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Genetic structure analysis and genetic fingerprinting of pomegranate cultivars (*Punica granatum* L.) by using SCoT molecular markers

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Research Article

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Abstract

Genetic diversity and genetic relatedness among 50 genotypes from eight countries, including Iran, Afghanistan, Turkmenistan, Syria, Lebanon, India, Yemen, and the United States located in two continents of Asia and the America, were assessed using SCoT markers. A total of 213 bands were produced; 100% of them were polymorphic; the average polymorphism information content (PIC) was 0.39. The mean Nei's gene diversity and Shannon's index were 0.33 and 0.49, respectively. Analysis of molecular variance suggested significant genetic differences within pomegranate populations. 99% of variance occurs within the populations, whereas 1% of the variation was recorded among the populations of pomegranate. Cluster analysis using SCoT markers able to group genotypes based on their geographical origins. Based on cluster analysis, the genotypes studied were divided into two main groups. The first group included most Asian genotypes, while American genotypes along with some Asian genotypes were in the second group. In the first group, Iranian genotypes were grouped with genotypes from Afghanistan and India. In the second group, the genotypes belonging to the America were in the same group as most of the genotypes of Turkmenistan. According to the present study, SCoT markers can be used to evaluate genetic diversity, identification and DNA fingerprinting pomegranate genotypes of different origins. This information can be used in breeding programs and the management of pomegranate collections.

Introduction

Pomegranate is one of the oldest edible fruits that have high nutritional value and medicinal properties. The genus *Punica* belongs to the family Lythraceae and has two species including *Punica granatum* L. and *Punica protopunica* Balf. (Morris, 2007). Earlier reported chromosome number of cultivated species Punica granatum was 2n = 2x = 16, 18 (Morton, 1987). Researchers believe that pomegranate is native to Iran and its surrounding countries, including Afghanistan, Pakistan, India and Oman, from where it diversified to other regions. The ability of pomegranate trees to adjust to different climatic conditions is reflected in the distribution of the genotype forms throughout Eurasia to the Himalayas (Sarkhosh *et al.*, 2006; Karimi *et al.*, 2020). Various studies show that Iran is considered the pomegranate centre of origin and possesses one of the richest pomegranate gene pools worldwide, which can be used in pomegranate breeding programs (Sarkhosh *et al.*, 2009). The existence of more than 760 pomegranate genotypes with different characteristics proves this claim (Parvizi *et al.*, 2016). Pomegranate cultivars are named independently of their geographical origin, while the characteristics based on the common genetics of the cultivars are contrary to the phenotypic differences (Karimi and Mirdehghan, 2013). In addition, due to the long history of pomegranate cultivation in various environmental conditions, the occurrence of spontaneous mutations and genetic diversity is not unexpected, which is one of the major problems in naming and classifying pomegranate genotypes. Due to the extraordinary importance of this valuable plant, the preparation of molecular identification for existing cultivars and genotypes to determine the identity and genetic ancestry and study of relationships will be a valuable step in identifying and conservation of genetic resources (Sarkhosh *et al.*, 2009). Careful study of plant phenotypic and phylogenetic relationships plays an important role in the development of breeding programmes to produce new cultivars with premium quality and greater resistance to adverse environmental conditions (Zarkti *et al.*, 2010). Morphological markers are used to identify cultivars and genetic resources of fruit trees, but due to the long growth of fruit trees and environmental factors affecting them, this method alone is not valuable. Today, modern biotechnology methods are used for the genetic fingerprinting and identification of the genetic diversity of plants (Yuan *et al.*, 2007; Jbir *et al.*, 2008; Sheidai *et al.*, 2008). A wide range of

molecular markers such as RAPD markers (Talebi Badaf *et al.*, 2003; Hasnaoui *et al.*, 2010), AFLP markers (Yuan *et al.*, 2007; Jbir *et al.*, 2008), SSR markers (Sinjare, 2015; Basaki *et al.*, 2016; Gunnaiah *et al.*, 2021), and ISSR markers (Almiahy and Jum'a, 2017) have been used to evaluate the genetic diversity of pomegranate cultivars and their wild genotypes. A novel molecular marker known as Start Codon Targeted (SCoT) polymorphism targets based on Polymerase Chain Reaction (PCR) which is designed based on the short conserved sequences in plant genes surrounding the ATG initiation codon (Collard and Mackill, 2009). SCoT marker is an effective technique for population studies, assessment of genetic variation and structure, identify cultivars and DNA fingerprinting (Collard and Mackill, 2009). SCoT markers are more reproducible in comparison with RAPD and ISSR (Amirmoradi *et al.*, 2012). The SCoT marker has been successfully used to study genetic diversity in mango (Luo *et al.*, 2012) and citrus fruits (Mahjbi *et al.*, 2015; Juibary *et al.*, 2021). Tabasi *et al.* (2020) reported that the SCoT marker is an effective tool for evaluating genetic diversity; identifying genotypes and DNA fingerprinting of Persian walnut populations. Guo *et al.* (2012) used SCoT polymorphic markers to assess genetic relationships among 64 grape varieties. In this study, a total of 434 loci were produced and 339 loci were polymorphism. Baghizadeh and Dehghan (2018) used SCoT and ISSR markers in the assessment of genetic diversity in some Iranian pistachio and reported that cluster analysis on SCoT and ISSR data discriminated the cultivars. Xiong *et al.* (2011) showed that start codon targeted polymorphism technique can be utilized to identify DNA polymorphisms and fingerprint cultivars in domesticated peanuts. In another study, 22 SCoT markers were used to detect the genetic relationship between male and female pistachio cultivars. The results showed that a total of 434 loci were produced that 339 loci were polymorphism (Malekzadeh *et al.*, 2018). Most previous studies have been on the genetic diversity of pomegranate cultivars in each country, and few comprehensive studies have been conducted on the origin and genetic relationships of pomegranate cultivars in different countries. Therefore, the present study was designed to evaluate the efficiency of SCoT marker in studying the genetic diversity and relationships of Iranian pomegranate cultivars with other countries and also to evaluate the correlation between geographical distance and genetic distance in the studied populations.

