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Characterization of ten date palm (*Phoenix dactylifera* L.) cultivars from Saudi Arabia using AFLP and ISSR markers

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ABSTRACT

Date palm is the most economically important plant in the Middle East due to its nutritionally valuable fruit. The development of accurate DNA fingerprints to characterize cultivars and the detection of genetic diversity are of great value for breeding programs. The present study explores the usefulness of ISSR and AFLP molecular markers to detect relationships among 10 date palm (*Phoenix dactylifera* L.) cultivars from Saudi Arabia. Thirteen ISSR primers and six AFLP primer combinations were examined. The level of polymorphism among cultivars for ISSRs ranged from 20% to 100% with an average of 85%. Polymorphism levels for AFLPs ranged from 63% to 84% with an average of 76%. The total number of cultivar-specific markers was 241, 208 of which were generated from AFLP analysis. AJWA cultivar had the highest number of cultivar-specific ISSR markers, whereas DEK, PER, SUK-Q, SHA and MOS-H cultivars had the lowest. RAB and SHA cultivars had the most and least AFLP cultivar-specific markers, respectively. The highest pairwise similarity indices for ISSRs, AFLPs and combined markers were 84% between DEK (female) and PER (female), 81% between SUK-Q (male) and RAB (male), and 80% between SUK-Q (male) and RAB (male), respectively. The lowest similarity indices were 65% between TAB (female) and SUK-Q (male), 67% between SUK-A (female) and SUK-Q (male), and 67% between SUK-A (female) and SUK-Q (male). Cultivars of the same sex had higher pairwise similarities than those between cultivars of different sex. The Neighbor-Joining (NJ) tree generated from the ISSR dataset was not well resolved and bootstrap support for resolved nodes in the tree was low. AFLP and combined data generated completely resolved trees with high levels of bootstrap support. In conclusion, AFLP and ISSR approaches enabled discrimination among 10 date palm cultivars of from Saudi Arabia, which will provide valuable information for future improvement of this important crop.

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1. Introduction

Date palm (*Phoenix dactylifera* L., 2 $n = 36$, Arecaceae) is the most economically important plant cultivated in the Middle East [1,2] with more than 450 cultivars or varieties in the Kingdom of Saudi Arabia alone and 2000 varieties worldwide [3]. Date palm is usually cultivated in arid and semi-arid regions due to its high tolerance to environmental stresses [4–6]. It is a perennial, arborescent, dioecious, monocotyledonous, highly heterozygous plant, with a very slow growth rate and a late reproductive phase [4–6]. The economic importance of date palm is due to its nutritionally valuable fruit, which consists of 72–88% sugar, minerals (i.e., iron, potassium, calcium, chlorine, copper, magnesium, sulfur and phosphorus), amino acids and vitamins [7]. Moreover, antioxidant and antimutagenic activities of date fruits have also been reported [8]. Date palm is also used for many industrial purposes, such as timber, furniture, rope and packing material [9]. Despite its economic importance, breeding programs to select for desirable traits are very limited. The development of accurate DNA fingerprints to characterize date cultivars and the detection of genetic diversity are of great value for the improvement of agricultural, horticultural, and nutritional value [10–13].

Previous efforts to develop molecular markers in date palm based on ISSR [4,14,15], RAPD [4,16,17], AFLP [5,6,18–22], and SSR [15] techniques are well documented. These markers are useful in cultivar identification and in studying genomic diversity and evolution. Sex-specific markers in date palm were also successfully detected based on RAPD and ISSR approaches [23,24]. These markers can be utilized in marker-assisted selection and breeding programs in date palm.

As little is known about the origin of date palm germplasm grown in Saudi Arabia, the present study aims at exploring the usefulness of ISSR and AFLP markers as the first step towards the assessing relatedness among 10 selected date palm cultivars and characterization of cultivar-specific markers to be utilized in the future breeding program in Saudi Arabia.

2. Materials and methods

2.1. Plant material

Ten date palm cultivars (five males and five females) from four different locations in Saudi Arabia were examined (Table 1). Two male Moshwaq (MOS-A and MOS-H) cultivars originated from different geographic locations. Leaf samples for each of these cultivars were kindly provided by Hada Al-Sham Station, King Abdulaziz University, KSA.

Table 2
List of ISSR primers and their nucleotide sequences.

No.	Name	Sequence	No.	Name	Sequence
1	814	$(CT)_8TG$	8	HB10	$(GA)_6CC$
2	844A	$(CT)_8AC$	9	HB11	$(GT)_6CC$
3	844B	$(CT)_8GC$	10	HB12	$(CAC)_3GC$
4	17898A	$(CA)_6AC$	11	HB13	$(GAG)_3GC$
5	17898B	$(CA)_6GT$	12	HB14	$(CTC)_3GC$
6	HB8	$(GA)_6GG$	13	HB15	$(GTG)_3GC$
7	HB9	$(GT)_6GG$			

ISSR: inter-simple sequence repeat.

2.2. Genomic DNA extraction and purification

Extraction of total DNA was performed separately from leaves of individual plants using the modified procedure of Gawel and Jarret [25]. To remove RNA contamination, RNase A (10 mg/mL, Sigma, USA) was added to the DNA solution and incubated at 37 °C for 30 min. Estimation of the DNA concentration in different samples was done by measuring optical density at 260 nm.

2.3. Inter-simple sequence repeat (ISSR)

Thirteen primers were utilized for ISSR analyses (Table 2). PCR was performed in a total reaction volume of 25 μL and amplification (Perkin Elmer 2400 thermocycler, Germany) was programmed to 40 cycles after an initial denaturation cycle for 4 min at 94 °C. Each cycle consisted of denaturation at 94 °C for 1 min, annealing at 40 °C for 2 min, and extension at 72 °C for 2 min, followed by a final extension cycle for 7 min at 72 °C. Reactions were done in three replicates per sample (e.g., bulk of five DNA extracts) to ensure reproducibility of the data.

2.4. Amplified fragment length polymorphism (AFLP)

AFLP analysis was performed using the AFLP Analysis System I (Invitrogen, Life Technologies, Grand Island, NY, USA) according to the manufacturer's protocol. Genomic DNA samples were digested with the restriction enzymes *Eco*RI and *Mse*I, followed by ligation of adapters to the digested DNA fragments. Pre-amplification was carried out

Table 1
Date palm cultivars examined along with their geographic locations, sex and fruit description.

