Journal of Plant Molecular Breeding (JPMB)
Vol. 3/Issue 1/ June 2015/ 18-34

Genetic analysis of castor (*Ricinus communis* L.) using ISSR markers

Farnaz Goodarzi[1], Reza Darvishzadeh[2,3*]& Abbas Hassani[1]

1. Department of Horticulture, Urmia University, Urmia, Iran.
2. Department of Plant Breeding and Biotechnology, Urmia University, Urmia, Iran.
3. Institute of Biotechnology, Urmia University, Urmia, Iran.

[*]Corresponding Author, Email: r.darvishzadeh@urmia.ac.ir

Received: January 2015 Accepted: June 2015

Abstract
Castor (*Ricinus communis* L.) is one of the most ancient medicinal oil crops in the world. It has been vastly distributed in different parts of Iran. In the present study, the inter simple sequence repeat (ISSR) markers were used to evaluate the molecular genetic diversity among and within 12 castor accessions collected from 6 regions of Iran. Totally, 16 ISSR primers amplified 166 loci, of which 116 loci (69.89 %) were polymorph, indicating high genetic variability in castor germplasm. An accession-specific ISSR band was detected in '80-29'accession. Genetic distance among accessions ranged from 0.2 to 0.056. Analysis of molecular variance revealed a higher level of genetic variation within (80%) than between (20%) accessions. A model-based Bayesian approach subdivided 60 genotypes from 12 accessions into 6 subgroups. UPGMA dendrogram based on Nei's genetic distance classified 12 accessions into 4 groups. The result indicates that there was no association between geographical origin and ISSR patterns. The results suggest that ISSR technique is a useful tool for studying genetic diversity in castor germplasm.
Keywords: Bayesian clustering, Genetic diversity, ISSR, *Ricinus communis*.

Introduction

Castor is an economically and industrially important oil seed plant from Euphorbiaceae family. Castor (*Ricinus communis* L.) belongs to a monotypic genus, *Ricinus* and subtribe, *Ricininae* with chromosome number of 2n=2x=20 (62). It has a mixed mating system generating both selfed and cross fertilized offspring. Under natural conditions, cross pollination in castor can exceed 80% (54). Among the Euphorbiaceae family, castor is the only species which has lowest DNA c-value at which genome size of castor is around 350 mega base pairs (6). Castor seed contains 45-55% oil with unique properties compared to other vegetable oils. More than 80% of its oil content is an unusual hydroxyl fatty acid, ricinoleic acid (12-hydroxy-9-octadecenoic acid), which makes it acceptable for different chemical reactions (45, 30, 63). Ricinoleic acid is not found in other oil seed crops (30). Castor oil is extensively utilized in different industries such as automotive, nylons, lubricants, paints, cosmetics and medicine (43). Pharmacological researches indicated that castor oil is commonly used as a laxative and for induction of labor in pregnant women (11). Castor seed is a rich source of natural poison called ricin that makes it

non-edible (4, 12). Castor is widely grown throughout tropical and subtropical regions of the world. It has been demonstrated that the greatest genetic diversity of castor is found in Ethiopia and east African regions (35, 7, 19). In western Asian, it is found in Iraq, Iran, Syria, Turkey and Armenia (3).

Availability of sufficient knowledge on genetic diversity and distributional pattern of genetic resources are necessary for plant breeding programs (28). The methods of genetic diversity study extend from analysis of morphological characters to biochemical and molecular traits (38). DNA markers have exhibited great ability for estimating genetic diversity in plant species, because they provide direct evaluation of distinct genetic material without environmental influences (42, 66). Different types of DNA based molecular techniques such as polymerase chain reaction (PCR)-based markers, hybridization-based markers and sequencing-based markers can be used to analyze the genetic diversity of plant materials (26, 69).

Molecular markers such as SSR and AFLP (2), SSR (5), SRAP (72), SNP (15), ISSR and RAPD (16) have been successfully used to estimate the genetic diversity in castor germplasm. ISSR is a semi-arbitrary molecular marker system with single forward primers of 16 to 18 nucleotide length comprising repetitive units either anchored with 2 to 4 arbitrary nucleotides at the 3' or 5' end or non-anchored (18, 70, 48). As no prior sequence knowledge is required, they are more easily applied than SSR markers, and are more reliable and robust than RAPD markers, possibly because the primers are longer and PCR conditions are more stringent (17, 39, 47). ISSR markers have been shown to be particularly useful in genetic fingerprinting and diversity analysis (1, 10, 34, 25, 67). ISSR is an ideal method for fingerprinting and a useful alternative to single-locus or hybridization-based methods because large numbers of DNA fragments are amplified per reaction, representing multiple loci from across the genome (1, 10, 34).

Although Iran is considered as a center of genetic diversity for castor, unfortunately, there is little information available on the genetic diversity of castor germplasms in Iran. The aim of the present study was to evaluate the level of genetic variation within and among castor accessions originating from different regions of country by ISSR markers. Actually, the results can generate basic information that could be useful for castor breeding programs, such as parental choice for developing heterotic hybrids or germplasm preservation.

Materials and methods

Plant materials

Seeds of 12 castor accessions originated from 6 different provinces were kindly provided by Seed and Plant Improvement Institute (SPII) Karaj, Iran (Table 1). The accessions were planted and grown in a randomized complete block design with three replications in Urmia Agricultural Research Center (37º 44´ N; 45º 10´ E). Considering the

mating system (54, 6) and sampling coast, as some other studies (22, 36, 27), five plants from each accession were randomly selected for ISSR analysis. Some apical fresh leaves from each individual per accession were taken and immediately fixed in liquid nitrogen. The samples were stored at -80ºC until DNA extraction.

Table 1. List of the 12 castor (*Ricinus communis* L.) accessions collected from various regions of Iran.

Number	Gene bank code	Location	Latitude	Longitude	Altitude (m)
P1	80-23	Tafresh (Markazi province)	34º 24´	49º 43´	1735
P2	80-31	Ashtian (Markazi province)	34º 30´	50º 04´	2450
P3	80-25	Arak (Markazi province)	34º 20´	49º 49´	1753
P4	80-12-1	Sahreza (Isfahan province)	32º 11´	51º 37´	1750
P5	80-29	Toyserkan (Hamedan province)	36º30´	48º 16´	1910
P6	80-18	Taft (Yazd province)	31º 32´	54º 15´	2000
P7	80-16-1	Fasa (Fars province)	28º 58´	51º 41´	1382
P8	80-17	Ashtian (Markazi province)	32º 24´	50º 14´	1775
P9	80-7	Mehriz (Yazd province)	30º 05	54º 17´	1550
P10	80-11-1	Sahreza (Isfahan province)	32º 14´	51º 32´	1750
P11	80-4	Jiroft (Kerman province)	28º 40´	57º 44´	685
P12	80-22	Tafresh (Markazi province)	34º 27´	49º 38´	1727

Genomic DNA extraction

Genomic DNA was extracted from the young leaves according to Cetyltrimethylammonium bromide (CTAB) method described by Murray and Thompson (37), with some modifications. DNA quality was checked by running 1 µl DNA in 0.8% (w/v) agarose gels (Invitrogen, Carlsbad, CA, USA) in 0.5× TBE (45mM Tris base, 45mM boric acid, 1mM EDTA pH 8.0) buffer. DNA samples showing a smear on the gel were discarded. The DNA samples were diluted to 30 ng μl^{-1} in TE buffer and stored at 4ºC for using as templates in polymerase chain reactions.