Materials and methods

Plant material

A total of 50 pomegranate genotypes from eight countries, including Iran, Afghanistan, Turkmenistan, Syria, Lebanon, India, Yemen and the United States located in two continents of Asia and the America, were used for SCoT analysis. The studied genotypes were geographically divided into two continents: Asia and the America. The characteristics of the populations studied are presented in Table 1.

DNA extraction

Total genomic DNA was extracted from the leaves using the cetyl trimethyl ammonium bromide (CTAB) method described by Murry and Thompson (1980) with minor modifications. DNA quality and quantity were measured using an ultraviolet spectrophotometer at 260 and 280 nm wavelength and 1% (w/v) agarose gel electrophoresis. DNA samples were diluted to 40 ng/µl with distilled water and stored at −20°C for further use.

Polymerase chain reaction

For this study, 36 SCoT primers were assayed for initial screening (Soriano *et al.*, 2010). 15 SCoT primers generated clear amplification products and polymorphisms and were used in further analysis (Table 2). The PCR reaction was performed using PCR Master Mix (2X PCR kit) prepared by Sinagen Company in a volume of 20 microliters. The amplification stages included initial denaturation at 94°C for 3 min, 40 cycles of denaturation at 94°C for 30 s, primer annealing for 30 s and at each primer's optimum temperature, extension at 72°C for 2 min and the final extension at 72°C for 7 min were all conducted in Thermal Cycler Bio-Rad, C1000tm. Amplified products were loaded into the wells of a 1.5% agarose gel in 0.5× TBE buffer, and electrophoresis was conducted at the voltage of 120 for 2 h. Then, gel imaging was performed using Gel Doc equipment (Malekzadeh *et al.*, 2018).

Data analysis

PCR-amplified fragments were scored as either one (1) or zero (0) based on the presence or absence of a band, respectively. To evaluate the efficiency of each primer, polymorphic information content (PIC) (Weising *et al.*, 2005), resolving power (RP) (Prevost and Wilkinson, 1999) and marker index (MI) (Powell *et al.*, 1996) were calculated separately. The similarity matrix was calculated using a Simple Matching coefficient, and cluster analysis was performed based on the complete linkage method in NTSYS pc 2.02e software (Rohlf, 2000).

The dissimilarity coefficients were used to perform principal coordinate analyses (PCoA) and construct Neighbor-Joining trees, analysis of molecular variance (AMOVA) and the relationship between genetic distance and geographical distance of the studied populations were performed by using GenAlex 6.4 (Peakall and Smouse, 2006).

Genetic diversity parameters, including percentage polymorphic loci, the effective number of alleles (ne), Nei's gene diversity (h) (Nei and Li, 1979) and Shannon's information index (I), were calculated by the POPGENE software version 1.32 software (Yeh *et al.*, 2000). The total genetic diversity (Ht), the mean genetic diversity within the population (Hs) and gene diversity among populations (GST) were calculated using POPGENE software version 1.32 (Yeh *et al.*, 2000). The Nei genetic distance (Weising *et al.*, 2005) was determined among the studied populations and was used for the grouping of the populations.

Results

Analysis of genetic diversity using SCoT polymorphic markers

The 15 SCoT primers generated a total of 213 polymorphic bands (100%) with an average of 14.20 bands per primer. The greatest number of SCoT markers was recorded by SCoT19 primer (21 bands), which the SCoT31 primer generates a lower number of polymorphic bands (5 bands). The number of effective alleles varied from 1.37 for SCoT31 primer to 1.74 for SCoT3 primer with an average of 1.55 bands per primer. Polymorphic information content (PIC) ranges from 0.34 to 0.44 with an average of

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Table 1. Details of pomegranate populations used in this study