Names	Abbreviations	Geographic locations	Sex	Fruit shapes	Fruit colors
Sukkariat Al-Madinah	SUK-A	Al-Madinah	Female	Oval	Brown
Dekhaini Al-Riyadh	DEK	Al-Riyadh	Female	Cylindrical	Yellow
Ajwa Al-Madinah	AJW	Al-Madinah	Female	Oval	Red
Tabzel Al-Riyadh	TAB	Al-Riyadh	Female	Oval	Yellow
Perny Al-Riyadh	PER	Al-Riyadh	Female	Oval	Brown
Sukkariat Qassim	SUK-Q	Qassim	Male	Oval	Brown
Rabia Al-Madinah	RAB	Al-Madinah	Male	Oval	Brown
Shalaby Al-Madinah	SHA	Al-Madinah	Male	Cylindrical	Yellow
Moshwaq Al-Riyadh	MOS-A	Al-Riyadh	Male	Cylindrical	Yellow
Moshwaq Hada Al-Sham	MOS-H	Hada Al-Sham	Male	Cylindrical	Yellow

using *Eco*RI primer plus one extension base at the 3′ position (A) and *Mse*I primer plus one extension base at the 3′ position (C) to amplify fragments that contain complementary sequences. Six combinations of *Eco*RI primers (plus three extension bases) and *Mse*I primers (plus three extension bases) were successfully used to selectively amplify the DNA fragments matching the primer-extension sequences. The six combinations were: E-AAC/M-CAA, E-ACA/M-CAG, E-ACC/M-CAT, E-ACT/M-CTC, E-AGC/M-CTG and E-AAG/M-CTT. Reactions were done in three replicates per sample and non-repeatable data were removed.

2.5. Detection of PCR products

ISSR products were visualized using agarose gel electrophoresis (1.2% in 1X TBE buffer) followed by staining with ethidium bromide (0.3 ug/mL). Amplicons were visually examined with an UV transilluminator and photographed using a CCD camera (UVP, UK). AFLPs were separated by capillary electrophoresis and amplicon sizes were estimated on ABI 3500 DNA sequencer (Applied Biosystems, Foster City, California, USA). Using the program Genemapper 4.1 (Applied Biosystems), a genetic fingerprint was produced for each sample by scoring the presence (1) or absence (0) of a standardized set of markers between 50 and 600 base pairs in size [26].

2.6. Data analysis

Fragments recovered by both techniques were considered reproducible and scorable based on the dataset generated from the three separate amplifications for each primer and primer combination. Data were scored as (1) for the presence and (0) for the absence of a given fragment, and sizes were estimated by comparison with a 100-bp ladder (Bioron, Germany) using Gel Works 1D advanced gel documentation system (UVP, UK). Binary data matrices were entered into TFPGA (version 1.3) and analyzed using qualitative routine to generate a similarity coefficient. Dissimilarity coefficients were used to construct dendrograms using unweighted pair group method with arithmetic average (UPGMA) and sequential hierarchical and nested clustering (Neighbor-Joining or NJ) routine using NTSYSpc (version 2.10, Exeter software). Principle component analysis (PCA) was performed using NTSYSpc (version 2.10) and 3D-plotted using GraphPad Prism (version 5.0).

2.7. Matrix comparison

Similarity matrices from ISSR and AFLP dataset were compared based on the TFPGA, the normalized Mantel statistics [27], and the PIC (polymorphism information content) was calculated using the following formula [28,29]:

$$\mathrm{PIC} = 1 - \sum f_i^2$$
$$i = 1 - n$$

where, f_i is the frequency of the ith amplicon. The number of amplicons refers to the number of scored fragments. The frequency of an amplicon was obtained by dividing the number of cultivars in which each fragment was found by the total number of cultivars. The PIC value provided an estimate of the discriminating power of a marker. Marker index (MI, the product of PIC and the number of polymorphic bands) was calculated for each marker type. In addition, average heterozygosity (He) and the effective multiplex ratio (E) were also calculated [28].

3. Results and discussion

ISSR and AFLP molecular markers were utilized to characterize 10 cultivars of date palm from four different geographic locations in Saudi Arabia (Table 1). Only clear, unambiguous and reproducible amplicons recovered through different techniques were considered for scoring. Each amplicon was considered a single locus. The optimal number of primers for ISSR or primer combinations for AFLP required in discriminating among genomic DNAs of different plant genotypes was estimated based on the reproducibility of data and the generated level of polymorphism ($75 \pm 10\%$). The required value of genetic distance to classify correlated plant accessions as distinct cultivars was discussed previously [30,31]. In the present study, 13 out of 30 primers for ISSR and six combinations for AFLP had informative patterns. Selection of ISSR primers and AFLP combinations was based on the number of the recovered amplicons and the reproducibility of the results. Less than 7% intra-plant polymorphism was detected across the two types of markers for the plants of the same cultivar (data available upon request). Since ISSR and AFLP markers are dominant, a pooling strategy (bulked DNA) is considered ideal for saturating intra-plant polymorphism with no effect on the accuracy of the results. Mengoni et al. [32] indicated that 10% intra-plant polymorphism is statistically insignificant.

3.1. Amplified products and polymorphism among different date palm cultivars

ISSR analysis is based on inter tandem repeats of short DNA sequences proven to be highly polymorphic even among closely related genotypes due to the lack of functional genetic constraints in these non-coding DNA regions [33]. Among 30 prescreened ISSR primers, 13 were selected for their scorability and reproducibility. Analysis of the 13 primers generated 135 amplicons across ISSR primers with a mean number of 10 amplicons per primer. The size of the ISSR amplified fragments ranged from 203 bp (for primer HB10) to 4596 bp (for primer HB15). The highest number of amplicons (15) was produced by primer HB10, whereas the lowest number (9) was revealed by primers 844B, 17898A, HB14 and HB15 (Table 3). The number of polymorphic amplicons was as high as 114 (85% polymorphism) and the average number of polymorphic fragments were 8.8 per primer (Table 3). The level of polymorphism for different ISSR primers ranged from 20% (for primer HB8) to 100% (for primers 814 and HB15). In this context, Hussein et al. [34] reported a low level of polymorphism (28.6%) in ISSR analysis of Egyptian date

Table 3
Total number of amplicons, monomorphic amplicons, polymorphic amplicons and percentage of polymorphism among the 10 date palm cultivars in ISSR and AFLP analyses.

