10.22092/cbj.2018.123510.1028

Characterization of strawberry (*Fragaria* × *ananassa* Duch.) cultivars using SCoT, ISSR and IRAP markers

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Received: 29 September 2018
Accepted: 20 December 2018

ABSTRACT

Badakhshan, H., M. S. Kamangar, M. S., and Mozafari, A. A. 2018. Characterization of strawberry (*Fragaria* × *ananassa* Duch.) cultivars using SCoT, ISSR and IRAP markers**. Crop Breeding Journal 8 (1 & 2): 61-72**

Genetic relationship of 36 strawberry cultivars was analyzed using three molecular marker systems; inter simple sequence repeats (ISSR), inter-retrotransposon amplified polymorphism (IRAP) and start codon targeted (SCoT) polymorphism. SCoT, IRAP and ISSR primers generated 238, 113 and 122 bands, respectively, of which 85.2, 91.5 and 89.35% were polymorphic. IRAPs with the highest values of expected heterozygosity (He), Shanonn index (I) and resolution power (Rp) were more powerful compared with SCoT and ISSR. However, the highest value of marker index was calculated for ISSRs. The genetic relationships were estimated using Dice similarity coefficient between different pairs of cultivars which varied from 0.577 to 0.901 for SCoT, 0.547 to 0.918 for IRAP, and 0.531 to 0.983 for ISSR. The UPGMA dendrograms using the SCoT and ISSR data classified cultivars into four major clusters; whereas based on the IRAP and combined data, the cultivars were divided into three major clusters. Approximately 50% of the cultivars with affiliation to pedigree and geographic origins were assigned to their major clusters. The results demonstrated that SCoT, ISSR and IRAP marker systems are useful for identification and genetic diversity analysis of strawberry cultivars.

Key words: Genetic diversity, genetic relationships, cluster analysis, molecular markers

INTRODUCTION

Strawberry (*Fragaria* × *ananassa* Duch.) is an octoploid (2n = 8x = 56) perennial plant belonging to the *Rosaceae* family (Debnath and Ricard, 2009; Peng *et al*., 2015). It is one of the most widely grown fruit plants in the world, and is consumed by millions of people (Debnath and Ricard, 2009). Strawberry is one of the richest natural source of vitamins and essential micronutrients, and also exhibits a high level of antioxidant capacity against free radicals which can be helpful for reducing the risk of cancer in humans and the reversal of age-related effects on memory (Giampieri *et al*., 2012; Wang and Lin, 2000).

Strawberry cultivars have been planted for long time in Iran, particularly in Kurdistan province (Eshghi *et al*., 2007; Tehranifar and Sarsaefi, 2002). A number of strawberry cultivars have been adapted to the cold semi-arid climatic conditions of Kurdistan (Manbari *et al*., 2012). Breeding and inbreeding have contributed to the reduction of genetic variation within strawberry germplasm. The consequences of such reduction in diversity are liability to biotic and abiotic stresses, lower selection gain and higher genetic erosion. Therefore, knowledge of the genetic diversity within germplasm can develop efficient and reliable breeding methods and helps to select parental materials for hybridization programs.

Identification of strawberry cultivars has been conducted using different methods (Degani *et al*., 2001). DNA-based markers are increasingly used in breeding programs of many plants allowing direct comparison

of different genetic materials independent of environmental influences (Lim *et al*., 2017). Different molecular tools such as RAPD (Degani *et al*., 2001), EST-SSR (Dong *et al*., 2011), ISSR (Arnau *et al*., 2002; Debnath and Ricard, 2009), SSR (Meng *et al*., 2015; Rugienius *et al*., 2015), AFLP (Degani *et al*., 2001; Rugienius *et al*., 2015) and DArT (Sanchez-Sevilla *et al.,* 2015) have been employed to analyze relationships between strawberry genotypes. These markers have proved to be effective tools for detection and assessment of genetic structures of strawberry germplasms. These molecular markers are different in some aspects such as reproducibility, resolution power and polymorphism detection. Inter-retrotransposon amplified polymorphism (IRAP) and inter- simple sequence repeats (ISSR) markers, respectively, amplify DNA segments lying between two long terminal repeats (LTR) sequences and replicable regions between two microsatellite repeats in any orientation. The start codon targeted polymorphism (SCoT) is designed based on the short conserved region in plant genes surrounding the ATG translation start codon (Collard and Mackill, 2009).

