Journal of Asian Scientific Research

journal homepage: http://www.aessweb.com/journals/5003

MOLECULAR CHARACTERIZATION OF SOME IRAQI DATE PALM CULTIVARS USING RAPD AND ISSR MARKERS

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ABSTRACT

Date palm (Phoenix dactylifera L.) is an important fruit tree in Iraq. Assessment of genetic relationships among date palm cultivars is of major importance for characterization of date palm germplasm, breeding programs, and conservation purposes. Genetic polymorphism in 17 well known date palm cultivars in Iraqi representing ten female and seven male cultivars was assessed using 30 RAPD universal primers and 12 ISSR primers. Results revealed that 86 polymorphic bands were detected by RAPD analysis while 85 bands were observed when ISSRs were used. Correlation fit with a 0.87 r matrix correlation value was observed between RAPD and ISSR data. Therefore, a combined data analysis of both markers was done to assess phylogenetic relationships among cultivars. A total of 2530 scorable bands were generated from both RAPD and ISSR primers with an average of 72.29 fragments per primer. RAPD and ISSR primers were annealed with 240 loci across all cultivar genomes with an average of 6.86 loci per primer. Among these loci scored, 171 loci (71.25%) were polymorphic for at least one of the cultivars with an average of 4.89 polymorphic bands per primer. Genetic distances were estimated using Jaccard's genetic similarity index and were ranged from 0.422 between the two males 'Risasy' and 'Ghanami Akhhder' to 0.789 between 'Hilawi' and 'Ghanami Ahmer' according to ISSR markers. Unweighted pair group method with arithmetic mean UPGMA ordered date palm cultivars into two main clusters independently of their origin and sex. Moreover, all primer combinations contributed to the discrimination of date palm cultivars, suggesting the efficiency of RAPD and ISSR methods in assessing genetic diversity in Iraqi date palm germplasm.

Keywords: *Phoenix dactylifera* L., Phylogenetic relationships, RAPD, ISSR, PCA analysis, Genetic diversity.

Contribution/ Originality

This study contributes in the existing literature deals with the assessment of genetic relationships among date palm cultivars using DNA molecular markers. It's important for characterization of date palm germplasm, breeding programs, and conservation purposes. The study comprises two DNA marker systems (RAPD and ISSR) making a comparison between them for assessing genetic diversity in Iraqi date palm cultivars.

1. INTRODUCTION

Iraq is the date palm birthplace. Historically, it was the domestication centre of this crop and considered one of a great socio-economic importance in the Arabian region. The tree has been, and is still, at the centre of the comprehensive agricultural development. It is believed that the tree originated in Mesopotamia [1]. The numbers of known date palm cultivars that are distributed all over the world are approximately 5000 out of which about 600 are found in Iraq. Biochemical studies, including isozyme and activity analyses of peroxidases have been used to characterize date palms in Morocco and Tunisia [2-6]. Such analysis doesn't reflect precisely polymorphisms may occur [7]. DNA marker analysis in Iraqi date palm cultivars is at developmental stage. The employment of DNA marker to identify cultivars started by Jubrael [8] at IPA center for agriculture research, Baghdad. They used Random Amplified Polymorphic DNA (RAPD) markers to identify 9 female cultivars. While his co-worker, Al-Khateeb and Jubrael [9] used the same analysis to identify eight male cultivars. Amplified fragment length polymorphism (AFLP) markers were also been used for genetic fingerprinting of 18 Iraqi date palm cultivars [10]. Khierallah, et al. [11], used AFLP fingerprinting to characterize 18 of Iraqi date palm cultivars. Microsatellite markers were also employed to assess genetic diversity in 30 Iraqi date palm cultivars [11]. ISSR ('anchored SSR-PCR') markers [12] are simple, rapid, inexpensive, and highly reproducible due to their primer length and to the high stringency achieved by the annealing temperature [13]. ISSR is believed to be one of the most efficient techniques that reveals high polymorphism and determines genetic diversity in date palm. Zehdi, et al. [14] and Karim, et al. [15] reported the employment of ISSR as informative markers to investigate the phylogenic relationships among a set of Tunisian date palm cultivars. Similarly, ISSR technique was proved efficient for determinating the molecular phylogeny of date palm cultivars grown in Saudi Arabia [16-18] and Egypt [19]. Munshi and Osman [18] suggested that utilization of RAPD and ISSR markers could potentially have a high priority for studying genetic diversity and molecular characterization of date palm germplasm. The aim of this study is the use of RAPD and ISSR markers in assessing genetic diversity in some Iraqi date palm cultivars and makes a comparison between those two DNA marker systems.