ISSR amplification

A total of 31 ISSR primers were examined for polymorphism on 2 individuals per accession. Sixteen primers out of 31 were selected based on clarity and reproducibility of bands for diversity analysis. PCR was carried out in a total volume of 20µl consisting of 30 ng DNA, 2µl 10× PCR buffer, 1 µM primer, 2.5 mM $MgCl_2$ (CinnaGen Co., Iran), 0.2 mM of each dNTP and 1 unit of *Taq* DNA polymerase (CinnaGen Co., Iran). Amplification was performed in a 96-well Mastercycler Gradient (Type 5331; Eppendorf AG, Hamburg, Germany). The amplification profile composed of an initial denaturation at 94°C for 4 min, followed by 36 cycles of 1 min

denaturation at 94°C, annealing temperature (Table 2) for 45 s, extension at 72ºC for 2 min, and a final extension at 72°C for 10 min. PCR products were electrophoresed on 1.6% agarose gels in 0.5× TBE buffer at 70 V for 3 h, stained with ethidium bromide (1.0 µg ml^{-1}) and photographed under UV light using a Gel-Doc image analysis system (Gel Logic 212 PRO, Carestream Health, Inc., Rochester, NY, USA). Molecular weight of amplified products was assessed with 100 bp DNA ladder.

Table 2. Nucleotide sequences, annealing temperature, total number of loci, percentage of polymorphic loci and PIC per primer for ISSR primers used in the present study.

Primer name	Sequence(5'→3')	Annealing temperature(°C)	Total number of loci	Polymorphic loci	Percentage of polymorphic loci	PIC per primer
812	5′-(GA)8A-3′	48	8	4	50	0.449
816	5′-(CA)8T-3′	54	10	6	60	0.449
818	5′-(CA)8G-3′	51	7	5	71.43	0.361
822	5′-(TC)8A-3′	42.5	12	4	33.33	0.441
825	5′-(AC)8T-3′	50	9	7	77.78	0.307
834	5′-(AG)8YT-3′	50.3	11	7	63.64	0.369
836	5′-(AG)8YA-3′	51.5	14	10	71.43	0.330
840	5′-(GA)8YT-3′	42	7	3	42.8	0.298
844	5′-(CT)8RC-3′	47.5	12	8	66.67	0.336
848	5′-(CA)8RG-3′	47	10	9	90	0.516
849	5′-(GT)8CG-3′	55	10	9	90	0.386
857	5′-(AC)8YG-3′	58	13	9	69.23	0.366
859	5′-(TG)8RC-3′	44.5	14	14	100	0.394
864	5′-(ATG)6-3′	48.5	11	6	54.55	0.343
885	5′-HBH(AG)7-3′	53.5	11	9	81.82	0.388
A12	5′-(GA)6CC-3′	47	7	6	85.71	0.431
Total	-	-	166	116	69.88	-
Mean	-	-	10.375	7.25		-

Y = (C, T); R = (A, G); H = (A, C, T); B = (C, G, T). PIC: Polymorphism information content

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 matrix was used for analysis. Number of markers per primer, percentage of polymorphic markers, number of marker with a frequency greater than or equal to 5%, number of private marker and number of less common marker with frequency lower than or equal to 25 and 50% (32) were calculated in the GenAlEx version 6 software (44). The PIC value for each ISSR primer was calculated as proposed by Roldan-Ruiz *et al.* (51):

$$PIC_i = 2f_i(1 - f_i)$$

where PIC is the polymorphism information content of marker i, fi is the frequency of marker fragments that were present, and 1-fi is the frequency of marker fragments that were absent. PIC was averaged over the fragments for each primer.

Genetic diversity within and among populations was measured as the percentage of polymorphic bands per

population, Nei's gene diversity (40), Shannon's information index (56), Gst (40) and gene flow (Nm) among populations (59) and Nei's unbiased genetic distance, all of which were measured using POPGENE program, (ver. 1.32) (68). AMOVA (14) was performed to estimate the variance within and among castor accessions in the GenAlEx software.

The genetic similarity between individuals (12 accessions×5 individuals per accession= 60 individuals) was calculated using Dic similarity coefficient (41). Dendrogram was constructed by complete linkage method using NTSYS-pc software (ver. 2.11) (50). The results of cluster analysis were corroborated by principal coordinate analysis (29) visualizing the relationships among the studied genotypes. Genetic distances between accessions were calculated by Nei's genetic distance in the POPGENE software. The genetic distances were employed for construction of phylogenetic tree in the POPGENE software.

Population structure was analyzed using a model-based Bayesian approach in the software package Structure 2.3.4 (46). Five independent runs of K= 1-10 were performed, assuming an admixture model and correlated allele frequencies with 5000 Markov chain Monte Carlo (MCMC) iterations and a burn-in period of 10,000. The K value was determined by the log likelihood for each K; Ln P(D)= L(K) (52). Since the distribution of Ln P(D) did not show a clear number of true K, delta K (ΔK) based on the second order rate of change in the likelihood (ΔK) (13) was used alternatively to identify a clear peak to represent the true K value. An individual was discretely assigned to a subpopulation when more than 70% of its genome composition came from that subpopulation (9).

Results

Sixteen out of 31 ISSR primers produced clear and reproducible fragment patterns across the studied accessions. Sixteen primers amplified a total of 166 loci, of which 116 (69.88%), were polymorph. The number of polymorphic markers per primer ranged from 3 to 14 with an average of 7.25. The highest level of polymorphism was observed by UBC-859 (100%), while the primer UBC-822 produced the lowest level of polymorphism (33.33%). Amplified fragments ranged from 230 to 2800 bp in size. PIC of primers ranged from 0.298 (840) to 0.516 (848) (Table 2).

Characteristics of the amplified ISSR markers in each accession were described in Table 3. The range of polymorphic loci was between 24.70% in '80-22' accession to 37.35% in '80-17'. One unique ISSR loci was observed in '80-29' accession. The mean observed number of alleles (Na) ranged from 1.247± 0.433 in '80-22' and '80-18' accessions to a maximum of 1.374± 0.485 in '80-17' accession. Values of the effective number of alleles (Ne) were less than those for (Na) with regard to every population and ranged from 1.163± 0.316 in '80-22' to 1.278 ± 0.393 in '80-17'. The heterozygosity among the accessions ranged from 0.094 in '80-22' to 0.154 in '80-17'. The mean Nei's gene diversity (H) ranged from 0.094 ± 0.173 in '80-22' to 0.154± 0.209 in '80-17' (Table 3).