Marker type	Primer	No. amplicons	No. monomorphic amplicons	No polymorphic amplicons	% polymorphism
ISSR	814	10	0	10	100
	844A	11	2	9	82
	844B	9	2	7	78
	17898A	9	1	8	89
	17898B	13	4	9	69
	HB8	5	4	1	20
	HB9	11	2	9	82
	HB10	15	2	13	87
	HB11	10	1	9	90
	HB12	14	1	13	93
	HB13	10	1	9	90
	HB14	9	1	8	89
	HB15	9	0	9	100
	Total	135	21	114	85
AFLP	E-AAC/M-CAA	112	33	79	71
	E-ACA/M-CAG	83	16	67	81
	E-ACC/M-CAT	210	40	170	81
	E-ACT/M-CTC	83	22	61	74
	E-AGC/M-CTG	109	17	92	84
	E-AAG/M-CTT	103	38	65	63
	Total	700	166	534	76

ISSR: inter-simple sequence repeat; AFLP: amplified fragment length polymorphism.

palm cultivars, while Adawy et al. [4] and Moghaieb et al. [24] reported high polymorphism in ISSR analysis (64.1% and 73%, respectively). In addition, Marsafari and Mehrabi [35] revealed a higher level of ISSR polymorphism (95.67%) when characterizing some Iranian date palm cultivars. The low level of polymorphism in ISSR analysis generated by Hussein et al. [34] was suggested to be due to a narrow genetic background of the Egyptian date palm cultivars analyzed.

AFLP analysis is based on the PCR amplification of selected restriction fragments from a total genomic DNA digest and this approach combines the reliability of RFLPs with the advantages of PCR methods. Therefore, AFLP permits the development of more accurate and comprehensive fingerprints [36]. In the present study, AFLP analysis of the six primer combinations generated a total of 700 amplicons with a mean number of 117 amplicons per primer combination. The size of the fragments ranged from 40 bp (for primer combinations E-AAC/M-CAA, E-ACA/M-CAG and E-AAG/M-CTT) to 591 bp (for primer combination E-AGC/M-CTG). The number of polymorphic amplicons was 534 (76% polymorphism) and the average

Table 4
List of positive and negative cultivar-specific markers of the 10 date palm cultivars detected in ISSR analyses. Type and number of markers along with their molecular weights (MW) in bp are indicated.

Marker type	Primer	Number (and MW in bp) of cultivar-specific markers										
		SUK-A[a]	DEK	AJW	TAB	PER	SUK-Q	RAB	SHA	MOS-A	MOS-H	Total
ISSR	814	-	-	-	-	-	-	-	-	1 (1540)	1 (2690)	2
	844A	1 (385)	1 (2075)	-	-	-	-	1 (910)	-	1 (580)	-	4
	844B	-	-	-	-	-	1 (940)	-	1 (2660)	-	-	2
	17898A	1 (3010)	-	-	-	-	-	-	-	-	-	1
	17898B	1 (1115)	-	1 (830)	-	-	-	-	1 (2320)	-	-	3
	HB8	-	-	-	-	-	-	1 (1020)	-	-	-	1
	HB9	-	-	-	-	-	-	-	-	-	-	-
	HB10	1 (205)	1 (375)	-	-	2 (1250, 2360)	1 (295)	-	-	1 (475)	-	6
	HB11	-	-	1 (325)	1 (1270)	-	-	2 (730, 910)	-	-	-	4
	HB12	-	-	1 (3265)	1 (370)	-	-	-	-	-	1 (780)	3
	HB13	1 (1270)	-	1 (790)	-	-	-	-	-	-	-	2
	HB14	-	-	3 (1100, 955, 800)	-	-	-	-	-	-	-	3
	HB15	1 (4600)	-	-	1 (650)	-	-	-	-	-	-	2
	Total	6	2	7	3	2	2	4	2	3	2	33

ISSR: inter-simple sequence repeat.
[a] See Table 1 for full names.

Table 5
List of positive and negative cultivar-specific markers of the 10 date palm cultivars detected in AFLP analyses. Type and number of markers along with their molecular weights (MW) in bp are indicated.

Marker type	Primer combination	Number (and MW in bp) of cultivar-specific markers										
		SUK-A[a]	DEK	AJW	TAB	PER	SUK-Q	RAB	SHA	MOS-A	MOS-H	Total
AFLP	E-AAC/M-CAA	8 (41, 69, 70, 104, 131, 149, 219, 422)	5 (109, 111, 126, 243, 368)	-	3 (217, 346, 566)	-	6 (59, 187, 228, 289, 258, 316)	-	1 (78)	3 (118, 123, 303)	-	26
	E-ACA/M-CAG	-	3 (114, 140, 296)	-	5 (44, 94, 252, 337, 365)	-	6 (191, 373, 375, 376, 440, 448)	-	3 (185, 214, 352)	2 (75, 250)	10 (68, 118, 137, 205, 222, 329, 364, 470, 471, 507)	29
	E-ACC/M-CAT	3 (48, 172, 354)	5 (59,72, 122, 149, 149, 345)	3 (180, 222, 362)	12 (146, 163, 166, 199, 225, 227, 230, 319, 320, 334, 351, 352)	4 (128, 147, 184, 218)	20 (97, 119, 120, 126, 136, 137, 223, 253, 271, 278, 289, 295, 312, 315, 342, 344, 390, 400, 446, 448)	3 (130, 185, 188)	1 (379)	5 (44, 60, 66, 328, 335)	5 (72, 202, 206, 231, 318)	61
	E-ACT/M-CTC	5 (72, 74, 198, 251, 284)	-	1 (60)	2 (99, 127)	1 (172)	6 (109, 125, 198, 261, 268, 334)	-	3 (151, 167, 247)	4 (48, 49, 50, 72)	2 (79, 177)	24
	E-AGC/M-CTG	9 (58, 82, 89, 121, 123, 138, 188, 189, 293)	2 (106, 173)	2 (97, 124)	1 (311)	15 (87, 146, 165, 169, 169, 196, 234, 242, 314, 315, 416, 366, 375, 379, 381, 391)	-	-	-	7 (126, 128, 170, 205, 264, 310, 327)	2 (226, 284)	38
	E-AAG/M-CTT	4 (93, 94, 253, 312)	2 (127, 128)	-	5 (71, 114, 126, 248, 363)	3 (105, 176, 246)	7 (79, 95, 96, 193, 230, 306, 324)	-	-	4 (85, 177, 313, 317)	5 (120, 327, 328, 355, 387)	30
	Total	29	17	6	28	23	45	3	8	25	24	208

AFLP: amplified fragment length polymorphism.
[a] See Table 1 for full names.

number of polymorphic fragments was 89 per primer combination (Table 3). The level of polymorphism ranged from 63% (for primer combination E-AAG/M-CTT) to 84% (for primer combination E-AGC/M-CTG). The highest number of amplicons (210) was detected by primer combination E-ACC/M-CAT, whereas the lowest number (83) was revealed by primer combinations E-ACA/M-CAG and E-ACT/M-CTC (Table 3). Cao and Chao [37] screened 32 primer combinations using two date palm cultivars and found that different primer combinations produced 50–70 fragments ranging in size from 50–700 bp. Diaz et al. [19] used five AFLP primer combinations on three date palm cultivars and generated 310 amplicons, 220 of which (71%) were polymorphic. El-Khishin et al. [38] profiled five Egyptian date palm cultivars using six AFLP primer combinations that generated a total of 433 amplicons with the highest level of polymorphism at 59.02%. Adawy et al. [5] employed 28 AFLP primer combinations to examine relationships among five Upper Egypt date palm cultivars and produced 1135 fragments with as low as 41.59% polymorphism. These discrepancies in the number of AFLP amplicons and the percentage of polymorphisms are likely due to the levels of divergence among different date palm cultivars and the use of different primer combinations.