2. MATERIALS AND METHODS

Plant materials and DNA extraction: A total of 17 well-defined reference Iraqi date palm cultivars were collected from two date palm stations belonging to the Ministry of Agriculture. Ten female cultivars were collected from Al-Mahaweel date palm station at Hilla Governorate south of Baghdad. The remaining nine cultivars (2 females and 7 males) were collected from Al-Zaafarania

Station in Baghdad. These cultivars originated from four different regions of Iraq, Baghdad, Diyala, Karbala and Basrah (Table 1). Total genomic DNA was extracted from young and healthy

Table-1. Name, gender and source of collection of 17 Iraqi date palm cultivars.

Cultivar	Gender	Sources of collection
Barhee	female	Al-Mahaweel Station
Qul Husaini	female	"
Deari	female	"
Helawi	female	"
Shwethi Ahmer	female	"
Baw Adem	female	"
Leelwi	female	"
Buliani	female	"
Um Al-Blaliz	female	Al-Za'afarania Station
Meer Haj	female	"
Risasy	male	"
Ghanami Akhder	male	"
Ghnami Ahmer	male	"
Khekri	male	"
Smeasmi	male	"
Ghulami	male	"
Greatli	male	"

leaves according to the procedure mentioned by Benito, et al. [20] with minor modifications. After purification, the resultant DNA was quantified using 1% agarose gel electrophoresis as described by Sambrook, et al. [21].

2.1. RAPD Analysis

Total DNA was extracted according to Benito, et al. [20] with some modifications. RAPD analysis was carried out according to Williams, et al. [22] with a few modifications. A total of 30 random decamer primers (Operon Technologies Inc., Alameda, California, USA) were used for RAPD amplification. PCR reactions were carried out in 25 µl volume containing 25 ng of total genomic DNA from each sample, 0.2 µl of a single primer, 100 mM of each dNTPs, 1X PCR buffer (10 mM Tris- HCl pH 8.3, 50 mM KCl, 2 mM $MgCl_2$) and 1 unit DNA *Taq* polymerase [23]. Amplification was performed using a thermocycler (PE 9600) programmed for RAPD: 1 cycle at 94ºC for 4 min, and 35 cycles with the following cycle profile: 1 min DNA denaturation step at 94 ºC, 2 min annealing step at 36 ºC, 2 min extension step at 72 ºC and last cycle at 72 ºC for 7 min, and an optional soak period at 4 ºC. Amplification products were loaded on 1% agarose gel and stained with ethidium bromide (0.5 mg/ml). Amplification for each primer was performed at least twice and only reproducible products were taken. DNA was visualized on a UV transilluminators (Gel documentation system ATTO, Japan). Fragment lengths were estimated by comparison with standard size markers (100bp DNA Ladder).

2.2. ISSR Analysis

Using 15 selected primers (Table 3), ISSR [24] was carried out on all date palm samples in a 25 µL reaction volume containing 7.5 mM Tris-HCl (pH 9 at 25 ºC), 50 mM KCl_2 mM $(NH_4)2SO_4$, 3 mM $MgCl_2$, 0.6 mM of each dNTP (Pioneer, Korea), 1.5 units of *Taq* DNA polymerase (Pioneer,

Korea), 20 ng of genomic DNA, and 200 pmol of each primer (Pioneer, Korea). Amplification reactions were subjected to a cycle of 94 ^{o}C for 5 min, followed by 40 cycles, each of which consisted of 94 ^{o}C for 10 sec, 50-58 ^{o}C (depending on the GC content of the primer, Table 2) for 10 sec, and 72 ^{o}C for 10 sec and a final extension step at 72 ^{o}C for 7 min. The PCR products were separated using 2.5% agarose gels in 0.5 × TBE buffer stained with ethidium bromide. A 100-bp DNA ladder (Biotools) was used to estimate the approximate molecular weight of DNA bands for each PCR product. Electrophoresis was performed at 85 V for 2.30 h. In order to reduce the possibility of cross contamination in RAPD and ISSR amplification reactions, a master reaction mixture was routinely prepared and a negative control was used. This control consisted of the reaction mixture excluding any DNA. Further, RAPD and ISSR analyses were repeated twice for all samples, and only clear bands produced in both replicates were scored as mentioned below.

2.3. Visualisation of Amplification Products and Data Analysis

Amplification profiles generated from RAPD and ISSR were photographed under UV light, screened, and compiled into a binary data matrix. Only distinct, reproducible and well-resolved fragments (bands) were recorded numerically as (1) when present or (0) when absent. Fragments with the same mobility were considered as identical, irrespective of fragment intensity. Genetic distances were estimated using Jaccard's genetic similarity index [25]. A dendrogram was generated by cluster analysis using the unweighted pair group method of the arithmetic averages (UPGMA). Principal coordinated analysis (PCA) was also carried out to display multiple dimension of the distribution of the accessions in a scatter-pot by PAST software version 2.17 [26]. The RAPD and ISSR matrices were subjected to the Mantel Test to verify the level of conformity between the data generated.

