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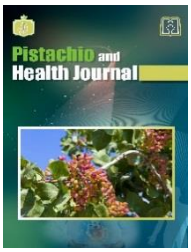

ORIGINAL ARTICLE

Efficacy of SCoT and ISSR markers in assessment of genetic diversity in some Iranian pistachio (*Pistacia vera* L.) cultivars

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Background: Start Codon Targeted (SCoT) and Inter-Simple Sequence Repeat (ISSR) markers were used to appraise genetic diversity existed in 20 samples of four Iranian pistachio (*Pistacia vera* L.) cultivars and relative efficiencies of the marker systems were compared.

Materials and Methods: 20 and 15 primers were used in SCoT and ISSR markers, respectively. Effective Multiplex Ratio (EMR), Marker Index (MI), Resolving Power (RP) and Polymorphic Information Content (PIC) of the primers were assessed for the two marker systems. Cluster analysis for molecular data was performed. Principal Coordinate Analysis (PCoA) was done too.

Results: The most of the parameters examined were found to be more suitable in ISSR system. The most remarkable result of the current research is that cluster analysis on SCoT and ISSR data clearly discriminated the cultivars in terms of their genetic characterizations. There was a high similarity between dendrogram derived from ISSR marker and dendrogram derived from both markers, in spite of some observed differences.

Conclusions: It is shown in the current research that there is remarkable genetic diversity among evaluated samples. SCoT and ISSR analysis produces adequate polymorphisms for DNA fingerprinting. This research reports the first application of the SCoT marker in characterization of Iranian pistachio cultivars.

Keywords: genetic diversity; ISSR marker; pistachio; SCoT marker

1. Introduction

Pistacia vera L. (pistachio) is a diploid (2n=2x=30) plant belonging to the Anacardiaceae family [1]. The most widely accepted classifications divide the family into five tribes: Anacardieae, Rhoeae, Semecarpeae, Spondiadeae, and Dobineae [2, 3]. *Pistacia vera* belongs to the Rhoeae tribe. The pistachio genus contains 13 or more species [4]. Research efforts have mainly focused on *P. vera*, and the other species were overlooked due to a large extent to the economic importance of *Pistacia vera* [5]. Two main diversity centers are found for Pistachio. The first one includes the Mediterranean region of Europe, Northern Africa, and the Middle Eastern countries. The second one is Caucasus regions and the Eastern part of Zagros Mountains from Crimea to the Caspian Sea [1]. The main cultivars grown in Iran are Fandoghi, Akbari, Kaleh-ghochi, Ahmad-Aghai, Badami, and Pustpiazi [6]. DNA-based markers provide accurate and useful information on the extent of diversity and relationships among plant species [4]. The first classification of Pistachio species at the molecular level was done according to chloroplast DNA profiles by Parfitt and Badenes in 1997 [4]. Pistachio has a high genetic diversity because of its dioecious and heterozygous nature [7]. Pistachio breeding programs were recently initiated for developing new cultivars [7]. DNA markers that are closely connected to important agronomic traits greatly contribute to practical crop improvement programs [8]. Since 1994, a new molecular marker technique named Inter Simple Sequence Repeat (ISSR) was available [8]. ISSRs are semi-arbitrary markers amplified by PCR in the attendance of one primer complementary to a target microsatellite [9]. The technique uses microsatellites, usually 16–25 bp long, as primers in a single primer PCR reaction targeting multiple genomic loci to amplify mainly the inter- SSR sequences of different sizes [8, 10]. In recent years, many new alternative and promising marker techniques have been developed in line with the rapid growth of genomic research [11]. Due to the enormous growth in public biological databases, the functional markers development that are placed in or near the candidate genes have become considerably easy [12]. Starting up a trend away from random DNA markers against gene-targeted markers, SCoT that is a novel marker system was developed according to the short conserved region flanking the ATG start codon in plant genes. SCoT markers are generally reproducible, and it offered that length of primer and annealing temperature are not the only factors that determine reproducibility. They are dominant markers like RAPDs and could be applied for genetic analysis, quantitative trait loci (QTL) mapping, and bulk segregation

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analysis [13]. In principle, SCoT has a similarity to RAPD and ISSR, its cause is that the same single primer was used as the forward and reverse primer [13, 14]. A limited number of researches have investigated the genetic diversity cultivars of P. vera. Therefore, our objectives in this study were evaluation of the genetic diversity of some *Pistacia vera* cultivars grown in Iran and to compare relative efficiencies of SCoT and ISSR markers with respect to their applicability in genetic diversity studies of *Pistacia vera* genotypes.