Table 3. Characteristics of amplified ISSR loci on the studied castor (*Ricinus communis* L.) accessions.

Accession	No. Bands	No. Bands Freq. >= 5%	No. Private Bands	No. LComm Bands (<=25%)	No. LComm Bands (<=50%)	Na ± (SD)		Ne ± (SD)		He ± (SE)		H ± (SD)		I ± (SD)		Number of polymorphic loci	Percentage of polymorphic loci
P1	144	144	0	4	6	1.307	0.463	1.207	0.350	0.117	0.015	0.117	0.189	0.189	0.271	51	30.72 %
P2	148	148	0	2	6	1.301	0.460	1.228	0.379	0.125	0.015	0.125	0.200	0.181	0.284	50	30.12 %
P3	142	142	0	2	3	1.307	0.463	1.202	0.342	0.116	0.014	0.116	0.186	0.186	0.186	51	30.72 %
P4	140	140	0	0	2	1.325	0.470	1.243	0.384	0.134	0.016	0.134	0.203	0.194	0.288	54	32.53 %
P5	152	152	1	2	9	1.283	0.452	1.197	0.353	0.110	0.015	0.110	0.187	0.162	0.268	47	28.31 %
P6	143	143	0	0	5	1.247	0.433	1.177	0.338	0.099	0.014	0.099	0.181	0.144	0.260	41	24.70 %
P7	152	152	0	0	9	1.349	0.478	1.232	0.357	0.133	0.015	0.133	0.193	0.196	0.279	58	34.94 %
P8	146	146	0	0	4	1.374	0.485	1.278	0.393	0.154	0.016	0.154	0.209	0.224	0.298	62	37.35 %
P9	148	148	0	2	5	1.337	0.474	1.244	0.379	0.136	0.016	0.136	0.201	0.198	0.287	56	33.73 %
P10	142	142	0	0	2	1.325	0.470	1.243	0.385	0.134	0.016	0.134	0.203	0.194	0.289	54	32.53 %
P11	149	149	0	2	6	1.265	0.443	1.174	0.326	0.100	0.014	0.100	0.177	0.148	0.256	44	26.51 %
P12	142	142	0	0	4	1.247	0.433	1.163	0.316	0.094	0.013	0.094	0.173	0.139	0.251	41	24.70 %

No. Bands = Number of different bands. No. Bands Freq. >= 5% = Number of different bands with a frequency >= 5%. No. Private Bands = Number of bands unique to a single population. No. LComm Bands (<=25%) = Number of locally common bands (Freq. >= 5%) found in 25% or fewer populations. No. LComm Bands (<=50%) = Number of locally common bands (Freq. >= 5%) found in 50% or fewer populations. na = Observed number of alleles. ne = Effective number of alleles. He = Expected heterozygosity = $2 \times p \times q$. H = Nei's gene diversity. I = Shannon's Information index. SD= Standard deviation. SE= Standard error.

Table 4. Overall genetic variability across all studied castor (*Ricinus communis* L.) accessions.

Sample size	Na ± (SD)	Ne ± (SD)	H ± (SD)	I ± (SD)	Ht ± (SD)	Hs ± (SD)	Gst	Nm
56	1.711 (0.457)	1.402 (0.389)	0.231 (0.202)	0.347 (0.284)	0.232 (0.037)	0.121 (0.015)	0.479	0.544

na = Observed number of alleles. ne = Effective number of alleles. H = Nei's (1973) gene diversity. I = Shannon's Information index. Ht = Total genetic diversity. HS = Gene diversity within populations. Gst = Coefficient of genetic differentiation among populations. Nm = Estimate of gene flow from Gst or Gcs. e.g., Nm = 0.5(1 - Gst)/Gst.

Table 5. Analysis of molecular variance (AMOVA) for Iranian castor (*Ricinus communis* L.) accessions based on ISSR data.

Source	df	SS	MS	Est. Var.	%	Stat	Value	P(rand >= data)
Among Accessions	11	516.967	46.997	4.963	18%	PhiPT	0.183	0.001
Within Accessions	48	1064.800	22.183	22.183	82%			
Total	59	1581.767		27.146	100%			

df: degree of freedom. SS: sum of square. MS: mean of square.

The Shannon's indices (I) ranged from 0.139± 0.251 in '80-22' to 0.224± 0.298 in '80-17'. Total gene diversity (Ht) and gene diversity within population (Hs) were 0.232± 0.037 and 0.121± 0.015, respectively. The coefficient of gene differentiation (Gst) among populations was 0.479. Based on the Gst value, the mean estimated number of gene flow (Nm) between populations was found to be 0.544 (Table 4). According to AMOVA, the genetic variation was mainly within accessions (82%) rather than among accessions (Table 5). Nei's genetic distances based on ISSR analysis were computed among accessions (Table 6). The genetic distance values ranged from 0.2 ('80-29' and '80-11-1' accessions) to 0.056 ('80-23' and '80-25' accessions).

Table 6. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) among 12 accessions of castor (*Ricinus communis* L.) based on ISSR data.

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
P1		0.898	0.946	0.923	0.824	0.844	0.859	0.851	0.860	0.853	0.826	0.844
P2	0.107		0.894	0.892	0.835	0.854	0.876	0.860	0.869	0.860	0.853	0.876
P3	0.056	0.112		0.927	0.834	0.855	0.878	0.867	0.879	0.870	0.824	0.859
P4	0.080	0.114	0.076		0.849	0.842	0.863	0.848	0.872	0.859	0.822	0.840
P5	0.194	0.180	0.181	0.164		0.843	0.845	0.835	0.831	0.819	0.893	0.837
P6	0.170	0.158	0.157	0.172	0.171		0.920	0.887	0.846	0.846	0.846	0.835
P7	0.152	0.133	0.130	0.148	0.169	0.084		0.905	0.907	0.867	0.862	0.868
P8	0.162	0.151	0.143	0.165	0.181	0.121	0.100		0.851	0.833	0.820	0.846
P9	0.151	0.141	0.129	0.137	0.185	0.168	0.097	0.162		0.913	0.860	0.887
P10	0.159	0.151	0.140	0.152	0.200	0.168	0.143	0.182	0.091		0.879	0.879
P11	0.191	0.159	0.194	0.197	0.113	0.167	0.149	0.199	0.151	0.129		0.867
P12	0.169	0.132	0.152	0.174	0.178	0.180	0.142	0.167	0.120	0.129	0.143	

The dendrograms representing relationships between 60 individuals (12 accessions × 5 individuals per accession = 60 individuals) and 12 accessions are shown in Figures 1 and 2, respectively. The dendrogram of individuals (Figure 1) did not divide the individuals into distinct groups resembling the geographically-defined accessions. Generally, individuals were evenly distributed along the dendrogram, revealing high intra-accession genetic diversity. According to population's dendrogram, all the castor accessions were categorized into 4 major groups (Figure 2). Group I included '80-23', '80-31', '80-25' and '80-12-1' accessions. Group II contained '80-7', '80-11-1' and '80-22' accessions. Group III comprised '80-18', '80-16-1' and '80-17' accessions. Group IV included '80-29' and '80-4' accessions from Hamedan and Kerman provinces, respectively. Principal coordinates analysis (PCoA) showed that the first three Eigen-values explained 26.45% of the cumulative variation which were then plotted to identify the diversity of the genotypes (Figure 3). The result of principal co-ordinate analysis (PCoA) was partially in accordance with the cluster analysis. In order to understand the genetic structure of the studied panel, a model-based Bayesian approach in the Structure software was used to assign each genotype to the corresponding subgroup. 21 out of 60 castor genotypes were partitioned into six subgroups and the remaining ones were categorized as mixed based on their Q values (the memberships of individuals to specific groups) (Figures 4).