3. RESULTS AND DISCUSSION

3.1. RAPD Analysis

Out of 30 selective RAPD universal primers tested, only 23 gave clear amplification. A total of 1608 scorable bands were generated from those primers with an average of 73 fragments per primer (Table 2). The number of amplified fragments per cultivar varied from 21 bands for the primer OP-C08 showing the lowest primer efficiency (1.31%) to 129 bands for the primer OP-W11 showing the highest primer efficiency (7.65%) with an average of 73 fragments per primer. RAPD primers were annealed with 150 loci across all cultivar genomes with average of 7 loci per primers. Among those loci scored, 86 loci (57.3%) were polymorphic for at least one of the cultivars with an average of 7 polymorphic bands per primer. The primers OPA.03 and OPA.11amplified only polymorphic bands (100% polymorphism). The primer OPA.03 showed the highest discrimination power (8.14%). While the primers OP-C08, OP-P07 and OP-R13 amplified only monomorphic bands showing no polymorphism with any of cultivars tested (Table 2).

3.2. ISSR Analysis

A total of 922 scorable bands were generated from ISSR primers (Table 3). The number of amplified fragments per cultivar varied from 47 bands for the primer DPISR10 showing the lowest

primer efficiency (5.1%) to 112 bands for the primer DPISR3 exhibiting the highest primer efficiency (12.15%) with an average of 62 fragments per primer. ISSR primers were annealed with 90 loci across all cultivar genomes with an average of 6 loci per primer.

Table-2. Number of fragments amplified, polymorphic bands, primer efficiency and discrimination power of primers used for RAPD analysis of 17 Iraqi date palm cultivars.

Primer	Sequence (5`-3`)	Total fragment (no.)	No. of loci	% Primer Efficiency	Polymorphic fragment (no.)	% Poly-morphsim	% Discriminatio n power
OP-A03	AGTCAGCCAC	73	7	4.54	7	100.0	8.14
OP-A05	AGGGGTCTTG	60	5	3.73	4	80.0	4.65
OP-A07	GAAACGGGTG	45	5	2.8	3	60.0	3.49
OP-A11	CAATCGCCGT	37	3	2.3	3	100.0	3.49
OP-B10	CTGCTGGGAC	104	10	6.47	6	60.0	6.98
OP-C02	GTGAGGCGTC	62	5	3.86	2	40.0	2.33
OP-C05	GATGACCGCC	72	6	4.48	3	50.0	3.49
OP-C08	TGGACCGGTG	21	2	1.31	0	0.0	0.00
OP-D20	ACCCGGTCAC	64	9	3.98	5	55.6	5.81
OP-E19	ACGGCGTATG	62	5	3.86	4	80.0	4.65
OP-F06	GGGAATTCGG	64	5	3.98	3	60.0	3.49
OP-I01	ACCTGGACAC	129	10	8.02	5	50.0	5.81
OP-I02	GGAGGAGAGG	108	9	6.72	5	55.6	5.81
OP-I04	CCGCCTAGTC	37	3	2.3	1	33.3	1.16
OP-I08	TTTGCCCGGT	102	10	6.34	7	70.0	8.14
OP-I10	ACAACGCGAG	60	5	3.73	3	60.0	3.49
OP-P06	GTGGGCTGAC	58	6	3.61	4	66.7	4.65
OP-P07	GTCCATGCCA	52	5	3.23	0	0.0	0.00
OP-P08	ACATCGCCCA	102	11	6.34	6	54.5	6.98
OP-P18	GGCTTGGCCT	73	6	4.54	3	50.0	3.49
OP-R13	GGACGACAAG	42	5	2.61	0	0.0	0.00
OP-W11	CTGATGCGTG	123	10	7.65	7	70.0	8.14
OP-X17	GACACGGACC	58	8	3.61	5	62.5	5.81
Total		1608	150		86		
Average		73	7	4.54	7	57.3	8.14

Table-3. Number of fragments amplified, polymorphic bands, primer efficiency and discrimination power of primers used for ISSR analysis of 17 Iraqi date palm cultivars.