2. Materials and Methods

2.1. Plant materials

Fresh leaf segments of pistachio common cultivars (Akbari, Ahmad-Aghaei, Kaleh-ghouchi, and Fandoghi, five samples for each one) were randomly harvested from five different regions with a large area under the cultivation of pistachio of Kerman province that are listed in Table 1. For preventing deterioration, the samples with three replications were immediately transferred to liquid nitrogen tanks. Then Young leaves tissues (100 mg) were ground under liquid nitrogen to obtain a fine powder. CTAB (the Doyle and Doyle) method (1987) with minor modifications was used for extracting genomic DNA from powdered leaves [4]. Spectrophotometry and gel electrophoresis methods were used for determining the quantity and quality of DNA. DNA samples were diluted to 40 ng/µL with distilled water and stored at -20°C for further use.

Table 1. List of 20 *Pistacia vera* samples used in this study and their origins

No.	Sample	Code
1	Ahmad-Aghaei (Rf)	A1
2	Kaleh-ghouchi (Rf)	B1
3	Akbari (Rf)	C1
4	Fandoghi (Rf)	D1
5	Ahmad-Aghaei (Za)	A2
6	Kaleh-ghouchi (Za)	B2
7	Akbari (Za)	C2
8	Fandoghi (Za)	D2
9	Ahmad-Aghaei (An)	A3
10	Kaleh-ghouchi (An)	B3
11	Akbari (An)	C3
12	Fandoghi (An)	D3
13	Ahmad-Aghaei (Ra)	A4
14	Kaleh-ghouchi (Ra)	B4
15	Akbari (Ra)	C4
16	Fandoghi (Ra)	D4
17	Ahmad-Aghaei (Ke)	A5
18	Kaleh-ghouchi (Ke)	B5
19	Akbari (Ke)	C5
20	Fandoghi (Ke)	D5

(An: Anar, Rf: Rafsanjan, Za: Zarand, Ke: Kerman, Ra: Ravar)

2.2. PCR primers, materials, and conditions

In the present research, which was conducted in Graduate University of Advanced Technology and Vali-e-Asr University of Rafsanjan in 2016, 35 available ISSR primers were assayed for initial screening. Of the 35 primers, 15 primer which gave the most informative patterns (in terms of repeatability and scorability) were selected for identification. Twenty single SCoT primers without any initial assessment and 15 ISSR primers were used for fingerprinting. Amplifications of SCoT and ISSR primers were done in a 25 µL reaction volume containing 1 µL DNA(40 ng), 12.5 µL Taq DNA Polymerase, 2x Master Mix Red Ampliqon (1.5 mM $MgCl_2$ final concentration), 1.1 µL of 10 µM primer, and 10.4 µL of distilled water. Amplification of SCoT primers was performed in a programmed thermocycler (Biorad Model T100) with an initial denaturation of DNA in 94°C for 3 min, followed by 35 cycles of 94°C for 1 min, between 50°C to 57°C for 1 min, and 72°C for 2 min about denaturation, annealing, and extension steps, respectively. The final extension at 72°C was held for 5 min. PCR reactions for amplification of ISSR primers were performed as described in SCoT analysis with the exception of primer volume (2.4 µL). The thermo cycler program for PCR was set to 2 min at 94°C, followed by 35 cycles of 94°C for 0.5 min, 1 min at between 45°C to 56°C and 2 min at 72°C about denaturation, annealing and extension steps, respectively. The final extension at 72°C was held for 5 min. All PCR amplification bands were separated on 1.5% agarose gel in Tris-Borate Buffer (TBA) stained with Ethidium-Bromide and documented using gel documentation system (UVTEC, UK). In order to investigate the repetitiveness of SCoT and ISSR bands, PCR operation was conducted on samples in the same conditions again; the repetitiveness of the obtained bands was confirmed. The set of ISSR and SCoT primers used in our investigation is shown in Table 2.