No.	Cultivar	Origin	Population	Code
1	Malas Mumtaz Saveh	Iran	Asia	AIMS
2	Tabrizi	Iran	Asia	AITA
3	Yousef Khani	Iran	Asia	AIYO
4	Taft Tabas soski	Iran	Asia	AITT
5	Bajestani	Iran	Asia	AIBA
6	Shirin Ghermeze Zabol	Iran	Asia	AIPA
7	Bihaste-e-Khafr Jahrom	Iran	Asia	AIBK
8	Voshik Malas Saravan	Iran	Asia	AIVO
9	Bargmordi	Iran	Asia	AIBG
10	Oude Pooste Ghermez	Iran	Asia	AIOU
11	Ashkezar	Iran	Asia	AIAS
12	Shirin Zodras	Iran	Asia	AISH
13	Alak	Iran	Asia	AIAL
14	Ghojogh Qom	Iran	Asia	AIGH
15	Shirin-e-Shahvar	Iran	Asia	AISS
16	Zhagh Aghda	Iran	Asia	AIZH
17	Poost Siah	Iran	Asia	AIPO
18	Bihaste Ravar	Iran	Asia	AIBR
19	Shishe Kabe	Iran	Asia	AISK
20	Anare Shekari	Iran	Asia	AIAS
21	Golbad	Iran	Asia	AIKO
22	Gol beh Behshahr	Iran	Asia	AIGO
23	Sefid Zodras Shirin	Iran	Asia	AISF
24	Ganesh	India	Asia	AIGA
25	Medovyi Vahsha	Turkmenistan	Asia	ATME
26	Ariana	Turkmenistan	Asia	ATAR
27	Desertnyi	Turkmenistan	Asia	ATDE
28	Cheranaya Roza	Turkmenistan	Asia	ATCH
29	Sirenevyi	Turkmenistan	Asia	ATSI
30	Austin	Syria	Asia	ASAU
31	Kandahari1	Afghanistan	Asia	AAK1
32	Kandahari2	Afghanistan	Asia	AAK2
33	Kandahari3	Afghanistan	Asia	AAK3
34	Red Angel	Lebanon	Asia	ALRA
35	Unknown	Yemen	Asia	AYUH
36	Balegal	United States America	America	AUBA
37	Crab	United States America	America	AUCR
38	Cranberey	United States America	America	AUCB
39	Granada	United States America	America	AUGR
40	Floischmons	United States America	America	AUFL
41	Purple Heart	United States America	America	AUPU
42	American River	United States America	America	AUAM
43	Sweet	United States America	America	AUSW

(*Continued*)

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Table 1. (*Continued.*)

No.	Cultivar	Origin	Population	Code
44	Hall	United States America	America	AUHA
45	Ever sweet	United States America	America	AUWV
46	Elf	United States America	America	AUEL
47	VKusnyi	United States America	America	AUVK
48	Sakerdze	United States America	America	AUSA
49	Eve	United States America	America	AUEV
50	Wonderful	United States America	America	AUWO

Table 2. Characteristics of SCoT primers used in this study

No	SCoT primers	'5→'3 primer sequence	Tm (°C)	% CG
1	SCoT-3	CAACAATGGCTACCACCG	54°C	55.55
2	SCoT-5	CAACAATGGCTACCACGA	54°C	50.00
3	SCoT-7	CAACAATGGCTACCACGG	52°C	55.55
4	SCoT-11	AAGCAATGGCTACCACCA	52°C	50.00
5	SCoT-12	ACGACATGGCGACCAACG	54°C	61.11
6	SCoT-13	ACGACATGGCGACCATCG	54°C	61.11
7	SCoT-14	ACGACATGGCGACCACGC	54°C	66.66
8	SCoT-19	ACCATGGCTACCACCGGC	54°C	66.66
9	SCoT-21	GCTACCACCACATGAACC	54°C	55.55
10	SCoT-22	AACCATGGCTACCACCAC	54°C	55.55
11	SCoT-23	CACCATGGCTACCACCAG	54°C	66.66
12	SCoT-30	CCATGGCTACCACCGGCG	56°C	72.22
13	SCoT-31	GCTACCACCGCCTATGCC	58°C	66.66
14	SCoT-32	CCATGGCTACCACCGCAC	56°C	66.66
15	SCoT-35	CATGGCTACCACCGGCCC	61°C	72.22

0.39. The maximum and lowest PIC values were for the SCoT3 and SCoT31 primers, respectively. The resolution power (Rp) ranged from 0.49 for the SCoT5 primer to 1.24 for the SCoT31 primer with an average of 0.97. The marker index (MI) ranged from 1.71 (SCoT31) to 8.32 (SCoT19) primer with an average of 5.70. H values (Nei's genetic diversity) ranged from 0.26 for SCoT31 to 0.41 for SCoT3, with an average of 0.33 for all primers. I value also showed a similar trend on an average of 0.49, with a maximum of 0.59 for SCoT3 and a minimum of 0.41 for SCoT11. The mean total heterozygosity observed (Ht) in 15 polymorphic markers was 0.33 (Table 3).

Genetic differentiation analysis among different populations of pomegranate

Among the populations from different continents, the average number of alleles in Asia and America were 0.42 and 0.52, respectively. The number of effective alleles ranged from 1.46 in American genotypes to 1.52 in Asian genotypes. Highest observed expected Heterozygosity (0.32) was observed in Asian genotypes, indicating higher genetic diversity compared to the genotypes from different continents. Mean heterozygosity within the population and gene diversity (Gst) among populations were 0.24 and 0.22, respectively (Table 4). No specific bands were observed in at least 25 or 50% of the studied populations, and only 3 specific bands were observed in the Asian population (Table 5).

Molecular analysis of variance (AMOVA)

AMOVA produced significant genetic differences based on SCoT data among the most studied populations. AMOVA analysis showed that the percentage of molecular variance among populations is 1% and within populations is 99%. Genetic differentiation parameters estimate also supported AMOVA and produced significant differences within most pomegranate populations studied. The pairwise genetic differentiation among the genotypes from different continents was compared based on F_{ST} values. F_{ST} values between the two populations were 0.108. The Nei genetic distance obtained for the studied populations based on SCoT data revealed that the distance between populations was 0.092.