Primer	Sequence (5`-3`)	Total fragment (no.)	No. of loci	% Primer Efficiency	Polymorphic fragment (no.)	% Poly-morphsim	% Discrimina-tion power
DPISR1	AGGAGGAGGAGGAGGAGG	62	6	6.72	6	100.0	7.06
DPISR2	AGAGAGAGAGAGAGAGAG AGG	76	7	8.24	7	100.0	8.24
DPISR3	AGAGAGAGAGAGAGAGAG AGC	112	10	12.15	8	80.0	9.41
DPISR4	AGAGAGAGAGAGAGAGAG AGT	59	9	6.40	9	100.0	10.59
DPISR5	CTCTCTCTCTCTCTCTCT A	78	8	8.46	8	100.0	9.41
DPISR6	CTCTCTCTCTCTCTCTCT G	83	10	9.00	8	80.0	9.41
DPISR7	CTCTCTCTCTCTCTCTCT T	111	9	12.04	9	100.0	10.59
DPISR8	GACACGACACGACACGAC AC	65	6	7.05	6	100.0	7.06
DPISR9	ACTGACTGACTGACTG	84	7	9.11	6	85.7	7.06
DPISR10	TGGATGGATGGATGGATGG A	47	5	5.10	5	100.0	5.88
DPISR11	AGAGAGAGAGAGAGAGAG AG	90	7	9.76	7	100.0	8.24
DPISR12	GACAGACAGACAGACA	55	6	5.97	6	100.0	7.06
Total		922	90		85		
Average		62	6	6.72	6	94.4	7.06

Among those loci scored, 85 loci (94.4%) were polymorphic for at least one of the cultivars with an average of 6 polymorphic bands per primer. All ISSR primers gave 100% polymorphism except DPISR3, DPISR6 and DPISR9 which amplified 8, 8 and 6 polymorphic bands giving 80, 80 and 85.7% polymorphism respectively. The primers DPISR4 and DPISR7 showed the highest discrimination power (10.59%). While the primers DPISR10 amplified only 5 bands recording the lowest discrimination power (5.88%) as shown in Table 3.

Table-4. Parameters revealed by RAPD, ISSR and both RAPD + ISSR markers analysis in 17 date palm cultivars grown in Iraq.

Parameters	RAPD	ISSR	RAPD+ISSR
Number of primers used	23	12	35.0
Number of total fragments	1608	922	2530
Average of fragment/ primer	73	62	72.29
Number of loci	150	90	240.0
Average of loci/ primer	7	6	6.86
Primer efficiency percentage	4.54	6.72	-
Number of polymorphic fragment	86	85	171.0
Average of polymorphic fragment /primer	3.74	7.08	4.89
Polymorphism percentage	57.3	94.4	71.25
Discrimination power percentage	8.14	7.06	-

The summary of the above results is shown in table 4. A total of 2530 scorable bands were generated from both RAPD and ISSR primers with an average of 72.29 fragments per primer. RAPD and ISSR primers were annealed with 240 loci across all cultivar genomes with an average of 6.86 loci per primer. Among scored loci, 171 loci (71.25%) were polymorphic for at least one of the cultivars with an average of 4.89 polymorphic bands per primer. Although RAPD primers generated more fragments per primer (73), number of loci (150) and discrimination power (8.14%) than ISSRs, it gave less primer efficiency (4.54%) and an average of polymorphic fragments per primer (3.74). ISSR markers revealed higher polymorphism (94.4%) than RAPD (57.3%) and both RAPD+ISSR (71.25%) (Table 4).

3.3. Genetic Distances and Cluster Analysis

The pair-wise Euclidean genetic distances index was calculated for the 86 RAPD polymorphic fragments, 80 ISSRs and both RAPD+ISSR polymorphic bands of the 17 cultivars (Tables 5, 6 and 7). The cultivar 'Shwethi Ahmer' was highly divergent from 'Ghnami Ahmer' with distances 0.672, 0.789 and 0.702 for the three analyses respectively. The cultivar ' Risasy ' is closely related to 'Ghnami Akhder' with distances 0.442, 0.422 and 0.436 for the three analyses respectively. According to RAPD analyses that revealed by 86 polymorphic loci, UPGMA ordered the date palm cultivars into two main clusters irrespective of their origin at similarity level of 0.56 (Figure 1-A).

The first one consisted of two male cultivars: 'Ghnami Ahmer' and 'Ghulami' and the second consisted of two sub-clusters, the first one consisted of five cultivars, four males: 'Risasy', 'Ghanami Akhder', 'Khekri' and 'Smeasmi' and the second sub-cluster consisted of two sub-