2.3. Data Analysis

The presence or absence of each of the observed bands for each of 35 primers was scored 1 and 0, respectively and then a matrix of 0 and 1 digits in Excel software was provided. Genetic association amongst samples was evaluated by the Jaccard's similarity coefficient [16]. The similarity matrix was subjected to cluster analysis of UPGMA method, and a dendrogram was generated using NTSYS software (version 2.02) [15]. Principal Coordinate Analysis (PCoA) was also done. Polymorphic Information Content (PIC) of each of the analyzed SCoT and ISSR was measured using the formula $PIC = 1- p^2-q^2$ [17], where p is frequency of present band, and q is frequency of absent band. The band in formativeness (Ib) was estimated as $Ib = 1 – (2 \times |0.5 - p|)$ [18], where p is the proportion of the varieties or genotypes containing the band. The Resolving Power of the primer (RP) was calculated in accordance with $RP = \Sigma Ib$. Marker Index (MI) was computed as EMR × PIC, where EMR (Effective Multiplex Ratio) was defined as the product of the total number of fragments per primer (n) and the fraction of polymorphic fragments (β). $EMR = n\beta$: n= total number of bands, β= total number of polymorphic bands. [18-20].

Table 2. The sequences of both SCoT and ISSR primers

SCoT Primers	5'→3' Sequence	Ta°C	ISSR primers	5'→3' Sequence	Ta°C
SCoT1	CAACAATGGCTACCACCA	50°C	UBC807	(GA)8CT	54°C
SCoT2	CAACAATGGCTACCACCC	53°C	UBC808	(AG)8C	52°C
SCoT3	CAACAATGGCTACCACCG	53°C	ISSR7	CCAG(GT)7	56°C
SCoT4	CAACAATGGCTACCACCT	50°C	UBC836	(AG)8CA	54°C
SCoT5	CAACAATGGCTACCACGA	50°C	ISSR4	(CA)7AG	56°C
SCoT6	CAACAATGGCTACCACGC	53°C	K10	(AC)8CG	50°C
SCoT7	CAACAATGGCTACCACGG	53°C	K11	(GT)8CC	50°C
SCoT8	CAACAATGGCTACCACGT	50°C	K13	(AG)8G	54°C
SCoT9	CAACAATGGCTACCAGCA	50°C	ISSR9	(AC)8T	55°C
SCoT10	CAACAATGGCTACCAGCC	53°C	ISSR10	(TG)8TT	45°C
SCoT11	AAGCAATGGCTACCACCA	50°C	K25	(GA)8A	50°C
SCoT12	ACGACATGGCGACCAACG	55°C	K26	(AG)8T	50°C
SCoT13	ACGACATGGCGACCATCG	55°C	K24A	(GA)8T	53°C
SCoT14	ACGACATGGCGACCACGC	57°C	ISSR6	GT(CAC)7	55°C
SCoT15	ACGACATGGCGACCGCGA	57°C	UBC851	(GT)8CG	56°C
SCoT16	ACCATGGCTACCACCGAC	55°C			
SCoT17	ACCATGGCTACCACCGAG	55°C			
SCoT18	ACCATGGCTACCACCGCC	57°C			
SCoT19	ACCATGGCTACCACCGGC	57°C			
SCoT20	ACCATGGCTACCACCGCG	57°C			

3. Results

This research reports the first application of the SCoT technique in pistachio characterization of *P. vera* cultivars. The genetic diversity indices of 20 SCoT primers are shown in Table 3. Using 20 selected SCoT primers, 151 bands were generated, of which 138 bands (91.3%) were polymorphic (Fig. 1). The maximum and lowest numbers of amplified bands were for SCoT13 with 13 bands and SCoT12 and SCoT20 with 5 bands, respectively. The values of polymorphism varied from 75% to 100%.