Cluster analysis

Cluster analysis was able to classify the studied genotypes based on geographical origin. A dendrogram was obtained by the UPGMA method using the total number of amplified SCoT fragments and grouped into two main clusters (Fig. 1). The first group (I) included most of the Asian genotypes, while the American genotypes and some Asian genotypes were in the second group (II). The first group (I) was further divided into two subgroups, Ia and Ib, segregating at a genetic distance level of 0.51. The first subgroup (Ia) the first subgroup included 19 Iranian genotypes and 2 genotypes from Afghanistan (AAK2 and AAK3) their genetic similarity is justifiable. In the second subgroup (Ib), genotypes from Iran and India (AIGA) were located in this subgroup. Among them, Golbad (AIKO) and Golbeh Behshahr (AIGO) were clustered more closely, indicating a higher similarity in their genetic background. The second group (II) was further divided into three subgroups, IIa; IIb and IIc, at a genetic distance of 0.62. The first subgroup (IIa) The first subgroup (IIa) included mostly American genotypes, as well as some Asian genotypes, but no Iranian genotypes, whereas all Iranian genotypes were found in the first group. Sirenevyi's (ATSI) genotype from Turkmenistan was located in this subgroup. The Red Angel genotype was also classified alongside the Ever sweet genotype. In this subgroup, the highest genetic similarity was related to Elf and Wonderful genotypes from the America. The second subgroup

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Table 3. Statistical summary of 15 polymorphic SCoT used for genetic diversity of 50 pomegranate genotypes

Primer	TAB	NPB	PPB	ne	PIC	Rp	MI	*h*	*I*	Ht
SCoT3	18	18	100	1.74	0.44	0.88	8.00	0.41	0.59	0.41
SCoT5	12	12	100	1.56	0.36	0.49	4.27	0.34	0.52	0.26
SCoT7	13	13	100	1.42	0.38	0.96	4.99	0.27	0.43	0.27
SCoT11	13	13	100	1.45	0.37	0.94	4.79	0.27	0.41	0.25
SCoT12	19	19	100	1.51	0.42	1.09	7.94	0.29	0.42	0.31
SCoT13	14	14	100	1.52	0.38	0.93	5.37	0.31	0.47	0.27
SCoT14	15	15	100	1.65	0.41	0.87	6.09	0.37	0.55	0.38
SCoT19	21	21	100	1.62	0.40	0.79	8.32	0.36	0.53	0.31
SCoT21	12	12	100	1.53	0.38	1.09	4.50	0.31	0.48	0.32
SCoT22	15	15	100	1.62	0.40	1.17	6.21	0.35	0.52	0.34
SCoT23	14	14	100	1.56	0.41	0.60	5.79	0.34	0.52	0.32
SCoT30	16	16	100	1.57	0.42	1.03	6.77	0.33	0.50	0.40
SCoT31	5	5	100	1.37	0.34	1.24	1.71	0.26	0.42	0.40
SCoT32	14	14	100	1.54	0.37	1.16	5.14	0.31	0.46	0.33
SCoT35	12	12	100	1.64	0.43	0.99	5.68	0.36	0.52	0.39
Mean	14.20	14.20	100	1.55	0.39	0.95	5.70	0.33	0.49	0.33

TAB, Total amplified bands; NPB, number of polymorphic bands; PPB, percentage of polymorphic bands; PIC, Polymorphic Information Content; Rp, Resolution Power; MI, Marker Index; ne, Effective number of alleles; *h*, Nei's gene diversity; *I*, Shannon's Information index; Ht, total heterozygosity.

Table 4. Genetic diversity analysis among three pomegranate populations by 15 SCOT markers

Population	*N*	Band Freq.	Na	Ne	*I*	He	UHe	Hs	Gst
Asian	35	0.42	2	1.52	0.49	0.32	0.32		
American	15	0.52	1.85	1.46	0.42	0.28	0.29		
Total population	50	2.46	8.61	7.12	1.96	1.28	2.54		
Mean	25	0.82	2.87	2.37	0.65	0.43	0.85	0.24	0.22

N, Observed number of alleles; Na, No of Different Alleles; *N*, No of Effective Alleles; I, Shannon's Information Index; He, Expected Heterozygosity; uHe, Unbiased Expected Heterozygosity; Hs, subpopulation heterozygosity; Gst, analogue of Fst.

Table 5. The SCoT bands pattern in pomegranate populations from different continents.

Population	Asian	American
No. Bands	213	210
No. Bands Freq. ⩾5%	213	210
No. Private Bands	3	0
No. L Comm Bands (⩽25%)	0	0
No. L Comm Bands (⩽50%)	0	0

(IIb) included the rest of the genotypes from America, three from Turkmenistan (ATME, ATDE, and ATAR) and one from Syria (ASAU) and Afghanistan (AAK1), thus confirming the relationship between the American and the Asian genotypes. Most of the genotypes of Turkmenistan belonged to this subgroup and were classified with the Cranberry genotype from the America. Genotypes from Asia including Kandahar 1 and Unknown from Yemen were located next to genotypes Crab and Balegal from America, respectively. The three subgroups (IIc) included only the genotype from America (AUVK), indicating this genotype is not closely related to the other genotypes in subgroups IIa, IIb, and all other major groups I genotype.