clusters, the first consisted of nine cultivars: 'Baw Adem', 'Buliani', 'Meer Haj', 'Um Al-Blaliz', 'Barhee ', 'Helawi', 'Shwethi Ahmer' and the male 'Greatli' while the second sub-cluster consisted of the last cultivar 'Qul Husaini' (figure 1-A). Eighty five ISSR polymorphic loci markers ordered the 17 date palm cultivars into two main clusters according to UPGMA analysis irrespective of their origin (Figure 1-B). The first one consisted of the male cultivar 'Ghnami Ahmer' and the second consisted of two sub-clusters, the first one consisted of the cultivar 'MeerHaj' while the second sub-cluster consisted of two sub-clusters, the first consisted of the cultivar 'Leelwi' and the second consisted of two sub-clusters, the first consisted of nine cultivars: 'Barhee', 'Qul Husaini', 'Baw Adem', 'Buliani', 'Deari', 'Helawi', 'Shwethi Ahmer' and the two males 'Greatli' and 'Ghanami Akhder'. The second sub-cluster consisted of the rest cultivars: the female 'Um Al-Blaliz', and four males 'Risasy', 'Khekri' 'Smeasmi' and 'Ghulami' as shown in Figure 1-B. Results shown in Figure 1-C revealed two main clusters with 0.44 similarity according to RAPD+ISSR markers. UPGMA analysis revealed by 171 polymorphic loci ordered the date palm cultivars into two main clusters irrespective of their origin. The first one consisted of the male cultivar:

Table-5. Genetic distance values among the 17 Iraqi date palm cultivars revealed by RAPD analysis.

Cultivar	Buliani	Qul Husaini	Baw Adem	Meer Haj	Barhi	Deari	Helawi	Shwethi Ahmer	Greatli	Risasy	Ghanami Akhder	Um Al-Blaliz	Ghanami Ahmer	Ghulami	Khekri	Smeas-mi	Leelwi
Buliani	0.000																
QulHusaini	0.537	0.000															
BawAdem	0.489	0.505	0.000														
MeerHaj	0.478	0.526	0.521	0.000													
Barhi	0.500	0.566	0.521	0.478	0.000												
Deari	0.505	0.590	0.547	0.483	0.483	0.000											
Helawi	0.516	0.590	0.594	0.537	0.460	0.511	0.000										
Shwethi Ahmer	0.521	0.566	0.561	0.521	0.521	0.448	0.460	0.000									
Greatli	0.511	0.526	0.542	0.511	0.552	0.557	0.537	0.511	0.000								
Risasy	0.561	0.576	0.571	0.580	0.532	0.566	0.585	0.590	0.552	0.000							
Ghanami Akhder	0.561	0.594	0.561	0.599	0.580	0.547	0.576	0.552	0.571	0.442	0.000						
UmAl-Blaliz	0.532	0.537	0.590	0.478	0.561	0.526	0.566	0.561	0.500	0.561	0.552	0.000					
Ghanami Ahmer	0.603	0.668	0.630	0.630	0.612	0.634	0.634	0.672	0.663	0.603	0.547	0.603	0.000				
Ghulami	0.566	0.599	0.537	0.612	0.585	0.580	0.668	0.612	0.566	0.576	0.547	0.547	0.532	0.000			
Khekri	0.547	0.580	0.537	0.576	0.526	0.532	0.590	0.594	0.537	0.483	0.483	0.537	0.626	0.571	0.000		
Smeasmi	0.566	0.599	0.603	0.566	0.566	0.532	0.571	0.576	0.547	0.505	0.557	0.526	0.651	0.590	0.489	0.000	
Leelwi	0.580	0.630	0.626	0.571	0.552	0.557	0.594	0.571	0.542	0.552	0.599	0.521	0.585	0.603	0.566	0.505	0.000

Table-6. Genetic distance values among the 17 Iraqi date palm cultivars revealed by ISSR analysis.

Cultivar	Buliani	QulHusaini	BawAdem	MeerHaj	Barhi	Deari	Helawi	Shwethi Ahmer	Greatli	Risasy	Ghanami Akhder	UmAl-Blaliz	Ghnami Ahmer	Ghulami	Khekri	Smeasmi	Leelwi
Buliani	0.000																
Qul Husaini	0.408	0.000															
Baw Adem	0.483	0.432	0.000														
MeerHaj	0.516	0.568	0.483	0.000													
Barhi	0.516	0.459	0.459	0.558	0.000												
Deari	0.587	0.537	0.558	0.624	0.527	0.000											
Helawi	0.577	0.568	0.641	0.667	0.494	0.506	0.000										
Shwethi Ahmer	0.537	0.506	0.527	0.577	0.516	0.506	0.471	0.000									
Greatli	0.516	0.483	0.459	0.596	0.516	0.587	0.537	0.471	0.000								
Risasy	0.624	0.577	0.577	0.658	0.587	0.577	0.587	0.568	0.527	0.000							
Ghanami Akhder	0.527	0.471	0.471	0.548	0.527	0.558	0.568	0.506	0.435	0.422	0.000						
UmAl-Blaliz	0.658	0.558	0.615	0.641	0.548	0.596	0.506	0.548	0.568	0.471	0.494	0.000					
Ghnami Ahmer	0.730	0.767	0.691	0.596	0.730	0.767	0.760	0.789	0.745	0.658	0.691	0.691	0.000				
Ghulami	0.624	0.615	0.596	0.624	0.606	0.615	0.641	0.606	0.606	0.537	0.577	0.516	0.675	0.000			
Khekri	0.548	0.537	0.537	0.624	0.568	0.577	0.568	0.548	0.506	0.516	0.447	0.494	0.641	0.471	0.000		
Smeasmi	0.587	0.577	0.596	0.658	0.548	0.577	0.568	0.568	0.548	0.494	0.537	0.471	0.641	0.537	0.422	0.000	
Leelwi	0.632	0.658	0.606	0.577	0.596	0.606	0.632	0.577	0.615	0.606	0.606	0.606	0.650	0.587	0.568	0.506	0.000