The SCoT, ISSR and IRAP methods have been reported to be more sensitive to low levels of genetic variations, and to produce more complex marker patterns than the other markers' systems which is advantageous when differentiating closely related cultivars. These markers are used for variety identification in varied plant species, such as barley (Fernandez *et al*., 2002), citrus (Mahjbi *et al*., 2015), wheat (Carvalho *et al*., 2010), banana (Nair *et al*., 2005) and Potato (Mousapour Gorji *et al*., 2011). These techniques are extremely reproducible, and can be used to analysis of nuclear genome in a single assay without any previous sequence information (AiFen *et al*., 2009). ISSR markers have been used for analyzing genetic diversity among various wild, domesticated and hybrid strawberry genotypes (Arnau *et al*., 2002; Debnath and Ricard, 2009; Kuras *et al.,* 2004; Morales *et al.,* 2011; Nunes *et al.,* 2013). The results suggested ISSRs are highly efficient markers in polymorphism detection as well as assigning strawberry cultivars in groups based on either pedigree or geographic regions. However, up to now, there is no report on IRAP or SCoT markers for analyzing genetic variation in strawberry germplasms.

The objectives of the present study were to analyze genetic diversity in different strawberry cultivars using IRAP, ISSR and SCoT markers, and to compare the usefulness of these three marker systems for analyzing genetic diversity among strawberry cultivars.

MATERIAL AND METHODS

Plant Materials and DNA Extraction

Leaves of 36 greenhouse planted strawberry (*F. ananassa*) cultivars (Table 1) were collected. The genomic DNAs were extracted by the CTAB method, as described by Doyle and Doyle (1990), with minor modifications including a phenol extraction to obtain DNA with good quality. The quality and quantity of the extracted DNAs were assessed by 0.8% agarose gel electrophoresis and spectrophotometry (NanoDrop 1000 Spectrophotometer, Wilmington, DE, USA), respectively.

DNA Markers

A set of 36 polymorphic DNA primers including 20 SCoT, eight IRAP and eight ISSR primers were selected for analyzing genetic variations among 36 strawberry cultivars. SCoT and IRAP primers were randomly chosen from Collard and Mackill (2009), Luo *et al*. (2010), Alavikia *et al*. (2008) and Kalendar *et al*. (1999). ISSR primers which were highly polymorphic in previous studies (Arnau *et al*. 2002; Hussein *et al*. 2008) were selected. Primer sequences are listed in Tables 2, 3 and 4. PCR amplification reaction programs were the same for three used markers systems.

Table 1. Name, origin and parentage of strawberry cultivars

No.	Cultivar	Origin	Parents	
			Female	Male
1	Zalushka	Russia	Senga	Festivalnaya
2	Karnaval	Russia	Raneplodnaya	Pamitnaya
3	Festivalnaya	Russia	Primer	Obilnaya
4	Allaya	Russia	Cyantstania	Rooshin skaya
5	Hybrid 280	Russia	Lochvira	Lidia
6	Dukat	Poland	Koralowa 100	Gorella
7	Tokai bus	Russia	Dukat	Zhemchuzhina
8	Rubinovyj kulon	Russia	Senga	Fairfax
9	Uonismaydes	Russia	Khavaland	-
10	Forton	Russia	Zenit	Iton
11	Paros	Italy	Marmolda	Irvine
12	Hybrid 700	Russia	Rane plodnaya	Bogota
13	Istochink	Russia	Grenadier	Pocahontas
14	Roosich	Russia	Vystavochnaya	Voskhod
15	Hybrid 141	Russia	Ranyaya plodnaya	Pamitnaya
16	Senga, Sengana	Germany	Markee	Sieger
17	Divnaya	Russia	Holiday	Festivalnaya
18	Aromatnaya	Russia	Kometa	Krasavitsa
19	Diamonte	USA	Cal.87.112-6	Cal.88.270-1
20	Camarosa	USA	Dougla	Cal 85.218-605
21	Queen Elisa	Italy	Miss	USB 35
22	Aromas	USA	Cal.87.112-6	Cal.88.270-1
23	Hybrid 98	Russia	Loch vira	Mamochka
24	Hybrid 521	Russia	Govarovskaya	Mamochka
25	Vigera	Russia	Ogonic	Mestnaya
26	Eros	Britain	Allstar	Elsanta
27	Vityaz	Russia	Surprise olympiad	Festivalnaya roomashka
28	Snejhana	Russia	Holiday	188-16-25
29	Bounty	Canada	Senga	Jersey belle
30	Honeoye	USA	Holiday	Vibrant
31	Kurdistan	Kurdistan (Iran)	Unknown	Unknown
32	Hybrid 510	Russia	Oso Grande	Pamitnaya
33	Bagema	Russia	Loch Vira	Hybrid 56
34	Sodaromka	Russia	Roksana	Festivalnaya
35	Karoda	Russia	Hybridnaya forma 138-16-25	Red Gauntlet
36	Orazhay C-G-L	Russia	Senga	Red Gauntlet