Principal component analysis

SCoT data were subjected to a principal component analysis (PCoA) to obtain an alternative view of the relationships between the genotypes. In the two-dimensional PCoA plot, in general, similar groupings were found as obtained with the UPGMA dendrogram. All the pomegranate genotypes from different countries were classified into two groups of the plot from PCoA. The two main axes explained 23.81% of the cumulative variance. The first two principal axes accounted for 15.87 and 7.93% of the total variation, respectively, indicating the complex multidimensional nature of SCoT variation. Group 1 included all Iranian genotypes as well as genotypes from India. Group 2 contained all American genotypes included in the analysis, but also 11 Asian genotypes from different countries. The two multivariate approaches, UPGMA and PCoA, used in the analysis of genetic

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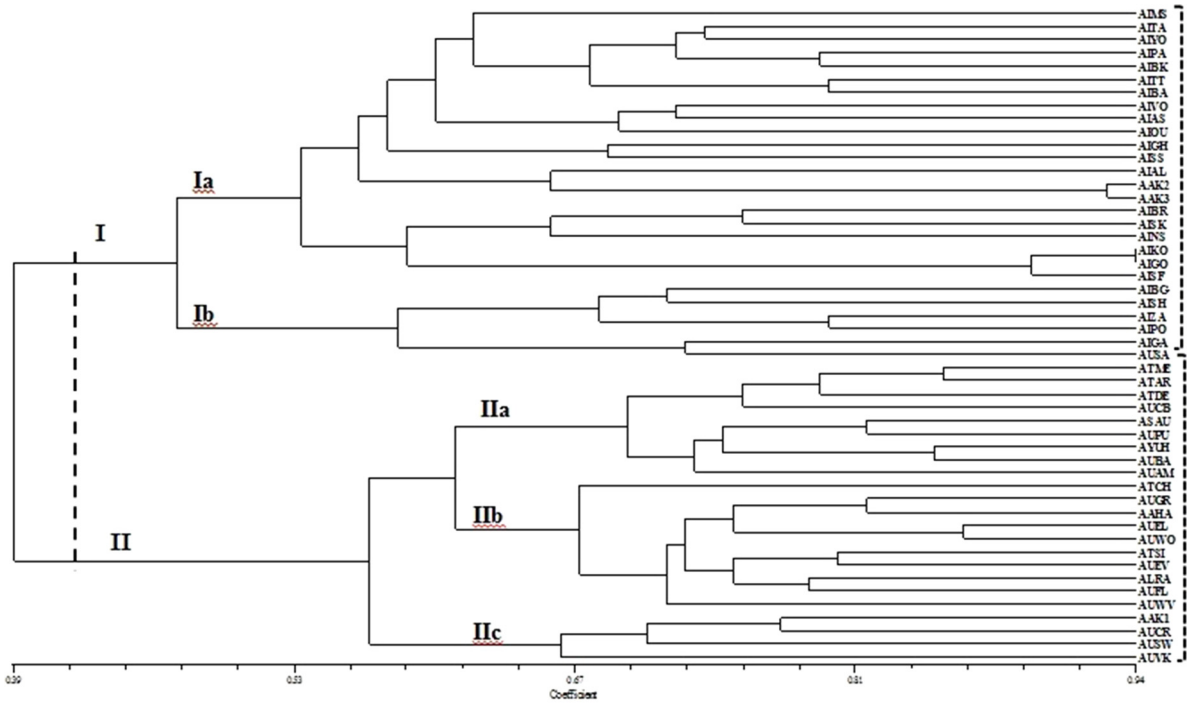

Figure 1. Dendrogram resulting from the analysis of data related to different pomegranate populations using the COMLETE algorithm and based the similarity simple matching (SM) coefficient: The first letter: the name of continent: The second letter: the name of the country origin: The third letter: the name of the cultivar in abbreviation.

relationships among the genotypes produced generally comparable results.

Discussion

The correct selection of genetic material by breeders requires the proper use of genetic diversity. Production of desirable and superior genotypes is possible only through the correct selection of genetic materials in a plant species with high genetic diversity and a comprehensive evaluation of superior genotypes in different environments (Garrido-Cardenas *et al.*, 2018). The present study provided comprehensive information on the genetic structure of pomegranate genotypes from eight countries across two continents: Asia and the America. The SCoT marker was regarded as suitable for studying genetic diversity in pistachios (Malekzadeh *et al.*, 2018), citrus (Mahjbi *et al.*, 2015), walnuts (Tabasi *et al.*, 2020) and grapes (Guo *et al.*, 2012). The 15 SCoT markers selected in this study are high in polymorphisms (100%) and can be used to differentiate pomegranate genotypes. SCoT markers have been used in previous studies to analyse the level of polymorphism in cultivars of different such as mango (73.82%) (Luo *et al.*, 2012), peanut (38.22%) (Xiong *et al.*, 2011), pistachios (92%) (Malekzadeh *et al.*, 2018) and grapes (93.1%) (Guo *et al.*, 2012). Mahjbi *et al.* (2015) reported a total of 132 amplified loci using 12 SCoT primers on 15 citrus species, and 93.9% of the loci displayed polymorphism. The present study showed that the percentage of polymorphic loci of each primer in pomegranate is higher as compared with those reported in other plant species. The average number of effective alleles (1.55) was lower than the total number of alleles. These observations show that few alleles contributed to the variation. However, the number of effective alleles in our study was lower than that reported for pomegranate genotypes from Iran (Alamuti *et al.*, 2012; Parvaresh *et al.*, 2012; Zarei and Sahraroo, 2018) and India (Singh *et al.*, 2015; Gunnaiah *et al.*, 2021). The disparity observed between the result of the present study and previous results might be due to the differences among individuals of the same population, size and of position the population used in each study. PIC is very helpful for marker informativeness and is an important factor to identify genotypic variation and population genetic diversity. The average PIC was equal to 0.39 ranging from 0.34 to 0.44 indicating moderate discriminating power of these markers. Similar to these results, Çaliskan *et al.*, (2017), Zarei and Sahraroo (2018) and Gunnaiah *et al.* (2021) previously reported PIC values of 0.2–0.50 for pomegranate cultivars. It has been reported that low PIC values indicate high genetic similarity of genotypes. Therefore, moderate PIC in this study is due to the vegetative reproduction of pomegranate. According to the PIC analysis, four studied SCoT primers (SCoT3, SCoT12, SCoT30, SCoT35) had higher PIC values and could contribute substantial information concerning pomegranate genetics and are useful for genetic diversity, mapping and breeding studies. Shannon's information index (I) and Nei's gene diversity (h) are one of the most widely used parameters to study genetic diversity in a population. Nei's gene diversity obtained for the studied populations varied from 0.26 to 0.41. This result shows a significant genetic diversity among pomegranate genotypes and supported the results of Shannon's information indicating a high level of genetic differentiation within the population's pomegranate. Among the studied populations, the highest diversity index was related to the Asian population, which indicates the richness of germplasms of this population compared to other populations. In Iranian genotypes, three private bands were observed that can be used to distinguish Iranian genotypes from other genotypes. In this study, two populations exhibited low heterozygosity (He = 0.43). Low heterozygosity of markers may be because the loci under consideration