Table-7. Genetic distance values among the 17 Iraqi date palm cultivars of as revealed by RAPD+ISSR analysis.

Cultivar	Buliani	QulHusaini	BawAdem	MeerHaj	Barhi	Deari	Helawi	ShwethiAhmer	Greatli	Risasy	GhanamiAkhder	UmAl-Blaliz	GhnamiAhmer	Ghulami	Khekri	Smeasmi	Leelwi
Buliani	0.000																
QulHusaini	0.498	0.000															
BawAdem	0.487	0.480	0.000														
MeerHaj	0.491	0.540	0.509	0.000													
Barhi	0.505	0.534	0.502	0.505	0.000												
Deari	0.534	0.573	0.550	0.534	0.498	0.000											
Helawi	0.537	0.583	0.610	0.583	0.472	0.509	0.000										
ShwethiAhmer	0.527	0.547	0.550	0.540	0.520	0.468	0.464	0.000									
Greatli	0.513	0.513	0.516	0.540	0.540	0.567	0.537	0.498	0.000								
Risasy	0.583	0.576	0.573	0.607	0.550	0.570	0.586	0.583	0.544	0.000							
GhanamiAkhder	0.550	0.557	0.534	0.583	0.563	0.550	0.573	0.537	0.530	0.436	0.000						
UmAl-Blaliz	0.576	0.544	0.598	0.537	0.557	0.550	0.547	0.557	0.523	0.534	0.534	0.000					
GhnamiAhmer	0.648	0.702	0.651	0.619	0.653	0.681	0.689	0.702	0.691	0.622	0.598	0.634	0.000				
Ghulami	0.586	0.604	0.557	0.616	0.592	0.592	0.659	0.610	0.579	0.563	0.557	0.537	0.583	0.000			
Khekri	0.547	0.567	0.537	0.592	0.540	0.547	0.583	0.579	0.527	0.495	0.472	0.523	0.631	0.540	0.000		
Smeasmi	0.573	0.592	0.601	0.598	0.560	0.547	0.570	0.573	0.547	0.502	0.550	0.509	0.648	0.573	0.468	0.000	
Leelwi	0.598	0.639	0.619	0.573	0.567	0.573	0.607	0.573	0.567	0.570	0.601	0.550	0.607	0.598	0.567	0.505	0.000

'Ghnami Ahmer' and the second consisted of two sub-clusters, the first one consisted of the male 'Ghulami' while the second consisted of two sub-cluster, the first one consisted of five cultivars, four males: 'Risasy', 'Ghanami Akhder', 'Khekri' and 'Smeasmi' and one female ' Um Al-Blaliz' while the second sub-cluster consisted of two sub-clusters, the first consisted of five cultivars: 'Baw Adem', 'Buliani', 'MeerHaj', 'Barhee', 'Qul Husaini' and the male 'Greatli' and the second sub-cluster consisted of the rest four cultivars 'Helawi', 'Shwethi Ahmer', 'Barhee' and 'Deari' as shown in Figure 1-C. In order to confirm the results of genetic relationships among date palm cultivars, the data were analyzed by Multivariate Principal Component Analysis (PCA) and shown in figure 2-A, B and C . Each scatter diagram of the first two (PC1 and PC2) based on 86, 85 and 171 polymorphic bands of RAPD, ISSR and both of them respectively exhibited the same clusters of cultivars as in each dendrogram.

Figure-1.A. Genetic relationships among the 17 Iraqi date palm cultivars estimated by RAPD analysis.