The PCR reactions were carried out in a final volume of 10 µL included 1 µL of genomic DNA as template, 0.25 µL dNTPs (10 mM), 0.5 µL $MgCl_2$ (25 mM), 1 µL 10 X PCR buffer (1 mM Tris-HCl, pH 8.8, 50 mM KCl and 0.1% Triton X-100), 0.2 µL *Taq* DNA polymerase (5 U l μL^{-1}), and 1 µL of each primers (10 mM). The PCR conditions were performed according to following program: 94°C for three minutes; followed by 35 cycles of 94°C for one minute, annealing temperature (45-58°C) for one minute and 72°C for two minutes (Tables 2, 3 and 4), and final extension at 72°C for five minutes. Amplified DNA fragments were electrophoresed on agarose gel (1.2%) stained with ethidium bromide (0.5 $\mu g ml^{-1}$), visualized, and photographed with UV light. The reproducibility of the bands was tested in three samples. A 100-3000 bp DNA ladder (Fermentas Co; Ltd) was used as molecular weight standard for bands scoring.

Data Analysis

Each sharp and prominent amplified fragment was scored manually as presence (1) and absence (0) for a given primer. Accordingly, total number of bands (NB), the number of polymorphic bands (NPB), the percentage of polymorphic bands (PPB) and the frequency of bands (FB) were

Table 2. Some characteristics of used SCoT primers. Total number of bands (NB), number of polymorphic bands (NPB), percentage of polymorphic bands (PPB), frequency of bands (FB), effective multiplex ratio (EMR), marker index (MI), resolving power (Rp), expected heterozygosity (He), and Shannon Index (I) obtained by each SCoT primer.

Primer ID	Sequence (5'→3')	Annealing Temperature	NB	NPB	PPB	FB	EMR	MI	Rp	He	I
SCOT1	CAACAATGGCTACCACCA	56	7	5	71.43	0.82	3.57	0.71	1.28	0.25	0.37
SCOT2	CAACAATGGCTACCACCC	51	12	11	91.67	0.66	10.08	3.02	5.94	0.38	0.55
SCOT7	CAACAATGGCTACCACGG	50	16	9	56.25	0.78	5.063	0.91	5.17	0.23	0.33
SCOT10	CAACAATGGCTACCAGCC	50	8	7	87.5	0.58	6.13	1.35	3.22	0.27	0.42
SCOT12	ACGACATGGCGACCAACG	55	11	10	90.91	0.6	9.09	2.36	6	0.32	0.49
SCOT14	ACGACATGGCGACCACGC	57	11	8	72.73	0.94	5.82	1.28	1.28	0.27	0.41
SCOT17	ACCATGGCTACCACCGAG	53	12	10	83.33	0.7	8.33	2.25	6.06	0.34	0.50
SCOT20	ACCATGGCTACCACCGCG	50	9	7	77.78	0.75	5.44	1.31	2.89	0.3	0.45
SCOT22	AACCATGGCTACCACCAC	53	8	6	75	0.71	4.50	1.13	3.72	0.33	0.47
SCOT23	CACCATGGCTACCACCAG	56	9	8	88.89	0.8	7.11	2.13	3.56	0.39	0.56
SCOT25	ACCATGGCTACCACCGGG	56	12	11	91.67	0.6	10.08	3.02	7.11	0.38	0.55
SCOT27	ACCATGGCTACCACCGTG	55	12	11	91.67	0.56	10.08	2.92	7.06	0.36	0.53
SCOT29	CCATGGCTACCACCGGCC	58	10	10	100	0.49	10.00	2.90	5.94	0.36	0.54
SCOT30	CCATGGCTACCACCGGCG	53	13	11	84.62	0.64	9.31	2.61	7.56	0.36	0.52
SCOT31	CCATGGCTACCACCGCCT	52	14	12	85.71	0.69	10.29	2.47	4.61	0.3	0.45
SCOT33	CCATGGCTACCACCGCAG	55	14	14	100	0.59	14.00	4.34	8.89	0.39	0.58
SCOT34	ACCATGGCTACCACCGCA	50	17	15	88.24	0.59	13.24	3.44	9.17	0.33	0.49
SCOT35	CATGGCTACCACCGGCCC	57	13	11	84.62	0.65	9.31	2.61	7.28	0.36	0.52
SCOT36	GCAACAATGGCTACCACC	50	14	14	100	0.5	14.00	3.78	6.56	0.33	0.5
SCOT70	ACCATGGCTACCAGCGCG	50	16	13	81.25	0.69	10.56	2.53	6	0.29	0.44
Total			238	203	1703.27	13.33	176.00	47.07	109.30	6.54	9.67
Mean			11.9	10.20	85.20	0.67	8.80	2.35	5.47	0.33	0.48