3.2. Cultivar-specific molecular markers for different date palm cultivars

The total number of cultivar-specific markers scored across species and marker type was as high as 241, 208 from AFLPs and 33 from ISSRs (Tables 4 and 5). The highest number of cultivar-specific markers from ISSRs (6) was scored for primer HB10, while no cultivar-specific markers were detected for primer HB9. The highest number of cultivar-specific markers from ISSRs for an individual cultivar (AJW) was seven, while the lowest was two for cultivars DEK, PER, SUK-Q, SHA and MOS-H (Table 4). The highest number of cultivar-specific AFLP markers for an individual primer combination was 61 (primer combination E-ACC/M-CAT), whereas the lowest number (24) was for primer combination E-ACT/M-CTC. The highest number of cultivar-specific AFLP markers for a single cultivar was 45 (RAB), while the lowest (3) was for cultivar SHA (Table 5). We recommend the use of primer combinations E-ACC/M-CAT and E-AGC/M-CTG in estimating distances among date palm species due to the generation of high number of amplicons, high percentage of polymorphism and high number of cultivar-specific markers.

Table 6
Similarity matrices based on molecular data for the 10 date palm cultivars. Bold numbers indicate the highest values, while italic ones indicate the lowest.

ISSR	SUK-A	DEK	AJW	TAB	PER	SUK-Q	RAB	SHA	MOS-A	MOS-H
SUK-A[a]	1.00									
DEK	0.76	1.00								
AJW	0.68	0.71	1.00							
TAB	0.75	0.75	0.74	1.00						
PER	0.75	**0.84**	0.73	0.76	1.00					
SUK-Q	0.69	0.69	*0.65*	0.70	0.69	1.00				
RAB	0.68	0.70	0.70	0.75	0.74	*0.76*	1.00			
SHA	0.72	0.70	0.71	0.75	0.70	0.72	0.75	1.00		
MOS-A	0.66	0.71	0.82	0.74	0.73	0.71	0.75	0.75	1.00	
MOS-H	0.68	0.79	*0.65*	0.74	0.78	0.75	0.74	0.73	0.74	1.00
AFLP	SUK-A	DEK	AJW	TAB	PER	SUK-Q	RAB	SHA	MOS-A	MOS-H
SUK-A	1.00									
DEK	0.77	1.00								
AJW	0.74	0.80	1.00							
TAB	0.74	0.76	**0.81**	1.00						
PER	0.75	0.77	0.79	0.77	1.00					
SUK-Q	*0.67*	0.70	0.70	0.68	0.70	1.00				
RAB	0.74	0.77	0.77	0.75	0.76	*0.80*	1.00			
SHA	0.72	0.76	0.78	0.75	0.77	0.70	0.78	1.00		
MOS-A	0.69	0.73	0.73	0.73	0.73	0.73	0.76	0.75	1.00	
MOS-H	0.71	0.74	0.77	0.74	0.75	0.69	0.72	0.78	0.70	1.00
Combined	SUK-A	DEK	AJW	TAB	PER	SUK-Q	RAB	SHA	MOS-A	MOS-H
SUK-A	1.00									
DEK	0.77	1.00								
AJW	0.73	0.78	1.00							
TAB	0.74	0.76	**0.80**	1.00						
PER	0.75	0.78	0.78	0.77	1.00					
SUK-Q	*0.67*	0.70	0.69	0.68	0.70	1.00				
RAB	0.73	0.76	0.75	0.75	0.75	*0.79*	1.00			
SHA	0.72	0.74	0.76	0.75	0.76	0.70	0.77	1.00		
MOS-A	0.68	0.73	0.75	0.73	0.73	0.73	0.76	0.75	1.00	
MOS-H	0.70	0.75	0.75	0.74	0.75	0.70	0.72	0.77	0.71	1.00

[a] See Table 1 for full names.