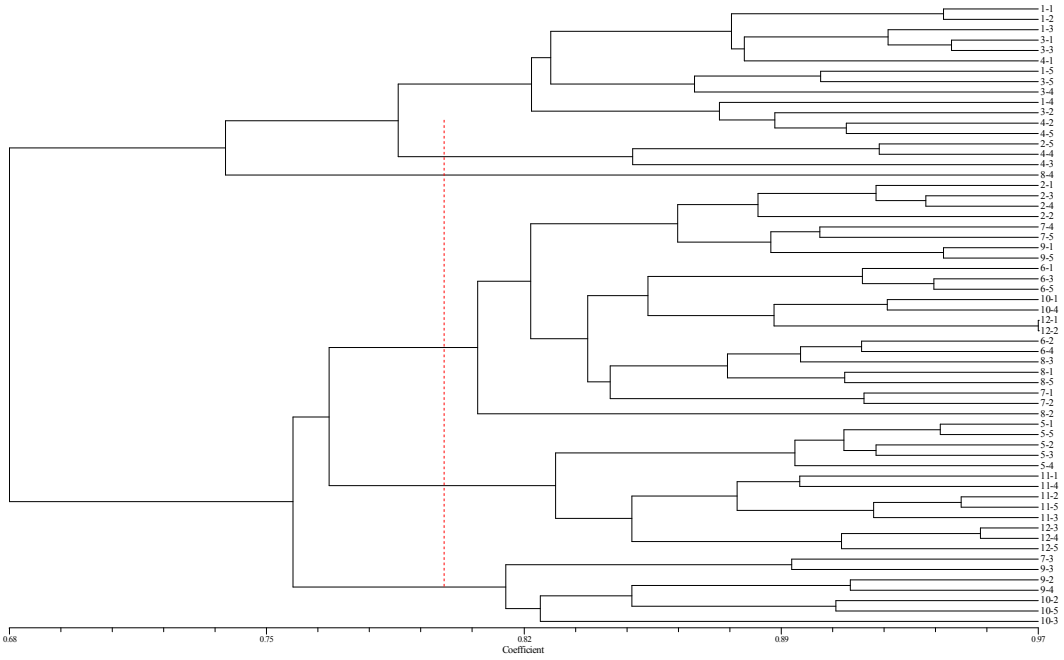

Figure 1. Dendrogram of 60 castor genotypes based on Dic similarity index.

Discussion

In the present study, the genetic diversity and relationships among 12 *R. communis* accessions coming from different regions of Iran were investigated using ISSR markers. The high reproducibility of ISSR markers may be because of using longer primers and higher annealing temperature than those used for RAPD. Based on its unique characters, ISSR technique can detect more genetic loci than isozyme and has higher stability than RAPD (72). ISSR markers have been used for studying genetic diversity in several plant species, such as *Coptis chinensis* (58), *Nelumbo nucifera* (8), *Asparagus acutifolius* (57), and *Solanum melongena* (24). The average level of polymorphism, revealed by 16 ISSR primers, was 69.88% indicating a high level of polymorphism. High level of genetic variability in *R. communis* germplasms has also been revealed by SSR (5), ISSR and RAPD (16) and SRAP (71) markers. In contrast to these results, Allan *et al.* (2) by using SSR and AFLP markers and Foster *et al.* (15) by using SNP markers identified low level of genetic variation in *R. communis* gremplasms.

Based on the Shannon's information index, genetic diversity in '80-17' accession is higher than the others and that the '80-22' accession had the lower one. The high level of heterozygosity was observed in '80-17' accession (from Ashtian/Markazi province) and the low level of heterozygosity was observed in '80-22' accession (from Tafresh/Markazi province).

The accession with high level of genetic diversity can be considered as a good genetic resource for parental selection in castor breeding programs. AMOVA was used for partitioning the total genetic variation among and within *R. communis* accessions (14). Based on AMOVA analysis, genetic variation within *R. communis* accessions was higher than among accessions. Allogamous, self-incompatible and cross-pollinated species are essentially explained by more within accessions level of genetic variation (20, 53), whereas self-pollinated plants or plants with vegetative reproduction are often presented by more among accessions level of genetic variation (61). Similar results have been reported in other cross-pollinated species such as *Rheum tanguticum* (23) and *Murraya koenigii* (64). Genetic variation is due to numerous factors, including mating system, gene flow, genetic drift, seed and pollen scattering, human activities, long-term evolutionary history, natural selection and breeding systems (55, 21). Considering the importance of medicinal plants in our country, we believe that human activities (seed transformation) and cross pollination are possible reasons in shaping Iranian *R. communis* genetic diversity and structure. In the present study, '80-29' accession from Toyserkan/Hamedan province produced unique band. ISSR marker is one of the available techniques for identification of unique band in plant materials (69). In several studies unique ISSR band in plant species like *Dendrobium* (65), *Citrus indica* (33), *Chimonanthus praecox*

(70), and *Populus cathayana* (31) has been identified. Generally, the unique bands could be transformed as distinct fingerprint into STS (sequence-tagged site) and SCAR (sequence characterized amplified regions) markers to develop the species specific marker for the best management and accurate identification of the plant materials. Reports on unique band using molecular methods are very little in the castor germplasm.