Table 3. Some characteristics of studied IRAP primers. Total number of bands (NB), number of polymorphic bands (NPB), percentage of polymorphic bands (PPB), frequency of bands (FB), effective multiplex ratio (EMR), marker index (MI), resolving power (Rp), expected heterozygosity (He), and Shannon Index (I) obtained by each IRAP primer.

Primer ID	Sequence (5'→3')	Annealing Temperature	NB	NPB	PPB	FB	EMR	MI	Rp	He	I
Gaga	GGGAACCAACCGTCACA	51	16	15	93.75	0.62	14.06	4.22	8.94	0.39	0.56
5′LTR1	ACAACCTTTATACGGGATCTCCGTT	48	18	17	94.44	0.56	16.06	4.66	10.28	0.37	0.53
5′LTR2	CTTAATACGGGATCTCCCTTACTA	48	17	12	70.59	0.70	8.47	1.95	6.61	0.30	0.43
LTR6149	CTCGCTCGCCCACACATCAACCGCGTTTATT	48	14	13	92.86	0.47	12.07	2.78	5.83	0.28	0.43
LTR6150	ATGTACACACCTATGTATCTGTACCCGGCTTGGTC	58	5	5	100	0.79	5.00	1.70	2.11	0.43	0.62
3′LTR	TGTTTCCCATGCGACGTTCCCCAACA	57	13	13	100	0.53	13.00	4.16	8.78	0.40	0.59
Sukkula	GATAGGGTCGCATCTTGGGCGTGAC	57	14	13	92.86	0.61	12.07	3.14	6.39	0.32	0.48
Nikita	CGCATTTGTTCAAGCCTAAACC	54	16	14	87.50	0.54	12.25	2.82	6.44	0.29	0.44
Total			113	102	732	4.82	92.98	25.42	55.38	2.78	4.08
Mean			14.13	12.75	91.5	0.60	11.62	3.18	6.92	0.35	0.51

Table 4. Some characteristics of studied ISSR primers. Total number of bands (NB), number of polymorphic bands (NPB), percentage of polymorphic bands (PPB), frequency of bands (FB), effective multiplex ratio (EMR), marker index (MI), resolving power (Rp), expected heterozygosity (He), and Shannon Index (I) obtained by each ISSR primer.

Primer ID	Sequence (5'→3')*	Annealing Temperature	NB	NPB	PPB	FB	EMR	MI	Rp	He	I
IS-2	(CA)6GG	45	20	18	90	0.48	16.2	3.73	9.28	0.28	0.43
IS-7	(CT)8AC	50	15	14	93.33	0.64	13.07	3.66	7.28	0.34	0.51
IS-11	(GAG)3GC	56	13	12	92.31	0.55	11.08	2.55	4.94	0.28	0.44
ISSR-1	VBV(AC)7	58	16	14	87.5	0.59	12.25	3.06	8.00	0.31	0.47
ISSR-2	BDB(CA)7	58	17	12	70.59	0.65	8.47	1.61	5.67	0.24	0.35
ISSR-4	GCV(TC)7	58	17	15	88.24	0.76	13.24	3.84	7.83	0.37	0.54
ISSR-5	VCG(TC)7	58	14	13	92.86	0.53	12.07	3.26	8.06	0.33	0.50
ISSR-7	HVH(TGT)5	57	10	10	100	0.57	10	3.30	6.67	0.42	0.61
Total			122	108	714.83	4.77	96.38	25.01	57.73	2.57	3.85
Mean			15.25	13.5	89.35	0.60	12.05	3.13	7.22	0.32	0.48