The maximum and lowest PIC values were for SCoT10 (0.407) and SCoT9 (0.211), respectively. The average RP value in SCoT marker was 4.18. The range of Marker Index was from 0.953 (SCoT9) to 4.62 (SCoT13). The average MI value in SCoT marker was 2.335. Cluster analysis based on UPGMA algorithm with SCoT marker grouped the 20 samples of four cultivars into three main clusters (Fig. 2). The first cluster, group I, consists of two cultivars. The second main cluster, group II, includes 17 cultivars. The third major cluster, group III, consists of one cultivar: Akbari Rafsanjan (C1). All of the groups include several samples except for Akbari Rafsanjan (C1) that builds a separate group in SCoT marker. The Cultivars Kaleh-ghouchi Ravar (B4) and Fandoghi Ravar (D4), which are genetically close cultivars, fell in same group in SCoT marker. Other cutting lines (Fig. 2) show subgroups and more details of classification. In the present research, the ability of 15 ISSR primers to generate polymorphic DNA fragments was investigated.

The genetic diversity indices of 15 ISSR primers are shown in Table 3. 15 selected ISSR primers amplified 131 bands with 124 (94. 6%) being polymorphic (Fig. 3). The maximum and lowest number of amplified bands was for UBC851 and K13 with 11 bands and UBC808 with four bands, respectively. The values of polymorphism ranged from 80% to 100%. The maximum and lowest PIC values were ISSR9 (0.47) and ISSR7 (0.187), respectively.

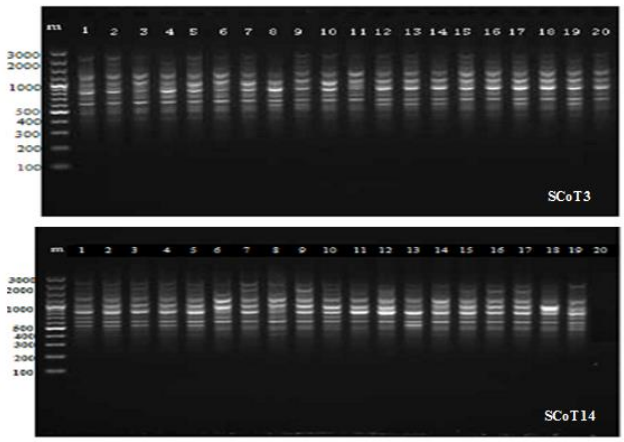

Fig. 1. Fingerprints of 20 *Pistacia vera* samples with two SCoT primers. M= 100 bp DNA ladder.
(The names of 1 to 20 numbers are listed in Table 1)

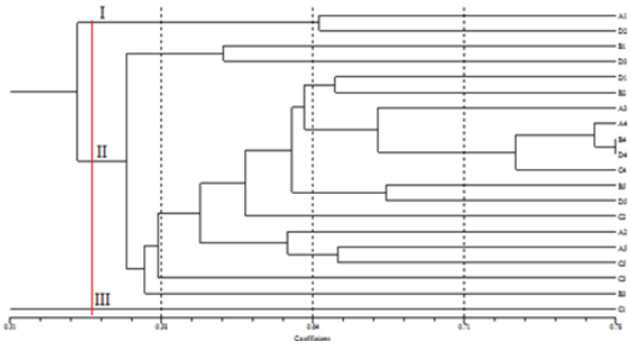

Fig. 2. Dendrogram of 20 samples of four pistachio cultivars based on SCoT marker clustered by UPGMA method

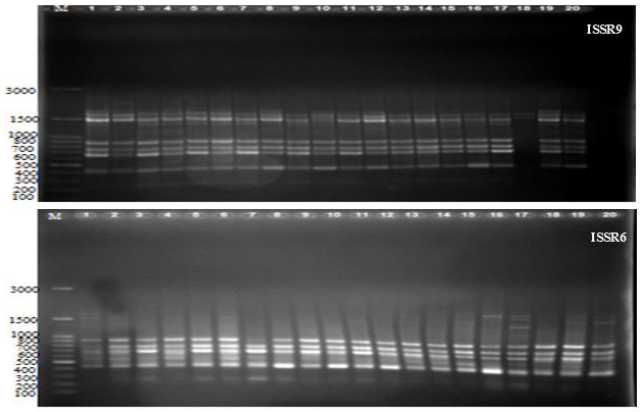

Fig. 3. Fingerprints of 20 *Pistacia vera* samples with two ISSR primers. M= 100bp DNA ladder.
(The names of 1 to 20 numbers are listed in Table 1)