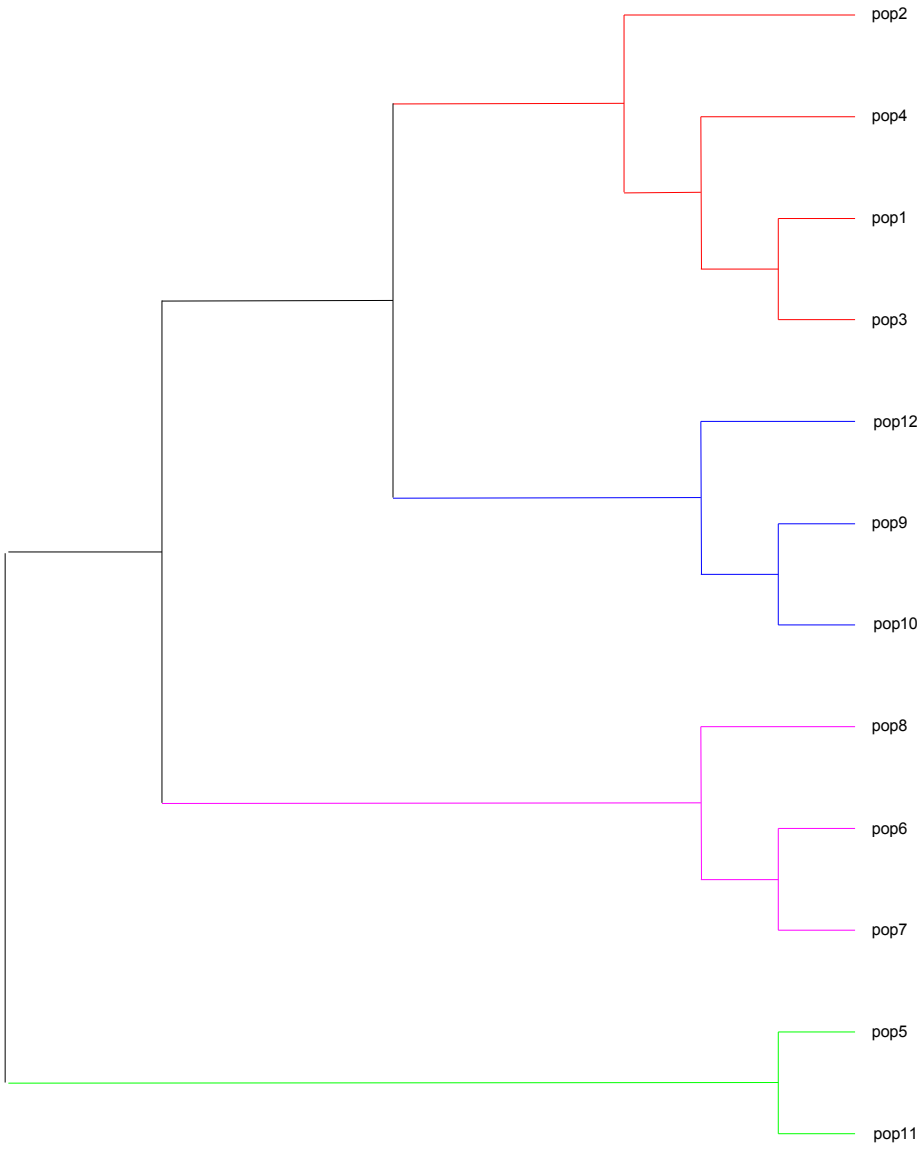

Figure 2. Dendrogram of 12 castor accessions based on Nei genetic distance calculated from ISSR data.

Allan *et al.* (2) reported two unique SSR alleles but no unique AFLP allele in some castor accessions from USDA collection.

The distribution of 60 individuals from 12 *R. communis* accessions across respective groups did not reflect the geographic origins. The high degree of genetic variation revealed in

dendrogram may be due to higher level of cross pollination. In view of population level, the studied castor accessions were categorized into 4 major clusters. The lowest genetic distance (0.056) was observed between '80-23' and '80-25' accessions both from Markazi province, which might be attributed to having similar ecological environment and short geographical distance.

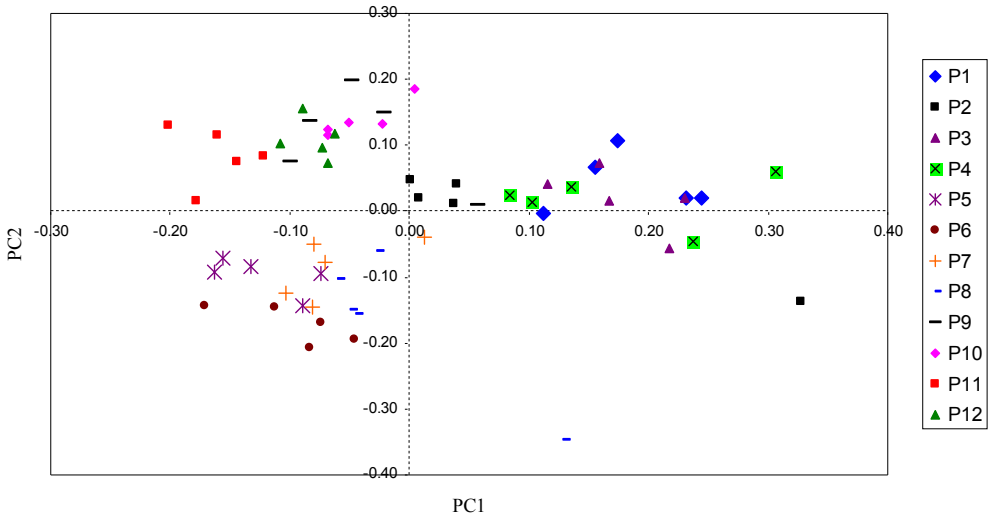

Figure 3. Two dimensional plot of the genetic relationship among 60 castor genotypes as revealed by principal co-ordinate analysis (PCoA).

.

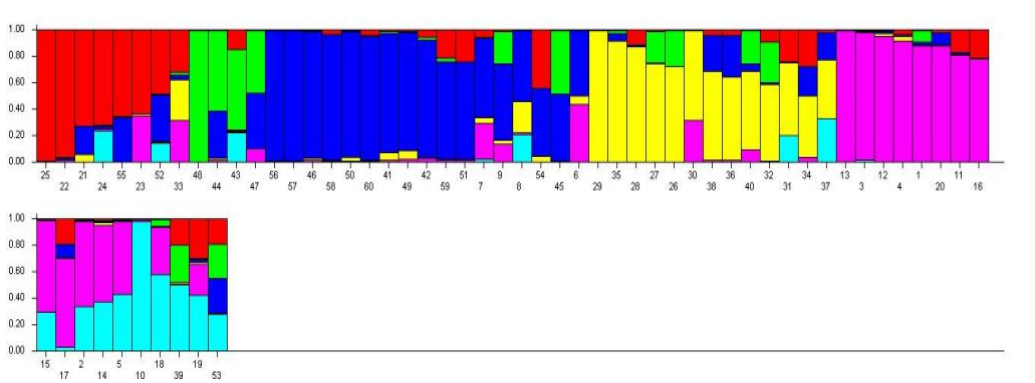

Figure 4. Genetic relatedness of 60 individuals from 12 castor (*Ricinus communis* L.) accessions with 16 inter simple sequence repeats (ISSRs) as analyzed by the Structure program. Numbers on the y-axis indicate the membership coefficient (Q) and on the x-axis indicate the individual's number. Genotypes 1-5 sampled from Tafresh accession (Markazi province); 6-10: Ashtian accession (Markazi province); 11-15: Arak accession (Markazi province); 16-20: Shahreza accession (Isfahan province); 21-25: Toyserkan accession (Hamedan province); 26-30: Taft accession (Yazd province); 31-35: Fasa accession (Fars province); 36-40: Ashtian accession (Markazi province); 41-45: Mehriz accession (Yazd province); 46-50: Shahreza accession (Isfahan province); 51-55: Jiroft accession (Kerman province); 56-60: Tafresh accession (Markazi province). Individuals with the same color belong to the same subgroup. Red: genotypes from Toyserkan accession from Hamedan province with cold and semidry climate; Green: genotypes from Shahreza accession (Isfahan province); Blue: genotypes from Tafresh accession (Markazi province with hot and dry climate); Yellow: genotypes from Taft and Fasa accessions (Yazd and Fars provinces, respectively); Pink: genotypes from Tafresh and Arak accessions (Markazi province); Aqua: genotypes from Ashtian accession (Markazi province).