* V= non T; B= non A; H= non G and D= non C

calculated using GenAlex 6.5 software (Peakall and Smouse, 2012). Cluster analysis based on Unweighted Pair Group Method with Arithmetic Averages (UPGMA) and Neighbor Joining (NJ) algorithms were carried out by using XLSTAT (version 2017) and DARwin (version 6.0.17) softwares to categorize overall variation to separate clusters.

The cophenetic correlation coefficients were calculated, and Mantel's tests (1967) were performed to check the justification of the cluster analysis to the similarity matrices, and consequently, only Dice Similarity Matrix was used in subsequent analyses. The degree of correlation between

$$PIC = 1 - \sum_{i=1}^{l} pi^2 - \sum_{i=1}^{l-1} \sum_{j=i+1}^{l} 2pi^2 qi^2$$

(Nagy *et al*., 2012)

where *pi* is the frequency of present band and *qi* is the frequency of absent band. Marker index (MI) was calculated to evaluate the usefulness of molecular markers based on PIC × EMR formula. The effective multiplex ratio (EMR) was measured by multiplying the proportion of polymorphic loci (β) and number of loci per assay unit (Powell *et al*., 1996).

RESULTS

SCoT amplification using 20 single primers produced 238 unambiguous, scorable bands (ranged from 150 to 3000 bp) of which 203 bands were polymorphic including one band ($SCoT_{31}$, 660 bp) which was distinctive of cv. Roosich. The average estimated PPB was 85.20%, and on average 11.9 bands were observed per primer. $SCoT_{29}$, $SCoT_{33}$ and $SCoT_{36}$ generated the maximum percentage of polymorphism (100%); while $SCoT_7$ produced the minimum percentage of polymorphism (56.25%). Maximum values of primer characteristics including EMR (14), MI (4.34), Rp (8.89), He (0.39) and I (0.58) were obtained by $SCoT_{33}$. The lowest values of the EMR, MI, RP, He and I were observed for $SCoT_{14}$, $SCoT_1$ and $SCoT_7$ primers, respectively. Conversely, the frequency of $SCoT_{14}$ (0.94), $SCoT_1$ (0.82) and $SCoT_7$ (0.78) bands were maximum (Table 2). FB was negatively correlated with He ($r = -0.474$, $p < 0.05$) and I ($r = -0.546$, $p < 0.05$), but positive correlation was found between He and I values.

Eight polymorphic IRAP primers generated 113 scorable bands ranged between 200 and 2900 bp. On average, each primer produced 14.13 bands and 102 bands were polymorphic (91.50%). One single band (5'LTR1, 1500 bp) was distinctive of cv. Aromas. The LTR6150 and 3'LTR produced the highest PPB (100%), whereas 5'LTR2 primer generated the lowest PPB (70.59%). Although the minimum values for EMR (5.00), MI (1.70), Rp (2.11) obtained by LTR6150, the calculated PPB (100%), FB (0.79), He (0.43) and I (0.62) were highest for this primer. The maximum values of NB (18), NPB (17), EMR (16.06), MI (4.66) and Rp (10.28) were obtained for $5'LTR_1$ primer (Table 3). FB positively correlated but not significantly with He and I.

Using ISSR primers, 122 distinct and scorable bands amplified of which 108 bands were polymorphic. Among the polymorphic bands one single band ($ISSR_2$, 320 bp) was distinctive of cv. Rubinovyj Kulon. The size of bands ranged from 100

to 3000 bp. The average of produced bands estimated as 15.25 for each primer. The highest (100) and lowest (70.59) values of the PPB were observed for ISSR7 and ISSR2, respectively. The ISSR7 created maximum values of He (0.42) and I (0.61); however, IS-2 primer generated the maximum values of EMR (16.20), MI (3.84) and Rp (9.28). The minimum values of EMR, MI, Rp, He and I were observed for ISSR2 primer (Table 4).