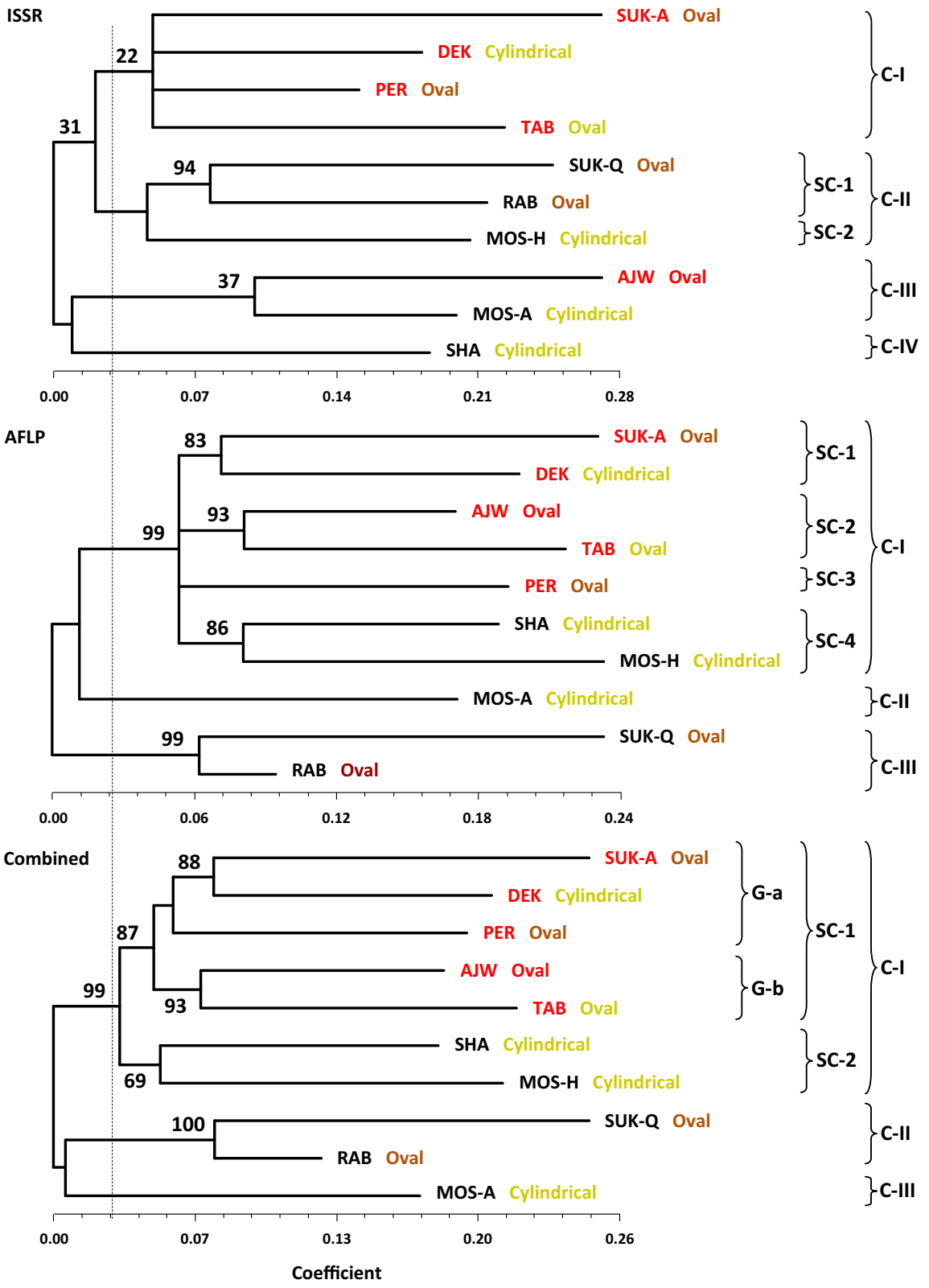

Fig. 1. (Color online.) Neighbor-Joining (NJ) trees based on inter-simple sequence repeat (a), amplified fragment length polymorphism (b), and combined (c) dataset of 10 date palm cultivars. Cultivar abbreviations are provided in Table 1. Cultivar acronyms in red and black are female and male plants, respectively. Fruit shape is indicated and acronym names are color coded by fruit color (yellow, red and brown). C: cluster; SC: subcluster; G: group.

3.3. Genetic relationships and cluster analysis

Genetic similarities among the 10 date palm cultivars based on Nei's method [39] within and across both types of markers are shown in Table 6 and Fig. 1. The highest pairwise similarity indices for ISSR, AFLP and combined datasets were 84% between DEK (female) and PER (female), 81% between AJW (female) and TAB (female), and 80% between AJW (female) and TAB (female), respectively. However, the highest pairwise similarity indices for ISSR, AFLP and combined datasets among male cultivars only were 76, 80 and 79%, respectively, all of which were between SUK-Q and RAB. The latter represents the most consistent results of ISSR, AFLP and combined data analysis. The lowest similarity indices across the three datasets were 65% between TAB (female) and SUK-Q (male), 67% between SUK-A (female) and SUK-Q (male), and 67% between SUK-A (female) and SUK-Q (male), respectively.

The dendrograms based on ISSR, AFLP and combined datasets were congruent with the similarity indices. The Neighbor-Joining (NJ) tree generated from ISSR data was not well resolved and bootstrap support for resolved nodes in the tree was low, except the node joining the two cultivars SUK-Q and RAB (94%). However, AFLP or combined dataset was resolved and bootstrap support for resolved nodes in both trees was high. The highest bootstrap for resolved nodes joining two cultivars was also scored for SUK-Q and RAB (99 and 100%, respectively), followed by AJW and TAB (93%) (Fig. 1). The topology of the ISSR tree is largely incongruent with the AFLP or the combined tree. The ISSR tree consisted of four clusters. Cluster I included four cultivars, all of which are females (SUK-A, DEK, PER and TAB). Cluster II consisted of two subclusters, the first included SUK-Q and RAB and the second included MOS-H. Cluster III included AJW and MOS-A, while cluster IV included SHA. The AFLP tree consisted of three clusters. Cluster I included the five female and two male cultivars, while cluster II included MOS-A, and cluster III included the two cultivars SUK-Q and RAB. Cluster I was divided into four subclusters, the two male cultivars (SHA and MOS-H) were in subcluster 4.