The highest genetic distance (0.2) was observed between '80-29' accession from Toyserkan/Hamedan province and '80-11-1' accession from Shahreza/Isfahan province which might be attributed to having long geographical distance. In constructed dendrogram *R. communis* accessions coming from the same geographical location (province) were distributed in various groups. For instance, the accessions from Markazi province were distributed into 3 distinct groups. Moreover, '80-29' accession from Hamedan was classified with '80-4' accession from Kerman which is unexpected because they are geographically far distant locations. Consequently, the clustering pattern was not obvious according to geographic origin of *R. communis* accessions.

Effective analysis of the population structure and accurately classifying of individuals to appropriate subpopulations were performed by Bayesian method in the Structure software. This clustering method is based on the allocation of individual genotypes to K clusters in such a way that Hardy–Weinberg equilibrium and linkage equilibrium are valid within clusters, whereas these kinds of equilibrium are absent between clusters. Maximum value of ΔK was observed in K=6, so the studied castor genotypes probably have 6 subpopulations (Figure 4). This finding indicates existence of acceptable genetic variation and ideality of studied germplasm for association mapping in caster.

The present study revealed the utility of ISSR technique as a molecular diagnosis tool to characterize the genetic diversity, and determined the pattern of genetic structure within and among different Iranian *R. communis* accessions. The results revealed rather high level of genetic polymorphism (68.88%) and wide genetic variation within accessions, which offer valuable information for conservation and management of genetic resources and utilizing them in castor breeding programs. The genetic distance between populations is a valuable parameter to conserve and use a given germplasm in breeding activities. It was proved that crosses between unrelated parents and genetically away will show more power hybrid than crosses between genotypes closely related (49, 60). Furthermore, our results revealed population structure in present germplasm that should be taken into account to carry out unbiased association mapping in castor improvement programs.

Acknowledgements

The authors are grateful for the support provided by Institute of Biotechnology, Urmia University, Iran.

References

1. Aghaei, M., Darvishzadeh, R. and Hassani, A. 2012. Molecular Characterization and similarity relationships among Iranian basil (*Ocimum basilicum* L.) accessions using inter simple sequence repeat markers. Rev Ciênc Agron, 43: 312-320.
2. Allan, G., Williams, A., Rabinowicz, P.D., Chan, A., Ravel, P. and Keim, J.P. 2008. World-wide genotyping of castor bean germplasm (*Ricinus communis* L.) using ALFP and SSRs. Genet Resour Crop Evol, 55: 365–378.
3. Anjani, K. 2012. Castor genetic resources: A primary gene pool for exploitation. Ind Crop Prod, 35: 1–14.
4. Audi, J., Belson, M., Patel, M., Shier, J. and Osterloh, J. 2005. Ricin poisoning—a comprehensive review. J Am Med Assoc, 294: 2342–51.
5. Bajay, M.M. 2010. Development of microsatellite markers and characterization of germplasm of castor (*Ricinus communis* L.). Dissertation, Universidade de Sao Paulo-USP, Brazil.
6. Chan, A.P., Crabtree, J., Zhao, Q., Lorenzi, H., Orvis, J., Puiu, D., Melake-Berhan, A., Jones, K.M., Redman, J., Chen, G., Cahoon, E.B., Gedil, M., Stanke, M., Haas, B.J., Wortman, J.R., Fraser-Liggett, C.M., Ravel, J. and Rabinowicz, P.D. 2010. Draft genome sequence of the oilseeds species *Ricinus communis*. Nat Biotechnol, 28(9): 951-956.
7. Carter, S. and Smith, A.R. 1987. Euphorbiaceous Flora of Tropical East Africa. A.A., Balkema Publishers, Rotterdam, Netherlands.
8. Chen, Y., Zhou, R., Lin, X., Wu, K., Qian, X. and Huang, S. 2008. ISSR analysis of genetic diversity in sacred lotus cultivars. Aquat Bot, 89: 311–316.
9. Courtois, B., Audebert, A., Dardou, A., Roques, S., Ghneim- Herrera, T., Droc G., Frouin, J., Rouan, L., Gozé, E., Kilian, A., Ahmadi, N. and Dingkuhn, M. 2013. Genome-wide association mapping of root traits in a Japonica rice panel. PLoS ONE, 8(11): e78037. doi: 10.1371/journal. pone.0078037.
10. Dashchi, S., Abdollahi Mandoulakani, B., Darvishzadeh, R. and Bernoosi I. 2012. Molecular similarity relationships among iranian bread wheat cultivars and breeding lines using ISSR markers. Not Bot Horti Agrobo, 40(2): 254-260.
11. Duke, J.A. 1998. *Ricinus communis*. from Purdue University New Crop Resource Online Program. http: //www.hort.purdue .edu/newcrop/duke_energy/Ricinus_communis.html.
12. Endo, Y.K., Mitsui, K., Motizuki, M. and Tsurugi, K. 1987. The mechanism of action of ricin and related toxic lectins on eukaryotic ribosomes: the site and the characteristics the modification in 28S ribosomal RNA caused by the toxins. J Biol Chem, 262: 5908–5917.
13. Evanno, G., Regnaut, S. and Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol, 14: 2611–2620.
14. Excoffier, L., Smouse, P.E. and Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genet, 131: 479-491.
15. Foster, J., Allan, T., Chan, G.J., Rabinowicz, A.P., Ravel, P.D., Jackson, J. and Keim, P.J.P. 2010. Single nucleotide polymorphism for assessing genetic diversity in castor (*Ricinus communis* L.). BMC Plant Biol, 10: 13.
16. Gajera, B.B., Kumar, N., Singh, A.S. and Punvar, B.S. 2010. Assessment of genetic diversity in castor (*Ricinus communis* L.) using RAPD and ISSR markers. Ind Crop Prod, 32: 491-498.
17. Godwin, L.A., Aitken, E.A. and Smith L.A. 1997. Application of inter simple sequence repeat (ISSR) markers to plant genetics. Electrophor, 18: 1524-1528.