The highest and the lowest average of MI value were obtained by IRAP (3.18) and SCoT (2.35) primers, respectively. Out of all used primers the 5'LTR2 generated the maximum MI (4.66) value. Mean Rp, as primer performance determinant, was highest for ISSR (7.22) and lowest for SCoT (5.47), respectively. While, the highest value of Rp (10.28) obtained by IRAP (5' LTR2) primer. The IRAP primers generated the highest average values of He (0.35) and I (0.51). However, the mean values of He and I were identical for ISSR and SCoT primers (Tables 2 and 4).

Genetic Similarity

The Dice similarity coefficients using IRAP data ranged from 0.547 to 0.918, therefore, Festivalnaya and Hybrid280 had more similarity whilst Roocich and Hybrid521 were less similar. The Dice similarity values calculated using ISSR data ranged from 0.531 to 0.983 for Roosich-Kurdistan, and Karnaval and Festivalnaya, respectively. Using SCoT data, similarity values ranged from 0.577 to 0.901 for Roosich and Vigera, and Hybrid521 andVigera, respectively. Significant correlation ($r = 0.345$, $p < 0.0001$) was obtained between Dice similarity matrices using Mantel test, estimated from 10000 permutations. Likewise, the mantel correlations were highly significant for pairwise SCoT and IRAP ($r = 0.434$, $p < 0.0001$), SCoT and ISSR ($r = 0.495$, $p < 0.0001$) as well as IRAP and ISSR ($r = 0.298$, $p < 0.0001$) data sets.

Genetic Relatedness

The UPGMA and Neighbor joining dendrograms generated from Dice similarity coefficient for each of SCoT, IRAP and ISSR primers. UPGMA was selected as suitable method due to its best pattern of clustering in accordance with the geographic origin and genetic relationships of cultivars. The cutting point of each dendrogram was determined according to pedigree information and the maximum variation between classes.

All of the strawberry genotypes were divided into four groups using SCoT data (Fig. 1, a). The Honeoye and Kurdistan cultivars were classified together in the second group. Honeoye has originated from USA, whereas no information is available about the origina and parentage of Kurdistan genotype. The third group with two subgroups was more consistent with pedigree (56%) and geographic origins (Table 1).

Genotypes were classified into four distinct groups using ISSR data in which approximately 38.09 percent of clusters were in agreement with pedigree (Fig. 1b). The cv. Kurdistan was grouped together with the Canadian cultivar (Bounty); while, most of the American and European cultivars formed the third group which comprised two subgroups with 57.14% accordance with pedigree (Table 1).

The cv. Roosich appeared as distinct from all other genotypes in SCoT and ISSR markers based on dendrograms (Fig. 1a and b).

Using IRAP data, genotypes were grouped into three clusters (Fig. 1c). Greater accordance (55.17%) between classifications of genotypes with the pedigree revealed in the second major group with five sub-groups in which cv. Honeye grouped with cv. Kurdistan.

Cultivars were classified into three groups with an average of 51.65% accordance with pedigree using combined SCoT, ISSR and IRAP data (Fig. 1d). Bounty, Honeoye and Kurdistan cultivars