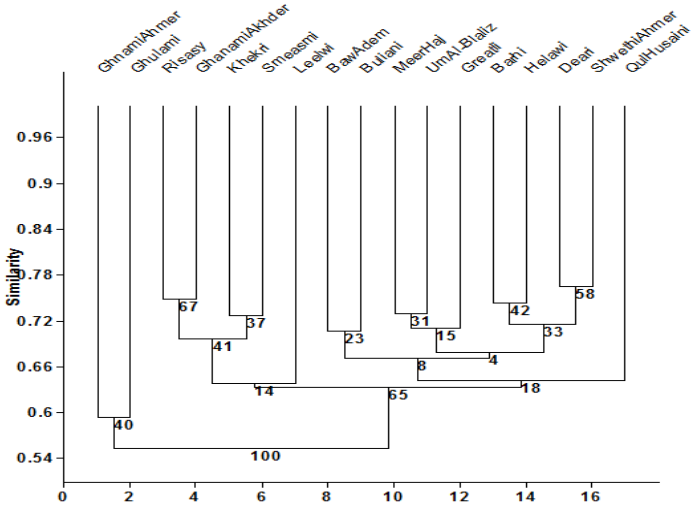


Figure-1.B. Genetic relationships among the 17 Iraqi date palm cultivars estimated by ISSR analysis.

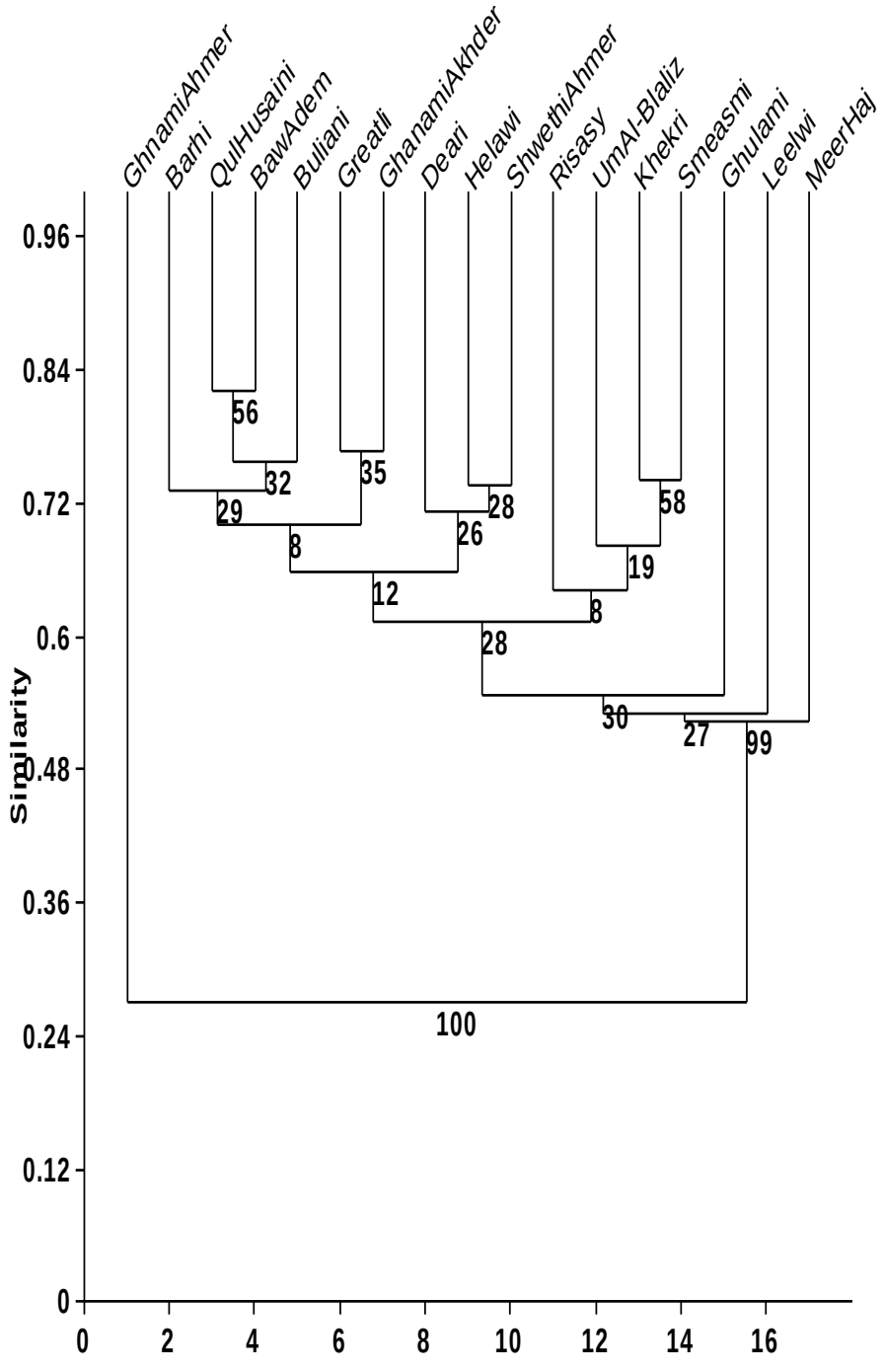
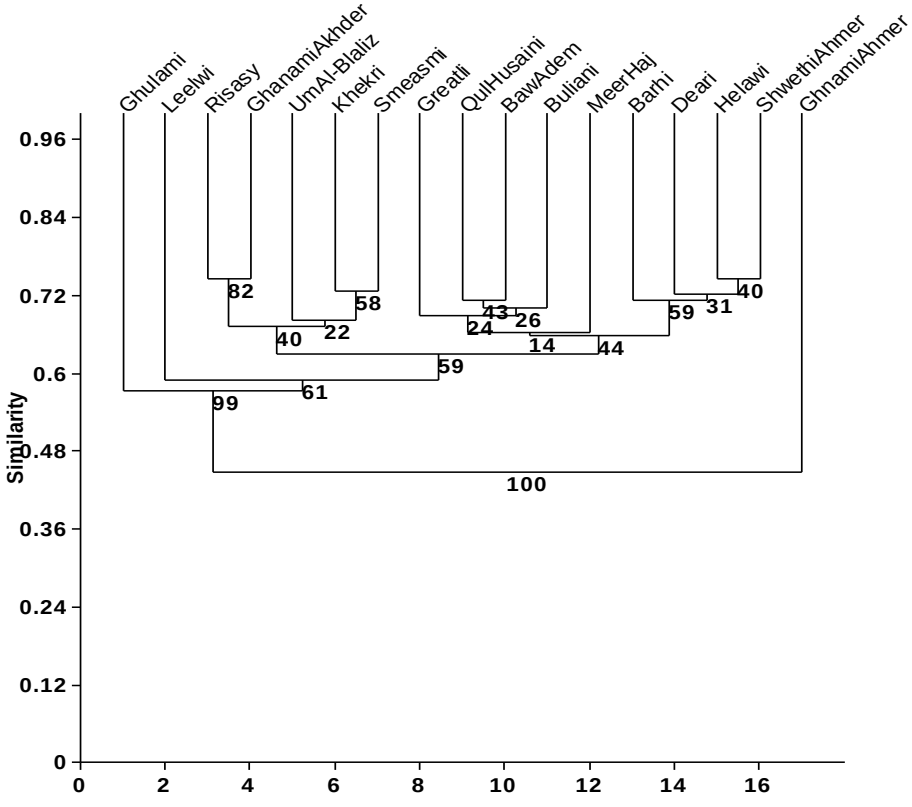