18. Gupta, M., Chyi, Y.S., Romero-Severson, J. and Owen, J.L. 1994. Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. Theor Appl Genet, 89: 998–1006.
19. Govaerts, R., Frodin, D.G. and Radcliffe-Smith, A. 2000. World checklist and bibliography of Euphorbiaceae (with Pandaceae). Redwood Books Limited, Trowbridge, Wiltshire.
20. Hamrick, J.L. and Godt, M.J.W. 1989. Allozyme diversity in plants species. In Plant Population Genetics, Breeding and Genetic Resources, pp. 43– 63. Eds A.H.D. Brown, M.T. Clegg, A.L. Kahler and B.S. Weir. Sunderland, MA, USA: Sinauer Associates.
21. Hamrick, J.L. and Godt, M.J.W. 1996. Effects of life history traits on genetic diversity in plant species. Philos Trans R Soc Lond B, 351: 1291-1298.
22. He, F., Kang, D., Ren, Y., Qu, L-J., Zhen, Y., and Gu, H. 2007. Genetic diversity of the natural populations of *Arabidopsis thaliana* in China. Hered, 99: 423-431.
23. Hu, Y., Wang, L., Xie, X., Yang, J., Li Y. and Zhang, H. 2010. Genetic diversity of wild populatio ns of Rheum tanguticum endemic to China as revealed by ISSR analysis. Biochem Sys Ecol, 38: 264–274.
24. Isshiki, S., Iwata, N. and Mizanur Rahim Khan, M.D. 2008. ISSR variations in eggplant (*Solanum melongena* L.) and related Solanum species. Sci Hort, 117: 186–190.
25. Jabbarzadeh, Z., Khosh-Khui, M., Salehi, H. and Saberivand, A. 2010. Inter simple sequence repeat (ISSR) markers as reproducible and specific tools for genetic diversity analysis of rose species. Afr J Biotechnol, 9: 6091- 6095.
26. Joshi, K., Chavan, P., Warude, D. and Patwardhan, B. 2004. Molecular markers in herbal drug technology. Curr Sci, 87(2): 159-165.
27. Kholghi, M., Darvishzadeh, R., Bernousi I., Pirzad, A. and Laurentin H. 2012. Assessment of genomic diversity among and within Iranian confectionery sunflower (*Helianthus annuus* L.) populations by using simple sequence repeat markers. Acta Agri Scand Sect B Soil Plant Sci, 62: 488-498.
28. Khush, G.S. 2002. Molecular genetics-plant breeder's perspective. In: Jain, S.M., Brar, D.S., Ahloowalia, B.S. (Eds.), Molecular Techniques in Crop Improvement. Kluwer Academic.
29. Kovach, W. 1999. MVSP-A Multivariate Statistical Package for Windows, ver. 3.1. Kovach Computing Services, Pentraeth, Wales, UK.
30. Lobb, K. 1992. Fatty acid classification and nomenclature. In: Chow, C.K. (Ed.), Fatty Acids in Foods and their Health Implications. Marcel Dekker, New York, pp. 1–15.
31. Lu, Z., Wang, Y., Peng, Y., Korpelainen, H. and Li, C. 2006. Genetic diversity of *populus cathayana* Rehd populations in southwestern china revealed by ISSR markers. Plant Sci, 170: 407–412.
32. Lynch, M. and Milligan B. 1994. Analysis of population-genetic structure using RAPD markers. Mol Ecol, 3: 91-99.
33. Marak, C.K. and Laskar, M.A. 2010. Analysis of phenetic relationship between *Citrus indica* Tanaka and a few commercially important citrus species by ISSR markers. Sci Hort, 124: 345–348.
34. Modareskia, M., Darvishzadeh, R., Hassani, A. and Kholghi M. 2012. Molecular diversity within and between Ajowan (*Carum copticum* L.) populations based on inter simple sequence repeat (ISSR) markers. J Plant MB, 1: 51-62.
35. Moshkin, V.A. 1986. Castor. Amerind Publishing Co. PVT Ltd, New Delh.

36. Muirhead, J. R., Gray, D. K., Kelly, D. W., Ellis, S. M., Heath, D. D., and Macisaac, H. J. 2008. Identifying the source of species invasions: sampling intensity vs. Genet Divers Mol Ecol, 17: 1020-1035.
37. Murray, M.G. and Thompson, W.F. 1980. Rapid isolation of high molecular-weight plant DNA. Nucleic Acids Res, 8: 4321-4325.
38. Muthusamy, S., S. Kanagarajan and S. Ponnusamy, 2008. Efficiency of RAPD and ISSR marker system in accessing variation of rice bean (*Vigna umbellate*) landrace. Electron J Biotechnol, Vol. 11.
39. Nagaoka, T. and Ogihara, Y. 1997. Applicabilty of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor Appl Genet, 94: 597–602.
40. Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA, 70: 3321-3323.
41. Nei, N. M. and Li, W. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA, 76: 5269-5273.
42. Nybom, H. 1994. DNA fingerprinting – a useful tool in fruit breeding. Euphytica, 77: 59-64.
43. Ogunniyi, D.S. 2006. Castor oil: a vital industrial raw material. Bioresour Technol, 97: 1086–1091.
44. Peakall, R. and Smouse, P.E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic sof tware for teaching and research. Mol Ecol Notes, 6: 288-295.
45. Piazza, G.J. and Farrell, H.M. 1991. Generation of ricinoleic acid from castor oil using the lipase from ground oat (*Avena sativa* L.) seeds as a catalyst. Biotechnol Lett, 13:179–184.
46. Pritchard, J.K., Stephanes, M., Rosenberg, N.A. and Donnelly, P. 2000. Association mapping in structured populations. Am J Hum Genet, 67:170–181.
47. Qian, W., Ge S. and Hong, D.Y. 2001. Genetic variation within and among populations of a wild rice Oryza granulata from China detected by RAPD and ISSR markers. Theor Appl Genet, 102: 440–449.
48. Ratnaparkhe, M.B., Tekeoglu, M. and Muehlbauer, F.J. 1998. Inter-simple-sequence-repeat (ISSR) polymorphisms are useful for finding markers associated with disease resistance gene clusters. Theor Appl Genet, 97: 515–519.
49. Reif, J.C., Gumpert, F., Fischer, S. and Melchiger, A.E. 2007. Impact of genetic divergence on additive and dominance variance in hybrid populations. Genet, 176: 1931-1934.
50. Rohlf, F.J. 1998. NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System. version 2.02. Exter Software, Setauket, New York, NY, USA.
51. Roldan-Ruiz, I., Dendauw, J., VanBockstaele, E., Depicker, A., and De Loose, M. 2000. AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* spp.). Mol Breed, 6: 125-134.
52. Rosenberg, N.A., Pritchard, J.K., Weber, J.L., Cann, H.M., Kidd, K.K., Zhivotovsky, L.A. and Feldman, M.W. 2002. The genetic structure of human populations. Sci, 298: 2381-2385.
53. Rossetto, M., Weaver, P.K. and Dixon, K.W. 1995. Use of RAPD analysis in devising conservation strategies for the rare and endangered *Grevillea scapigera* (Proteaceae). Mol Ecol, 4: 321–329.
54. Savy Filho, A. 2005. Castor bean breeding. In: Borém A. (Ed.) Improvement of Cultivated Species. Viçosa: Federal University of Viçosa.
55. Schall, B.A., Hayworth, D.A., Olsen, K.M., Rauscher, J.T. and Smith, W.A. 1998. Phylogeographic studies in plants: problems and prospects. Mol Ecol, 7: 465– 474.