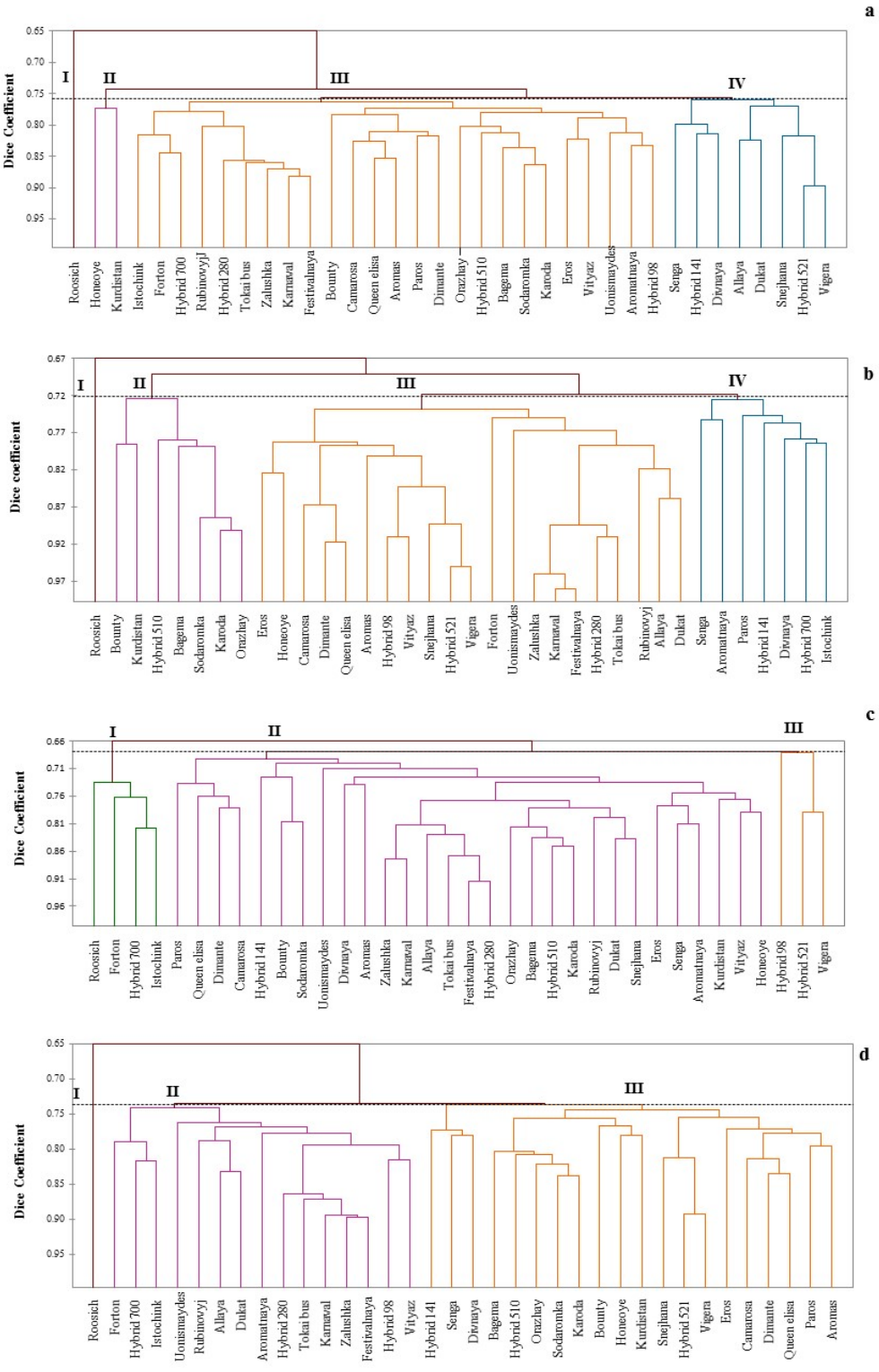

Fig. 1. Dendrograms for 36 cultivars of strawberry with UPGMA based on Dice coefficient. (a) SCoT data-based dendrogram, (b) ISSR data-based dendrogram, (c) IRAP data-based dendrogram, and (d) the combination SCoT, ISSR and IRAP data-based dendrogram.

were classified once again in the third group.

DISCUSSION

Three DNA fingerprinting techniques, SCoT, ISSR and IRAP markers, were used to analyze the genetic variation among 36 strawberry cultivars. Additionally, the usefulness of three different types of DNA markers was demonstrated for characetrizing genetic variation and their efficiency to reveal the extent of polymorphism in octoploid strawberry. SCoT, ISSR and IRAP markers exhibited high polymorphism among analyzed strawberry cultivars. SCoT primers were designed based on conserved regions flanking the ATG initiation codon sequence of genes. Thus, these markers are often considered as functional markers (Collard and MacKill, 2009). ISSRs target the microsatellite repeats of the genome, whereas IRAPs reveal information from the retrotransposon regions of the genome.