The average RP value in ISSR marker was 5.13. The maximum and lowest MI indices were K13 (4.43) and UBC808 (1.145). The average MI value in ISSR marker was 2.916. Cluster analysis with UPGMA algorithm based on ISSR marker grouped the 20 samples of four cultivars into three major clusters (Fig. 4). The first cluster (group I) further divided into two sub-clusters. The first sub-cluster consisted of Ahmad-Aghaei Rafsanjan (A1). The second sub-cluster contained seven samples. The second main cluster (group II) further divided into two sub-groups. The first sub-group consisted of seven cultivars. The second sub-group consisted of 4 samples. All of the groups include several samples except for Akbari Rafsanjan (C1) that builds a separate group in ISSR marker. Other cutting lines (Fig. 4) show subgroups and more details of classification. A possible interpretation for the difference in the resolution of SCoT and ISSR primers is that the two-marker techniques target various parts of the genome. These differences may also be attributed to marker sampling errors and/or the percent polymorphism detected by different markers, highlighting the importance of the number of loci and their coverage of the overall genome in obtaining reliable estimates of genetic relationships among cultivars [21, 22].

Table 3. Characteristics of SCoT and ISSR banding profiles produced in 20 samples of four cultivars

Primer code	Total No. bands	No. poly bands	% of poly. bands	PIC values	RP	MI	EMR
SCoT1	67	56	83.3	0.35	3.3	1.48	4.16
SCoT2	127	113	88.9	0.32	4.3	2.31	7.10
SCoT3	138	125	90.9	0.35	5.8	3.22	9.09
SCoT4	78	78	100	0.40	3.8	2.41	6.00
SCoT5	99	99	100	0.39	4.3	2.98	7.60
SCoT6	141	141	100	0.37	6.1	4.16	11.0
SCoT7	85	85	100	0.39	5.1	3.19	8.00
SCoT8	86	72	83.3	0.35	3.4	1.48	4.16
SCoT9	133	100	75	0.21	2.5	0.95	4.50
SCoT10	93	93	100	0.40	4.7	2.85	7.00
SCoT11	94	81	85.7	0.34	3.8	1.75	5.14
SCoT12	74	74	100	0.35	2.6	1.76	5.00
SCoT13	168	168	100	0.35	6.8	4.62	13.0
SCoT14	98	84	85.7	0.36	4.2	1.89	5.14
SCoT15	143	127	88.9	0.27	3.7	1.97	7.11
SCoT16	98	98	100	0.39	4.2	2.77	7.00
SCoT17	120	105	87.5	0.31	4.0	1.93	6.12
SCoT18	153	139	90.9	0.33	5.3	3.07	9.09
SCoT19	105	90	85.7	0.28	3.1	1.44	5.14
SCoT20	70	56	80	0.33	2.8	1.05	3.20
UBC807	131	105	80	0.22	3.5	1.42	6.40
UBC808	51	51	100	0.28	1.5	1.14	4.00
UBC836	87	87	100	0.44	5.3	3.13	7.00
UBC851	149	149	100	0.40	7.1	4.41	11.0
K24A	113	113	100	0.36	4.7	3.32	9.00
ISSR4	141	127	90	0.34	5.1	2.80	8.10
ISSR6	115	115	100	0.39	4.5	3.12	8.00
ISSR7	144	115	80	0.18	7.2	1.19	6.40
ISSR9	94	94	100	0.47	6.4	3.76	8.00
ISSR10	112	112	100	0.38	4.8	3.07	8.10
K10	135	121	90	0.37	5.7	3.10	8.10
K11	101	101	100	0.46	7.1	4.17	9.00
K13	146	146	100	0.40	6.6	4.43	11.0
K25	127	111	87.5	0.30	3.3	1.85	6.12
K26	115	115	100	0.35	4.5	2.87	8.00

PIC= Polymorphic Information Content, RP = Resolving Power, MI= Marker Index EMR = Effective Multiplex Ratio.