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are least exposed to evolutionary forces such as mutation, selection or gene flow and asexual reproduction methods in pomegranate (Gunnaiah *et al.*, 2021). The genetic diversity within and between populations was 0.24 and 0.22, respectively. Low genetic differentiation among the genotypes from different countries suggests restricted gene flow among populations, which happens due to the large geographical distance between the populations and the clonal selection. The results obtained from AMOVA analysis and pairwise Fst test showed significant molecular diversity within most populations studied. Therefore, *in situ* conservation is important to protect and preserve genetic resources. The result obtained had not agreed with the previous observations that different pomegranate cultivars maintain most of their variation within the population (Parvaresh *et al.*, 2012; Guyana *et al.*, 2021). Guyana *et al.* (2021) reported that gene flow and clonal selection have caused molecular diversity between pomegranate germplasms of different countries. The Nei genetic distance obtained for the studied populations based on SCoT data revealed that the distance between populations was 0.092, which indicates the gene flow between these two populations. This result also shows that the genotypes of two populations (Asian and American) differ in some degree in their genetic content and may be used in hybridization and breeding programs (Bussell, 1999). Based on the clustering results, Iranian genotypes were a high level of genetic diversity. Among Iranian genotypes, Golbad and Golbeh Behshahr had a closer relationship genetic. Iran is considered the pomegranate centre of origin and possesses one of the richest pomegranate gene pools worldwide. Hence, the identification, collection as well as conservation of different cultivars of pomegranate from different geographical regions of Iran would be of great importance. Allelic richness among Iranian pomegranates is the reason for the distribution of pomegranates in different regions and adaptation to different weather conditions. Due to the long history of cultivation and diversity, pomegranate cultivars have a similarity of names or similar genotypes in different regions. Therefore, identifying morphological traits and knowing the genetic structure of genotypes is important. A closer examination of the origin of the cultivars may explain their inclusion in the different clusters. Thus, Iranian genotypes appeared in cluster I far away from all other American genotypes probably due to their different origin. The three main groups include three different continents, which strongly indicates that geographical isolation played an important role in the genetic structure and distribution of the pomegranate genotype. In the present study, some Asian genotypes were located closest to American genotypes. The pomegranate's place of origin is Central Asia, where it has spread to the rest of the world (Mediterranean Basin, Southern Asia and several countries of North and South America) (Sarkhosh *et al.*, 2006). It is probable that a cutting from one genotype in a geographical area is relocated and associated with another name in the new region. American genotypes showed close relationships to Turkmenistan genotypes, which are likely to have originated in Turkmen genotypes. It is reported that some of the cultivars available in America were imported from Turkmenistan by Dr Gregory and cultivated there (Volk and Preece, 2021). The placement of different cultivars with different geographical origins in a group to factors such as evolutionary processes, migration (Koehmstedt *et al.*, 2011), selection based on Morphological traits (Belaj *et al.*, 2010) and finally breeding was attributed using foreign and domestic genetic resources (Sarri *et al.*, 2006).

Conclusions

In this study, 50 pomegranate genotypes from eight different countries were analysed using SCoT to assess their genetic diversity and population structure. Genetic diversity indices showed a relatively high level of diversity in the studied pomegranate genotype. The results show that the primers SCoT3, SCoT12, SCoT30 and SCoT35 are efficient for studying the genetic diversity of pomegranates. Hence, these four primers may also be of potential value for further research on genetic Mapping, linkage analysis and marker-assisted selections in pomegranate. The high level of genetic diversity observed within the studied populations is due to the variety of genetic backgrounds or the different genetic origins of the genotypes. Among the studied genotypes, Iranian genotypes had higher genetic diversity compared with other genotypes. These genotypes have undergone genetic changes in terms of some genes and even alleles during the evolutionary period and adaptation to their growth areas, and parallel to that, the allelic diversity in the genotypes has increased. Iranian pomegranate genotypes can be a source of variation for many traits of interest in breeding, in particular tolerance to abiotic and biotic stresses. Therefore, a component of germplasm characterization, management and conservation is necessary to prevent genetic erosion. The obtained results can be used in the breeding programs of this valuable plant, especially the selection and hybridization programmes to produce hybrid cultivars, as well as the selection of parents for maximum heterosis.

Author contributions. HRK designed the experiments; ESS performed the experiments and AMM analysis data. All authors read and approved the final manuscript.