Each technique, though differing in the underlying principle, was informative with regard to the amount of detected polymorphism. The polymorphism generated with IRAP primers (average He = 0.35, I = 0.51) was found to be higher than SCoT and ISSR primers (average He = 0.33, I= 0.48). This attributes to variable average number of polymorphic bands per markers data ranged from 85.2% for SCoT to 91.5% for IRAP markers. This analysis also implied potential utilization of primers LTR6150, 3'LTR, Gaga, ISSR7, ISSR4, SCoT2, SCoT23, SCoT25 and SCoT33 (with higher He and I) for detecting polymorphism among strawberry cultivars (Tables 2, 3 and 4). In general, our results showed that SCoT markers were less efficient than IRAP and ISSR assay in respect to polymorphism detection (Table 2), as they detected 85.2% polymorphism compared to 91.5% for IRAP markers and 89.35% for ISSR markers (Tables 3 and 4).

Marker index (MI) and resolution power (Rp) used for comparison of the efficiency of three marker systems showed that IRAP and ISSR markers, with slightly differences, were more informative than SCoTs in the assessment of genetic diversity among strawberry cultivars. The higher performance and discriminativer power of ISSR and IRAP markers was due to primers designation based on the abundant number of the repetitive DNA sequences in plants including microsatellites and retrotransposon repeats. Some bands were specific to only one genotypes enabling immediate identification of that genotype as concluded earlier by Arnau *et al*. (2002). $SCoT_{31}$ (660 bp), $ISSR_2$ (320 bp) and 5'LTR1 (1500 bp) bands were distictive for Roosich, Rubinovyj and Kulon, and Aromas cultivars, respectively. The distinctive bands provide the possibility of PCR- based SCAR markers designation to develop breeding schemes through marker-assisted selection (MAS) methods.

To date, molecular markers have been widely used for the identification of genetic relationships in wild and cultivated strawberry germplasm, but this is the first report of genetic characterization of octaploid strawberry using IRAP and SCoT markers. Arnau *et al*. (2002) results with ISSR markers showed that the ISSR technique has a high discriminative potential and revealed real relationships between cultivars. However, low levels of polymorphism (30%) between strawberry varieties were reported in comparison with our results. On the other hand, generated levels of polymorphism using SCoT, ISSR and IRAP primers were nearly similar with the variation observed by Meng *et al*. (2015),using SSR markers (88.9%).

Mean similarity coefficient obtained using IRAP and ISSR data was 0.73, while the mean similarity coefficient using SCoT data was 0.74 (Fig. 1). The Mantel correlation between SCoT, IRAP and ISSR Dice's similarity coefficient was significant. This showed that these techniques demonstrated harmony in

distinguishing powers among the strawberry cultivars. High significant correlations between ISSR, SCoT and IRAP markers has been reported by Rahmani *et al*. (2014) and Rahmani *et al*. (2015).

Two clustering methods including UPGMA and NJ were used to demonstrate genetic relationships among strawberry cultivars. The results were generally consistent, but due to more congruency of the results of UPGMA with pedigree, this method was chosen for further analysis. All cultivars were grouped in four, three and four clusters based on SCoT, IRAP and ISSR assessments, respectively. A doable rationalization for distinctive resolution of SCoTs, IRAPs and ISSRs is that, they have distinct targets in the genome. However, based on the three assays the Russian strawberry cultivars were separated from American and European cultivars. This observation may result from differences in the procedures of plant propagation and breeding programs in these regions. The groups obtained by IRAP marker were relativity more consistent with pedigree information than ISSR and SCoT primers due to high potential of the IRAP primers in detection of cultivars. Therefore, these markers are appropriate markers to discriminate strawberry genotypes based on their paternity relationships and ancestors.

The cv. Kurdistan with unknown origin and parentage has been introduced and commercialized in Kurdistan province since more than 50 years ago. In all generated dendrograms using SCoT, IRAP, and combined SCoT, ISSR and IRAP data the cv. Kurdistan was classified together with the American cv. Honeoye which support the conclusion that these two cultivars might be more closely related. However, reliable and accurate conclusions warrant DNA sequencing analysis.

In conclusion, characterization of strawberry cultivars is necessary to promote and improve its cultivation. Additionally, strawberry breeding programs needs more genetic information to be able to develop new competitive cultivars. The present study using IRAP, SCoT and ISSR fingerprinting techniques revealed adequate genetic variation among starwberry cultivars. IRAP markers were more efficient than SCoTs and ISSRs in detecting genetic diversity as well as revealing genetic relationships among starwberry cultivars.

ACKNOWLEDGMENTS

The authors are grateful to the University of Kurdistan, Iran, for financial support.

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