56. Shannon, C.E. and Weaver, W. 1949. The Mathematical theory of communication, Urbana (Illinois), University of Illinois Press.
57. Sica, M., Gamba, G., Montieri, S., Gaudio, L. and Aceto, S. 2005. ISSR markers show differentiation among Italian populations of *Asparagus acutifolius* L. BMC Genet, 6: 17.
58. Shi, W., Yang, C.-F., Chen, J.-M. and Guo, Y.-H. 2007. Genetic variation among wild and cultivated populations of the Chinese medicinal plant *Coptis chinensis* (Ranunculaceae). Plant Biol, 10: 485-491.
59. Slatkin, M., and Barton, N. H. 1889. A comparison of three methods for estimating average levels of gene flow. Evol, 43: 1349-1 368.
60. Solomon, K.F., Labuschagne, M.T. and Viljoen, C.D. 2007. Estimates of heterosis and association of genetic distance with heterosis in durum wheat under different moisture regimes. J Agric Sci, 145: 239-248.
61. Tachida, H. and Yoshimaru, H. 1996. Genetic diversity in partially selfing populations with the stepping-stone structure. Heredity, 77(5): 469–475.
62. Tomar Rukam, S., Parakhia, M.V., Kavani, R.H., Dobariya, K.L., Thakkar, J.R., Rathod, V.M., Dhingani, R.M. and Golakiya B.A. 2014. Characterization of castor (*Ricinus communis* L.) genotypes using different markers. Res J Biotech, 9(2): 6-13.
63. Velasco, L., Rojas-Barros, P. and Fernández-Martínez, J.M. 2005. Fatty acid and tocopherol accumulation in the seeds of a high oleic acid castor mutant. Ind Crop Prod, 22: 201–206.
64. Verma, S. and Rana, T.S. 2011. Genetic diversity within and amon g the wild populations of Murraya koenigii (L.) Spreng., as revealed by ISSR analysis. Biochem Syst Ecol, 39: 139–144.
65. Wang, H.Z., Feng, S.G., Lu J.J., Shi, N.N. and Liu, J.J. 2009. Phylogenetic study and molecular identification of 31 DendrObium species using inter-simple sequence repeat (ISSR) markers. Sci Hort, 122: 440–447.
66. Weising, K., Nybom, H., Wolff, K. and Meyer, W. 1995. DNA fingerprinting in plants and fungi. CRC Press, Inc Boca Raton, FL. 322 pp.
67. Yao, M.Z., Chen, L. and Liang, Y.R. 2008. Genetic diversity among tea cultivars from China, Japan and Kenya revealed by ISSR markers and its implication for parental selection in tea breeding programmes. Plant Breed, 127: 166 -172.
68. Yeh, F.C., Yang, R.C., Boyle, T.J., Ye, Z.H. and Mao, J.X. 1999. Popgene ver. 1.32, the user-friendly shareware for population genetic analysis. Edmonton, Canada: Molecular Biology and Biotechnology Centre, University of Alberta.
69. Yip, P.Y., Chau, C.F., Mak, C.Y. and Kwan, H.S. 2007. DNA methods for identification of Chinese medicinal materials. Chin Med, 2(9): 1-19.
70. Zhao K.G., Zhou M.Q., Chen L.Q. 2007. Genetic diversity and discrimination of *Chimonanthus praecox* (L.) Link germplasm using ISSR and RAPD markers. Hortscience, 42(5): 1144–1148.
71. Zheng L., Qi J., Fang P., Su J., Xu J., Tao A. 2010. Genetic diversity and phylogenetic relationship of castor germplasm as revealed by SRAP analysis. Plant Sci J, 28(1): 1-6.
72. Zietkiewicz, E., Rafalski, A. and Labuda, D. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics, 20: 176–183.

تجزیه ژنتیکی کرچک (*Ricinus communis* L.) با استفاده از نشانگرهای ISSR

فرناز گودرزی[۱]، رضا درویش‌زاده[۲،۳]*، عباس حسنی[۱]

۱. گروه علوم باغبانی دانشکده کشاورزی دانشگاه ارومیه

۲. گروه اصلاح و بیوتکنولوژی گیاهی دانشکده کشاورزی دانشگاه ارومیه

۳. گروه علوم باغبانی، دانشکده کشاورزی دانشگاه ارومیه

*نویسنده مسوول: r.darvishzadeh@urmia.ac.ir

چکیده

کرچک (*Ricinus communis* L.) یکی از قدیمی‌ترین گیاهان دارویی روغنی در جهان است. این گیاه به طور گسترده در قسمت‌های مختلف ایران پراکنده شده است. در مطالعه حاضر تنوع ژنتیکی در بین و درون ۱۲ توده کرچک جمع‌آوری شده از ۷ ناحیه کشور با استفاده از نشانگرهای بین ریزماهواره‌ای ارزیابی شد. در کل با ۱۶ آغازگر ISSR تعداد ۱۶۶ مکان تکثیر شد که از آنها ۱۱۶ مکان (۶۹/۸۹ درصد) پلی‌مورف بودند که نشان‌دهنده تنوع ژنتیکی بالا در ژرم پلاسم کرچک است. در توده "۲۹-۸۰" یک نوار ISSR"ویژه‌ی توده" شناسایی شد. فاصله ژنتیکی بین توده‌ها در دامنه بین ۰/۲ تا ۰/۰۵۶ متغیر بود. تجزیه واریانس مولکولی نشان داد ۸۰ درصد از واریانس کل مربوط به درون توده‌ها و ۲۰ درصد مربوط به بین آنهاست. بر اساس روش بیزی ۶۰ ژنوتیپ متعلق به ۱۲ توده در ۶ زیر گروه قرار گرفتند. در تجزیه کلاستر با روش UPGMA بر اساس فاصله ژنتیکی Nei تعداد ۱۲ توده مورد مطالعه در ۴ زیر گروه قرار گرفتند. نتایج نشان می‌دهد که ارتباطی بین الگوی ISSR و منشاء جغرافیایی توده‌ها وجود ندارد. نتایج بدست آمده حاکی از آن است که تکنیک ISSR یک ابزار مفید برای مطالعه تنوع ژنتیکی در ژرم پلاسم کرچک است.

کلمات کلیدی: تجزیه کلاستر به روش بیزی، تنوع ژنتیکی، ISSR، *Ricinus communis*