was also corroborated by the allele frequency divergence estimated between pairs of groups (data not shown) and mingles of the accessions from the different origins in the same cluster (Figure 2). The membership coefficient estimates (Q) for each accession for the inferred clusters with maximum log-likelihood probability did not confidentially assign some accessions to the identified clusters based on UPGMA method. Additionally, the Bayesian analysis was performed to assess the genetic relationships among accessions and the occurrence of gene flow among them. This analysis revealed that the chickpea collection studied here is not genetically structured (Figure 3). The high level of diversity within origins (91%) compared to among origins confirmed the occurrence of the gene flow among accessions, as well. However, although, RTN-based markers were able to some extent separate the chickpea accessions but the measured relative genetic similarity among accessions were not correlated with geographical distances between places of their origins. Since, the most genetic distance was found between accessions TJ84 and Ba4, these accessions could be used as crossing parents in chickpea breeding programs.

In conclusion, although RTN-based markers were able to differentiate the chickpea accessions in some extent but the measured relative genetic similarity among accessions were not correlated with geographical distances between places of their origins. Since different RTN elements have various insertional patterns and history, it is advisable to use a set of diverse RTN elements to have complete genome coverage. However, the ubiquitous presence of LTR RTNs in chickpea genome and the variable nature of these elements should make them excellent sources of polymorphic markers and suggests that the use of these techniques in combination would allow chickpea breeders to obtain markers close to virtually any important agronomical trait. Besides, the use of the strong and complementary statistical methods such as Bayesian analyses proved to be useful for the determination of genetic relationships among chickpea accessions and for the definition of the genetic structure of this collection. These data might be useful in the future for designing chickpea breeding programs and defining strategies for germplasm conservation.

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IRAP I REMAP PRIMENA U UTVRĐIVANJU GENETIČKE DIVERGENTNOSTI KOLEKCIJE LEBLEBIJE U IRANU

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Izvod

Retrotranspozoni (RTN) značajno doprinose veličini, organizaciji i genetičkoj divergentnosti genoma domaćina. Identifikovano je nekoliko familija RTN kod leblebije (*Cicer arietinum* L.) i drugih srodnih biljnih vrsta. U ovom radu su prikazani rezultati activnosti integracije i insercioni polimorfizam RTNs CARE1, Tms1Ret1, TPS i 64 LORE u 64 genotipa kolekcionisanih u Iranu. Ispitivan je polimorfizam umnožavanjem inter retro transpozona (IRAP) i polimorfizma umnoženih retrotranspozon – mikrosatelita (REMAP). Dobijeni rezultati pokazuju das u svi ispitivani retrotranspozoni (RTN) aktivni u transpoziciji u genomu leblebija i izazivaju visok stepen polimorfizma. Među RTN familijama koje su korišćene, najveći procenat polimorfnih lokusa (PPL) je utvrđen kod TPS familija (81.82%). Umnoženo je ukupno 129 lokusa korišćenjem 18 IRAP i REMAP prajmer sekvenci od kojih su 83 (64.34%) bili polimorfni. Koeficijent genetičke sličnosti (Dice) je varirao od 0.84 do 0.98, u proseku 0.93. Parametri očekivane heterozigotnosti (He) Shanonov indeks informacije (I) I broj efektivnih alela su bili najvši u delu kolekcije iz Urmie. Svi ispitivani uzorci su se na osnovu UPGM algoritma I koeficijenta sličnosti grupisali u 7 grupa (klastera). Srednje Fst vrednosti su za sve izuzev IV I VII grupe bile niže od 0.20 na osnovu čega je zaključeno da ne postoji jasna diferencijacija između grupa i ukazano da je verovatno u pitanju protok gena. Mada je korišćenjem RTN- markera dobijena merena relativna genetička sličnost nije bila u korelaciji sa geografskom distancom između mesta porekla

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