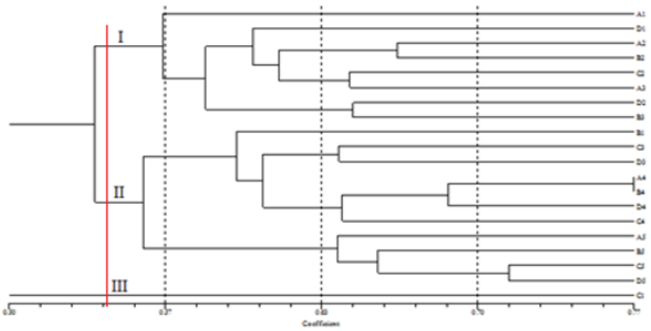

Fig. 4. Dendrogram of 20 samples of four pistachio cultivars based on ISSR-marker clustered by UPGMA method

Cluster analysis with UPGMA algorithm based on ISSR and SCoT markers grouped the 20 samples of four cultivars into three major clusters (Fig. 5). The first cluster, group I, further divided into two sub-groups. The first sub-group consisted of one cultivar, Ahmad-Aghaei Rafsanjan (A1). The second sub-group contained seven cultivars. The second main cluster, group II, further divided into two sub-groups. The first sub-group contained seven cultivars. The second sub-cluster consisted of four cultivars. All of the groups include several samples except for Akbari Rafsanjan (C1) that builds a separate group in ISSR and SCoT markers. Other cutting lines (Fig. 5) show subgroups and more details of classification. The dendrogram obtained using the combined data of the two sets of molecular markers is approximately similar to ISSR marker dendrogram (for instance, group of Ahmad-Aghaei Ravar and Kaleh-ghouchi Ravar, Fandoghi Ravar; Kaleh-ghouchi Kerman, Fandoghi Kerman; Fandoghi Rafsanjan, Kaleh-ghouchi Zarand; Kaleh-ghouchi Rafsanjan, Fandoghi Anar; Fandoghi Zarand, Kaleh-ghouchi Anar; Akbari Rafsanjan and Ahmad-Aghaei Rafsanjan). According to Fig 5, the strongest similarity was between Kaleh-ghouchi Ravar and Fandoghi Ravar and the weakest similarity was observed in Akbari and Ahmad-Aghaei Rafsanjan. The cophenetic coefficient was acceptable in both molecular marker systems, indicating a good fit for clustering.

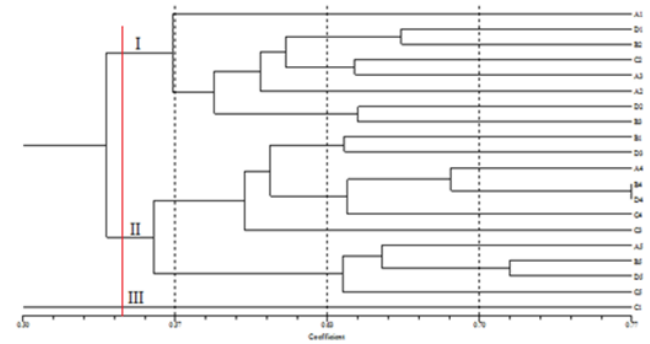

Fig. 5. Dendrogram of 20 samples of four cultivars of *Pistacia vera* based on SCoT and ISSR-marker clustered by UPGMA method

The values of mantel test correlation determined a positive and significant correlation between the SCoT and ISSR. Based on PCoA analysis, all Pistachio samples fell in three distinct groups (Fig. 6). The results of SCoT and ISSR markers Dendrogram and PCoA analysis are relatively consistent with each other.

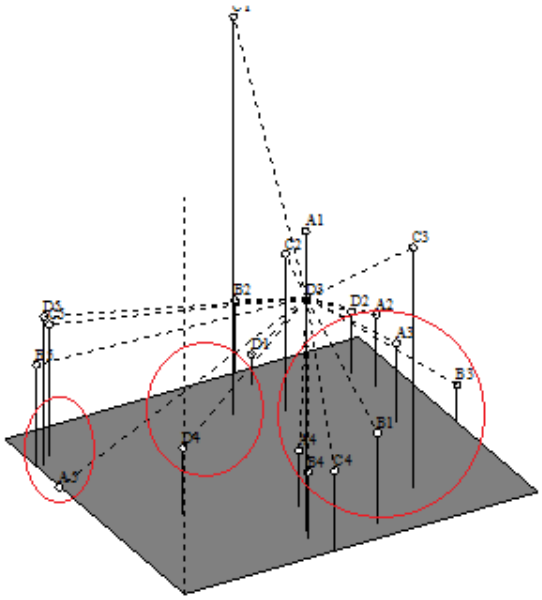

Fig. 6. Three-dimensional plot of principal coordinate analysis (PCoA) of 20 samples of four cultivars of *Pistacia vera* using SCoT and ISSR- marker