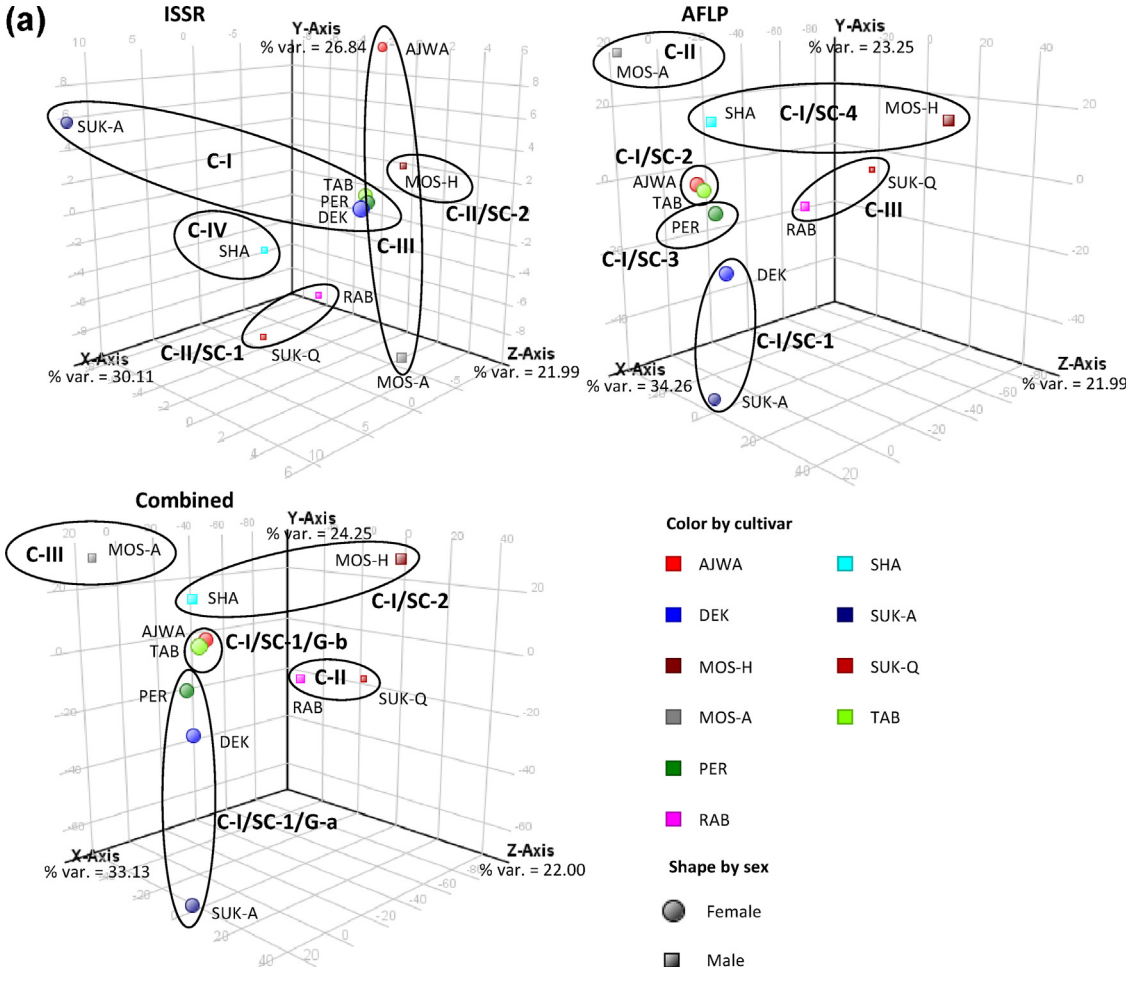

Fig. 2. (Color online.) Principle component analysis (PCA) plots based on inter-simple sequence repeat, amplified fragment length polymorphism and combined datasets of the 10 date palm cultivars. Plots were constructed based on cultivar (a), geographic location (b), fruit shape (c) and fruit color (d). Clustering was based on the Neighbor-Joining (NJ) trees of Fig. 1. C: cluster; SC: subcluster; G: group.

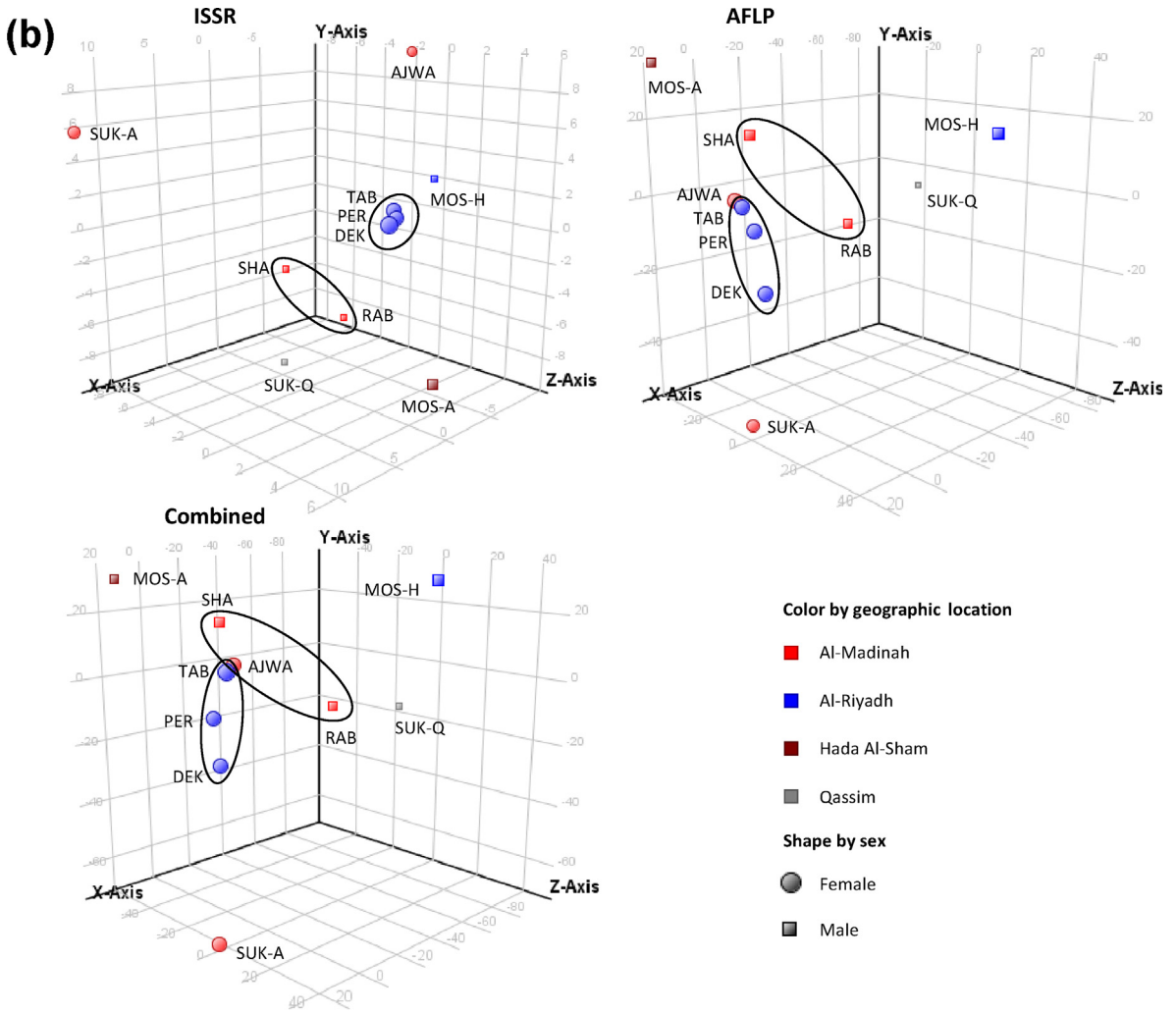

Fig. 2. (*Continued*)

The combined tree was congruent with the AFLP tree, largely due to the fact that most of the data in the combined analysis is derived from AFLPs. However, subcluster I of cluster I of the combined tree was further divided into two groups, one included SUK-A, DEK and PER, while the second involved the other two female cultivars AJW and TAB. The dendrograms from AFLP and combined markers indicated a partial separation of cultivars based on sex, which is also reflected in the pairwise similarity indices. There is no correspondence between the tree topology and fruit shape or color. The high pairwise relatedness between SUK-Q (male) and RAB (male) (79%) may be explained by the similarity in sex (male), fruit shape (oval) and fruit color (brown), while the similarity between AJW and TAB (80%) can be explained by the similarity in sex (female) and fruit shape (oval). Unexpectedly, pairwise similarity between MOS-A and MOS-H was low (71%), although they are the same sex (male), fruit shape (cylindrical) and fruit color (yellow). These results suggest that more detailed genomic and genetic analysis of these cultivars is required to characterize their relatedness.