Conflict of interest. The authors declare that they have no conflict of interest.

Data archiving statement. All relevant data are within the paper and tables and figures.

References

Alamuti MK, Zeinalabedini M, Derazmahalleh MM, Shojaie TR, Irandoost HP, Zahravi M, Vazifehshenas M, Ebrahimi MA, Nekouei SMK and Salekdeh GH (2012) Extensive genetic diversity in Iranian pomegranate (*Punica granatum* L.) germplasm revealed by microsatellite markers. *Scientia Horticulturae* **146**, 104–114.

Almiahy FH and Jum'a F (2017) Evaluation of the genetic diversity of pomegranate accessions some Iraqi pomegranate (*Punica granatum* L.) genotypes using ISSR marker. *Journal of Agriculture and Veterinary Science* **10**, 44–49.

Amirmoradi B, Talebi R and Karami E (2012) 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* **298**, 1679–1688.

Baghizadeh A and Dehghan E (2018) Efficacy of SCoT and ISSR markers in the assessment of genetic diversity in some Iranian pistachio (*Pistacia vera* L.) cultivars. *Pistachio and Health Journal* **7**, 37–43.

Basaki T, Khayam Nekouei M, Choukanand R and Mardi M (2016) Evaluation of Iranian pomegranate collection using simple sequence repeat and morphological traits. *Crop Breeding Journal* **1**, 67–78.

Belaj A, Munoz-Diez C, Baldoni L, Satovic Z and Barranco D (2010) Genetic diversity and relationships of wild and cultivated olives at regional level in Spain. *Scientia Horticulturae* **124**, 323–330.

Bussell JD (1999) The distribution of random amplified polymorphic DNA (RAPD) diversity amongst populations of *Isotoma petraea* (Lobeliaceae). *Molecular Ecology* **8**, 775–789.

Çaliskan O, Bayazit S, Oktem M and Ergul A (2017) Evaluation of the genetic diversity of pomegranate accessions from Turkey using new microsatellite markers. *Turkish Journal of Agriculture and Forestry* **41**, 142–153.

https://doi.org/10.1017/S1479262123000394 Published online by Cambridge University Press

Collard BY and Mackill D (2009) Start Codon Targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. *Plant Molecular Biology Reports* **27**, 86–93.

Garrido-Cardenas JA, Mesa-Valle C and Manzano-Agugliaro F (2018) Trends in plant research using molecular markers. *Planta* **247**, 543–557.

Gunnaiah R, Jagadeesha RC, Cholin S, Prabhuling G, Govindaswamy Babu A, Fakrudin B, Pujer P and Murthy SB (2021) Genetic diversity assessment and population structure analysis of pomegranate cultivars from different countries and Himalayan wild accessions. *Journal of Horticultural Science and Biotechnology* **5**, 614–623.

Guo DL, Zhang JY and Liu CH (2012) Genetic diversity in some grape varieties revealed by SCoT analyses. *Molecular Biology Reports* **39**, 5307–5313.

Hasnaoui N, Mars M, Chibani J and Trifi M (2010) Molecular polymorphisms in Tunisian pomegranate (*Punica granatum* L.) as revealed by RAPD fingerprints. *Journal Diversity* **2**, 107–114.

Jbir R, Hasnaoui N, Mars M, Marrakchi M and Trifi M (2008) Characterization of Tunisian pomegranate (*Punica granatum* L.) cultivars using amplified fragment length polymorphism analysis. *Scientia Horticulturae* **115**, 231–237.

Juibary PL, Seyedmehdi FS, Sheidai M, Noormohammadi Z and Koohdar F (2021) Genetic structure analysis and genetic fingerprinting of sweet orange cultivars (*Citrus sinensis* L. Osbeck) by using SCoT molecular markers. *Genetic Resources and Crop Evolution* **68**, 1645–1654.

Karimi HR and Mirdehghan SH (2013) Correlation between the morphological characters of pomegranate (*Punica granatum* L.) traits and their implications for breeding. *Turkish Journal of Botany* **37**, 355–362.

Karimi HR, Zari F, Mirdehghan SH, Mohamadi Mirik AA and Sarkhosh A (2020) Correlation among some biochemical compound in fruit, leaf and shoot bark of pomegranate trees for breeding programs. *International Journal of Fruit Science* **20**, 805–824.

Koehmstedt AM, Aradhya MK, Soleri D, Smith JL and Polito VS (2011) Molecular characterization of genetic diversity, structure, and differentiation in the olive (*Olea europaea* L.) germplasm collection of the United States Department of Agriculture. *Genetic Resources and Crop Evolution* **58**, 519–531.

Luo C, He XH, Chen H, Hu Y and Ou SJ (2012) Genetic relationship and diversity of *Mangifera indica* L.: revealed through SCoT analysis. *Genetic Resources and Crop Evolution* **59**, 1505–1515.

Mahjbi A, Baraket G, Oueslati A and Salhi-Hannachi A (2015) Start Codon Targeted (SCoT) markers provide new insights into the genetic diversity analysis and characterization of Tunisian *Citrus* species. *Biochemical Systematics and Ecology* **61**, 390–398.

Malekzadeh KH, Mahmoodnia Meimand M and Amirebrahimi F (2018) Analysis of genetic diversity among male and female pistachio genotypes using start codon targeted (SCoT) makers. *Journal of Plant Science and Molecular Breeding* **6**, 10–18.

Morris J (2007) *A molecular phylogeny of the Lythraceae and inference of the evolution of heterostyly* (Ph.D. thesis). Kent State University, Ohio.