4. Discussions

Using more than one marker has always been recommended for a better analysis of genetic homogeneity of plants [23]. Recently, Simple Sequence Repeat (SSR) marker has been applied to distinguish 17 cultivars of pistachio by their nuts collected from the markets in the U.S.A and in Europe [24], and in another research, SSR primers were applied to analyze four commercially important pistachio rootstocks grown in California [25]. Genetic relationships among thirty one samples of Iranian pistachio were assessed by six ISSR primers. Good amplification products were obtained from primers based on GA, CA, and GAA repeats. The range of genetic similarity was from 0.84 to 1. The cluster analysis divided the samples into 11 groups [9]. In the present research, a comparative assessment of SCoT and ISSR markers for the evaluation of Pistachio genetic diversity was done. Not all ISSR primers were suitable. 20 out of 35 ISSR primers did not produce any bands. SCoT and ISSR markers generated high numbers of polymorphic bands that can be used in diagnostic fingerprinting of *Pistacia vera*. Based on the data obtained from these two molecular markers, i.e., polymorphism percentage, PIC values, RP values, and marker index, the efficiency of ISSR for fingerprinting of cultivars was more than other marker. These two techniques could be used in conjunction with each other for diagnostic fingerprinting of *Pistacia vera* cultivars. Pakseresht *et al.* (2013) performed a comparative evaluation of ISSR, DAMD, and SCoT markers for the assessment of genetic

diversity and protection of chickpea genotypes and reported that the SCoT and DAMD markers are more effective in fingerprinting of chickpea genotypes [26]. Application of SCoT marker in other plants such as mango [27], potato [28], and Cicer [29] showed that this marker is efficient.