The results of pairwise similarity indices and dendrograms constructed for different cultivars from ISSR, AFLP and combined datasets were consistent with those of PCA analyses. Percentages of variation in PCA plots of ISSR, AFLP and combined datasets were 30.11, 34.26 and 33.13% on the X axis, 26.84, 23.25 and 24.25% on the Y axis and 21.99, 21.99 and 22.00% on the Z-axis (Fig. 2a). PCA indicated that pairs of cultivars SUK-Q and RAB and AJWA and TAB are closely related. Based on geographic location, PCA plots of ISSR, AFLP or combined data (Fig. 2b) consistently indicated relatedness among two cultivars from Al-Madinah (SHA and RAB) and three cultivars from Al-Riyadh (DEK, TAB and PER). The other cultivars showed no relatedness based on their geographic locations. The PCA plot of ISSR data based on fruit shape showed the lowest level of consistency with that of AFLP or combined dataset. Accordingly, the PCA plot of combined dataset was partially inconsistent with that of AFLP dataset (Fig. 2c).

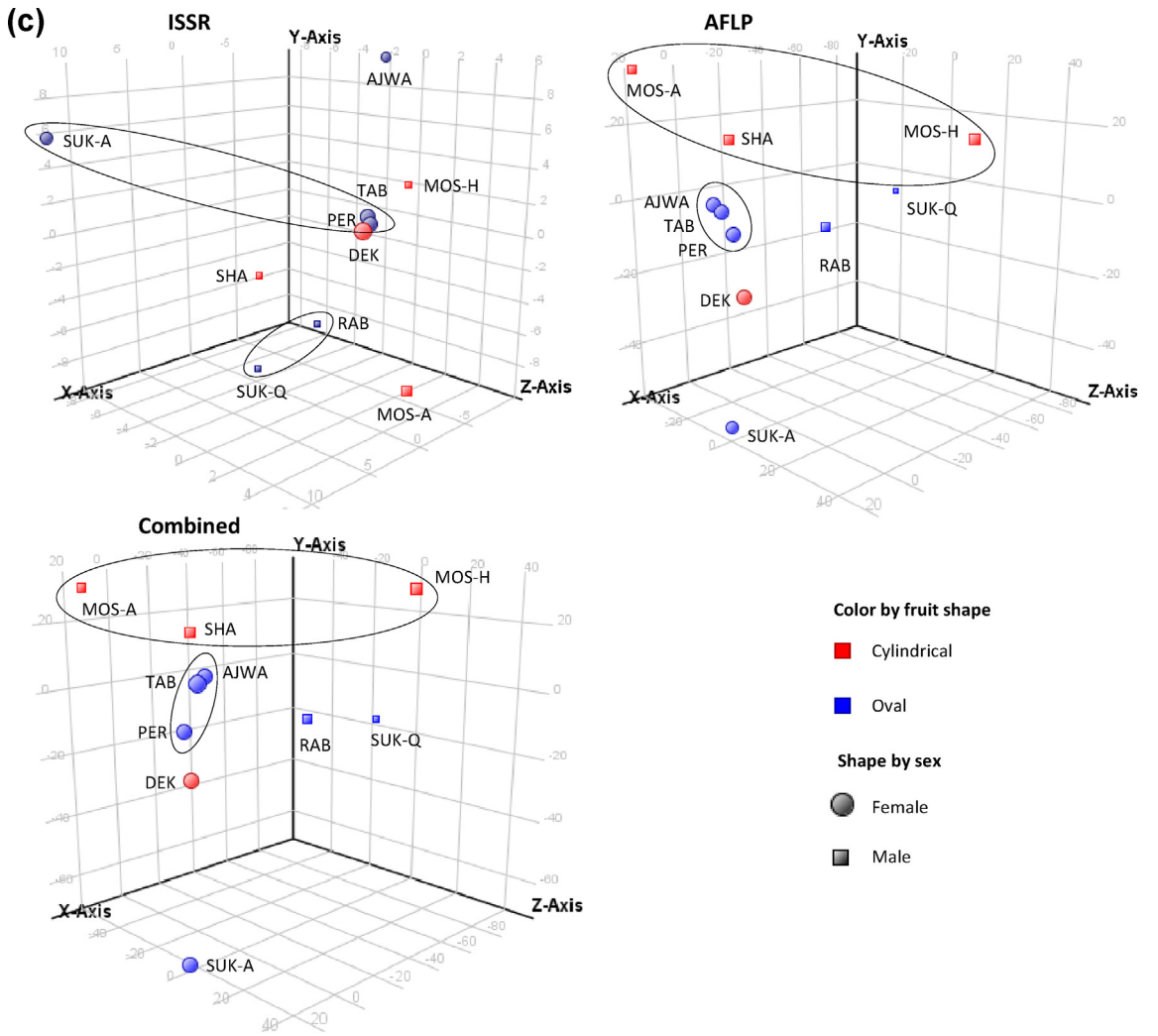

Fig. 2. (*Continued*)

SUK-Q and RAB in ISSR plot were the most closely related cultivars, as they share the same fruit shape (oval) and color (brown). In addition, SUK-A, PER and TAB share the same fruit shape (oval) and TAB and DEK share fruit color (yellow) (Fig. 2c, d). In AFLP or combined PCA plots, two sets of three cultivars (AJW, TAB and PER with oval fruits, and SHA, MOS-A and MOS-H with cylindrical fruits) showed relatedness based on fruit shape (Fig. 2c). In the PCA plots of AFLP or combined data based on fruit color, three sets of cultivars; i.e., SHA, MOS-A and MOS-H (yellow), TAB and DEK (yellow) and PER and SUK-A (brown) were most closely related (Fig. 2d).

The polymorphism information content (PIC), average heterozygosity (He), effective multiplex ratio (E), and marker index (MI) were computed based on experimental data (Table 7). AFLP data revealed higher PIC, He, E and MI values (Table 7) compared to ISSR indicating that AFLP is more effective in detecting polymorphism among date

Table 7
Polymorphism information content (PIC), expected heterozygosity for polymorphic products (He), effective multiplex ratio (E) and the marker index (MI) of each marker type used across different date palm cultivars.

Marker type	PIC	He	E	MI
ISSR	0.32	0.48	113	54.2
AFLP	0.37	0.50	534	266.6

ISSR: inter-simple sequence repeat; AFLP: amplified fragment length polymorphism.