Morton J (1987) Fruits of warm climates. Miami FL, USA. 550p.

Murry M and Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. *Journal of Nucleic Acids* **8**, 4321–4325.

Nei M and Li W (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the United States of America* **76**, 5269–5273.

Parvaresh M, Talebi M and Sayed-Tabatabaei BE (2012) Molecular diversity and genetic relationship of pomegranate (*Punica granatum* L.) genotype using microsatellite markers. *Scientia Horticulturae* **138**, 244–252.

Parvizi H, Sepaskhah AR and Ahmadi SH (2016) Physiological and growth responses of pomegranate tree (*Punica granatum* L. cv. Rabab) under partial root-zone drying and deficit irrigation regimes. *Agricultural Water Management* **163**, 146–158.

Peakall R and Smouse PE (2006) GENALEX 6: genetic analysis in excel population genetic software for teaching and research. *Molecular Ecology Resources* **6**, 288–295.

Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S and Rafalski A (1996) The comparison of RFLP, RAPD, AFLP, and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding* **2**, 225–238.

Prevost A and Wilkinson M (1999) A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theoretical and Applied Genetic* **98**, 107–112.

Rohlf FJ (2000) *NTSYS-PC Numerical Taxonomy and Multivariate Analysis System Version 2.2*. New York: Exeter Software Setauket.

Sarkhosh A, Zmani Z, Fatahi R and Ebadi A (2006) RAPD Markers reveal polymorphism among some Iranian Pomegranate (*Punica granatum* L.) genotypes. *Scientia Horticulturae* **111**, 24–29.

Sarkhosh A, Zamani Z, Fatahi R and Ranjbar H (2009) Evaluation of genetic diversity among Iranian soft-seed pomegranate accessions by fruit characteristics and RAPD markers. *Scientia Horticulturae* **3**, 313–319.

Sarri V, Baldoni L, Porceddu A, Cultrera NGM, Contento A, Frediani M, Belaj A, Trujillo I and Cionini PG (2006) Microsatellite markers are powerful tools for discriminating among olive cultivars and assigning them to defined populations. *Genome* **49**, 1606–1615.

Sheidai M, Sadeghi A, Shahreiyari ZH, Noormohammadi Z, Farahanei F and Tabatabaei Ardakanei S (2008) RAPD and cytogenetic study of some pomegranate (*Punica granatum* L.) cultivars. *Caryologia* **6**, 68–73.

Singh NV, Abburi VL, Ramajayam D, Kumar R, Chandra R, Sharma KK, Sharma J, Babu KD, Pal RK, Mundewadikar DM, Saminathan T, Cantrell R, Nimmakayala P and Reddy UK (2015) Genetic diversity and association mapping of bacterial blight and other horticultural important traits with microsatellite markers in pomegranate from India. *Molecular Genetic and Genomics* **290**, 1393–1402.

Sinjare D (2015) Application of microsatellite SSR markers in a number of pomegranates (*Punica granatum* L.) cultivars in Kurdistan region/Duhok province. *International Journal of Chemical Sciences* **3**, 117–122.

Soriano JM, Zuriaga E, Rubio P, Llacer G, Infante R and Badenes ML (2010) Development and characterization of microsatellite markers in pomegranate (*Punica granatum* L.). *Molecular Breeding* **27**, 119–128.

Tabasi M, Sheidai M, Hassani D and Koohdar F (2020) DNA fingerprinting and genetic diversity analysis with SCoT markers of Persian walnut populations (*Juglans regia* L.) in Iran. *Genetic Resources and Crop Evolution* **67**, 1437–1447.

Talebi Badaf M, Bahar M and Sharif N (2003) Study of pomegranate genetic variation using RAPD markers. 3th Iran Biotechnology Congress, 345–346.

Volk GM and Preece GE (2021) Field Tour of the USDA National Clonal Germplasm Repository for Tree Fruit, Nut Crops, and Grapes in Davis, California. Colorado State University Publishing, 570p.

Weising K, Nybon H, Wolff K and Kahl G (2005) *DNA Fingerprinting in Plants: Principles, Methods, and Applications*. Boca Raton, USA: CRC Press.

Xiong F, Zhong R, Han Z, Jiang J, He L, Zhuang W and Tang R (2011) Start codon targeted polymorphism for evaluation of functional genetic variation and relationships in cultivated peanut (*Arachis hypogaea* L.) varieties. *Molecular Biology Reports* **38**, 3487–3494.

Yeh F, Yang R, Boyle T, Ye Z and Mao J (2000) Popgene. The user friendly shareware for population genetic analysis. Version 1.32. Molecular Biotechnology Center, University of Alberta, Canada.

Yuan Z, Yin Y, Qu J, Zhu L and Li Y (2007) Population genetic diversity in Chinese pomegranate (*Punica granatum* L.) cultivars revealed by fluorescent-AFLP markers. *Journal of Genetic and Genome* **34**, 1061–1071.

Zarei A and Sahraroo A (2018) Molecular characterization of pomegranate (*Punica granatum* L.) accessions from Fars province of Iran using microsatellite markers. *Horticulture, Environment, and Biotechnology* **59**, 1–11.

Zarkti H, Ouabbou H, Hilali A and Udupa SM (2010) Detection of genetic diversity in Moroccan durum wheat accessions using agro-morphological traits and microsatellite markers. *African Journal of Agricultural Research* **5**, 1837–1844.

https://doi.org/10.1017/S1479262123000394 Published online by Cambridge University Press