5. Conclusions

The present research showed that there is remarkable genetic diversity among evaluated samples. ISSR and SCoT analysis produces sufficient polymorphisms for DNA fingerprinting. This research reports the first application of the SCoT marker in the characterization of Iranian pistachio cultivars. Generally, ISSR and SCoT markers could be used in conjunction with each other for diagnostic fingerprinting of *Pistacia vera* cultivars.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Zohary M. A monographical study of the genus Pistacia. Palestine Journal of Botany (Jerusalem Series). 1952;5(4):*187-228.*
2. Mitchell J D, Mori SA. The Cashew and Its Relatives: Mem. New York Bot. Gard; 42. 1987.
3. Wannan B S, Quinn C J. Floral structure and evolution in the Anacardiaceae. Botanical Journal of the Linnean Society. 1991;107(4): *349-385*.
4. Kafkas S, Ozkan H, Ak E, Acar I, Atli H S, Koyuncu S. Detecting DNA polymorphism and genetic diversity in a wide pistachio germplasm: Comparison of AFLP, ISSR, and RAPD markers. Journal of the American Society for Horticultural Science. 2006; 131(4):*522-529.*
5. Whitehouse W E. The pistachio nut-a new crop for the western United States. Economic Botany. 1957; 11(4): *281-321*.
6. Esmail-Pour A. Effect of Bane rootstocks on quantitative and qualitative traits of Commercial Product pistachio cultivars. Second National Conferences of Pistacia. Shiraz. 2001.
7. Tajabadipur A. Identification of some pistachio cultivars (Doctoral dissertation, M. Sc. thesis). 1997.
8. Zietkiewicz E, Rafalski A, Labuda D. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics.1994;20 (2):*176-183*.
9. Noroozi S, Baghizadeh A, Jalali M. The genetic diversity of Iranian pistachio (*Pistacia vera* L.) cultivars revealed by ISSR markers. Biol Diver Conserv. 2009;2: *50-56.*
10. Gupta P K, ChyiY S, Romero-Severson J, OwenJ L. Amplification of DNA markers from evolutionary diverse genomes using single primers of simple-sequence repeats. Theoretical and Applied Genetics. 1994; 89: *998–1006*.
11. Gupta P K, Rustgi S. Molecular markers from the transcribed/expressed region of the genome in higher plants. Functional & integrative genomics. 2004;4(3):*139-162*.
12. Andersen J R, Lübberstedt T. Functional markers in plants. Trends in plant science. 2003;8(11): 554-560.
13. Collard B C, Mackill D J. Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. Plant molecular biology reporter.2009; 27(1):*86-93.*
14. Gupta M, Chyi Y S, Romero-Severson J, Owen J L. Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. TAG Theoretical and Applied Genetics.1994; 89(7): *998-1006.*
15. Rohlf F J. NTSYS-pc, numerical taxonomy and multivariate system. version 2.0. Exeter Software, New York. 1998.
16. Jaccard P. Nouvelles recherches surla distribution florale. Bull Soc Vaud Sci Nat. 1908; 44: 223–270.
17. Shahlaei A, Torabi S, Khosroshahli M. Efficacy of SCoT and ISSR markers in assessment of tomato (*Lycopersicum esculentum* Mill.) genetic diversity. Int. J. Biol. Sci.2014; *5*: *14-22.*
18. Ghislain M, Zhang D, Fajardo D, Huamán Z, Hijmans R J. Marker-assisted sampling of the cultivated Andean potato *Solanum phureja* collection using RAPD markers. Genetic Resources and Crop Evolution.1999; 46(6):*547-555.*
19. Prevost A, Wilkinson M J. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theoretical and Applied Genetics.1999; 98(1): *107-112.*
20. Tiwari S, Tiwari R, Srivastava R, Kumari P, Singh S K. Molecular genetic analysis of *Eucalyptus tereticornis* by using RAPD markers. European Journal of Experimental Biology.2013; 3(6): *103-110.*
21. Nagaraju J, Reddy K D, Nagaraja G M, Sethuraman B N. Comparison of multilocus RFLPs and PCR-based marker systems for genetic analysis of the silkworm, Bombyx mori. Heredity. 2001; 86(5):*588-597.*
22. Gajera B B, Kumar N, Singh A S, Punvar B S, Ravikiran R, Subhash N, Jadeja G C. Assessment of genetic diversity in castor (*Ricinus communis* L.) using RAPD and ISSR markers. Industrial Crops and Products.2010;32(3):*491-498.*
23. Lakshmanan V, Reddampalli V S, Neelwarne B. Molecular analysis of genetic stability in long-term micropropagated shoots of banana using RAPD and ISSR markers. Electronic Journal of Biotechnology.2007;10(1): *106-113.*
24. Ahmad R, Ferguson L, Southwick S M. Identification of pistachio (*Pistacia vera* L.) nuts with microsatellite markers. Journal of the American Society for Horticultural Science.2003;128(6), *898-903.*
25. Ahmad R, Ferguson L, Southwick S M. Molecular marker analyses of pistachio rootstocks by simple sequence repeats and sequence-related amplified polymorphisms. The Journal of Horticultural Science

and Biotechnology.2005;80(3): *382-386.*

26. Pakseresht F, Talebi R, Karami E. Comparative assessment of ISSR, DAMD and SCoT markers for evaluation of genetic diversity and conservation of landrace Chickpea (*Cicer arietinum* L.) genotypes collected from North- West of Iran. Physiol Mol Biol Plants.2013; 19(4):*563-574.*
27. Luo C, He X H, Chen H, Ou S J, Gao M P, Brown J S, Schnell R J. Genetic diversity of mango cultivars estimated using SCoT and ISSR markers. Biochemical Systematics and Ecology.2011; 39(4):*676-684.*
28. Pour Gorgi A, Rahimi M, Hajianfar R, Hassanpanah D, Vedadi S, Noori M, Neshan N. Creation of genetic diversity using reciprocal crossing method and amp; mutation in order to select a superior plant of potato (CV. Kennebec, Agria, Buren, Clone 397007 (Savalan) and Banba). Seed and Plant Improvement institute. 2011. 59 p.
29. Amirmoradi B, Talebi R, Karami E. Comparison of genetic variation and differentiation among annual Cicer species using start codon targeted (SCoT) polymorphism, DAMD-PCR, and ISSR markers. Plant systematics and evolution.2012;298(9): *1679-1688.*