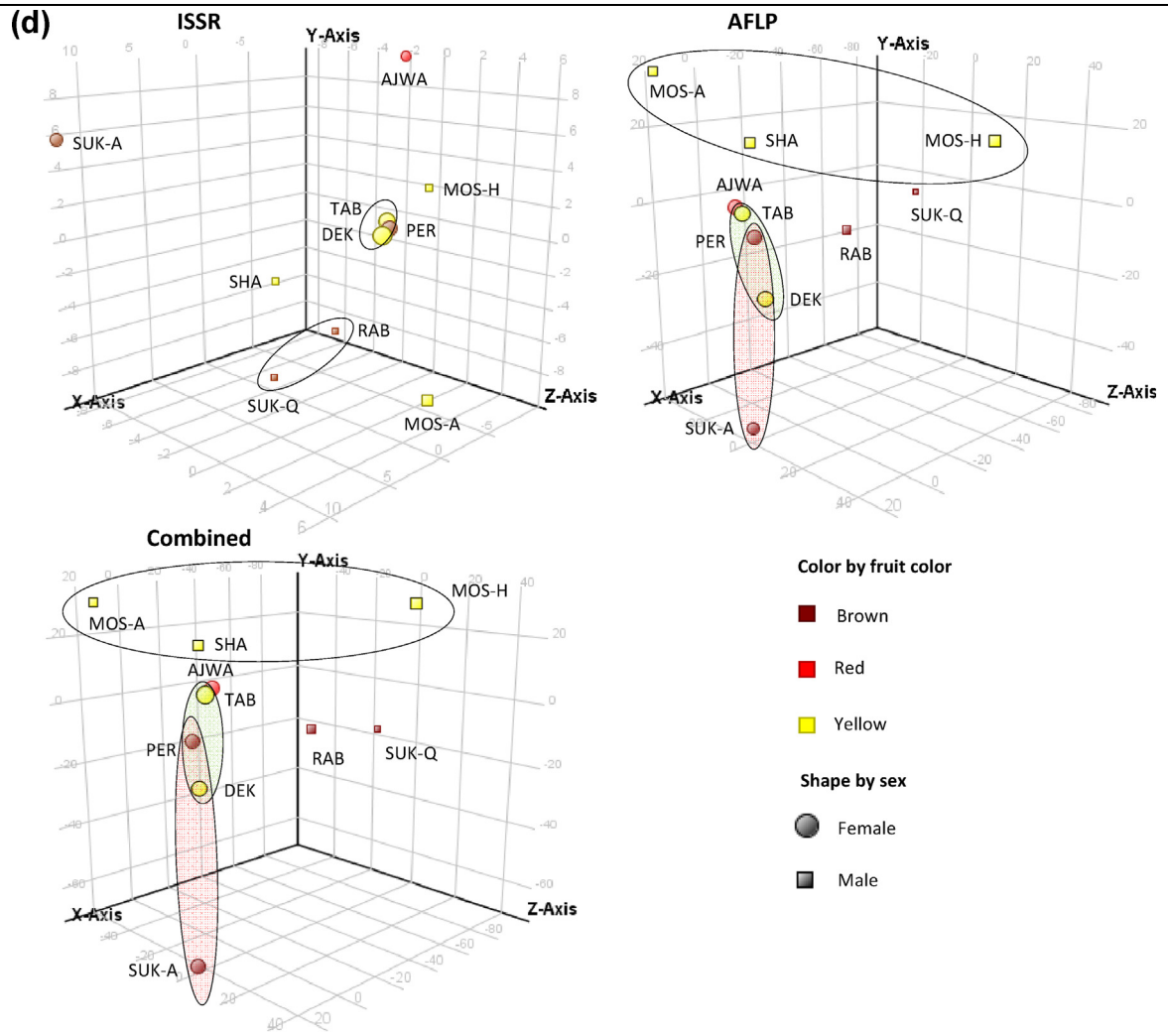

Fig. 2. (*Continued*)

palm cultivars. Forty-eight of the 114 ISSR markers (42%) and 267 of the 534 AFLP markers (50%) exhibited PIC values ranging from 0.4 to 0.6 (Fig. 3a, b). Effective multiplex ratio (E) of the AFLP depends on the fraction of polymorphic markers (β) as many of the fragments obtained by one primer combination are polymorphic across the examined date palm cultivars. These results highlight the distinctive nature of the AFLP marker compared to ISSR marker as a powerful procedure to survey the genetic diversity of date palm cultivars.

These results are in agreement with those of Powell et al. [28] (for both types of markers in soybean). In the present study, ISSR markers were found less reliable for detecting genetic relatedness among date palm cultivars than AFLP markers. The use of more ISSR primers may improve the reliability of this approach for characterizing cultivars at the molecular level. AFLP markers have recently been considered suitable for genomic diversity and cultivar fingerprinting [40–42]. In a number of studies, AFLP markers were also identified for economically important traits [43–45], some of which can be utilized in marker-assisted selection programs.

More recently, a genomic sequence analysis interestingly demonstrated that *P. dactylifera* experienced a genome-wide duplication and genetic diversity analysis indicated that stress resistance and sugar metabolism-related genes tend to be located in chromosomal regions with low density of single-nucleotide polymorphisms [46].

In conclusion, AFLP and ISSR markers differed in their ability to differentiate individuals and for detecting polymorphisms. They can complement each other, although this was not the case in the present study. However, these markers did provide sufficient variation to identify date palm cultivars from Saudi Arabia. Some of the AFLP markers generated through this work (e.g., sex marker) can be utilized in the future in breeding programs of date palm.

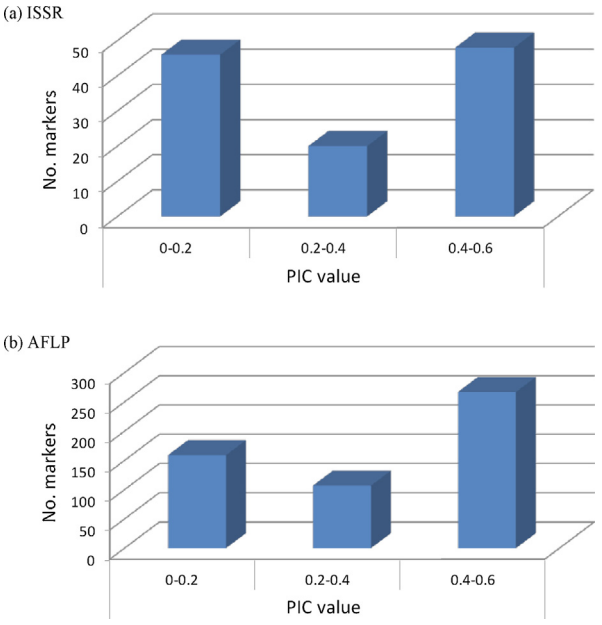

Fig. 3. Distribution of the polymorphism information content (PIC) obtained from inter-simple sequence repeat (a) and amplified fragment length polymorphism (b) datasets.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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