Figure-1.C. Genetic relationships among the 17 Iraqi date palm cultivars estimated by RAPD + ISSR analysis.



Date palm domestication and the nature of date palm culture may have played a vital role in the composition of date palm genomes [27]. Continuous selection was carried following asexual reproduction implemented by farmers may resulted in new cultivars Emergence. Exchange of propagules, which are a mixture of vegetative and seed-propagated materials, has been conducted among farmers. All these processes together may result in a mixed genome within the same country [27]. ISSR analysis showed a high level of polymorphism among Iraqi cultivars. However, the cultivar nomenclature and classification is still remaining to be based on fruit characters, including morphological, physical and chemical traits. Results obtained in this study demonstrated the efficiency of the two markers for assessment of genetic diversity of Iraqi date palm cultivars. Genetic diversity value was higher in ISSR markers with high primer efficiency compared with RAPD markers or RAPD+ISSR. The high level of polymorphism detected in date palm cultivars by ISSR markers compared with RAPDs revealed the discriminating capacity of the former. The co-dominant nature of ISSR markers permits the detection of a high number of loci and contributes to higher levels of expected heterozygosity being reached than would be possible with RAPDs. The phylogenetic tree constructed in this study revealed that the examined date palm cultivars are not monophelic and provide evidence of divergence among all tested genotypes since they were grouped in clusters.

This confirms the findings of Trifi, et al. [28], Hamama, et al. [29], and Hussein, et al. [16] and Haider, et al. [30]. However, a close relationship among cultivars was observed and the genetic polymorphism among them was found to be narrow. Genetic similarity index estimated by two DNA markers used in this study indicated the presence of very close cultivars to each other (0.422) 'Ghanami Akhdhar' and 'Resasy' . Our findings confirmed the closeness of male cultivars according to the tested markers as they were mostly in the

Figure-2.A. Principle component analysis of the 17 Iraqi date palm cultivars estimated by RAPD markers.

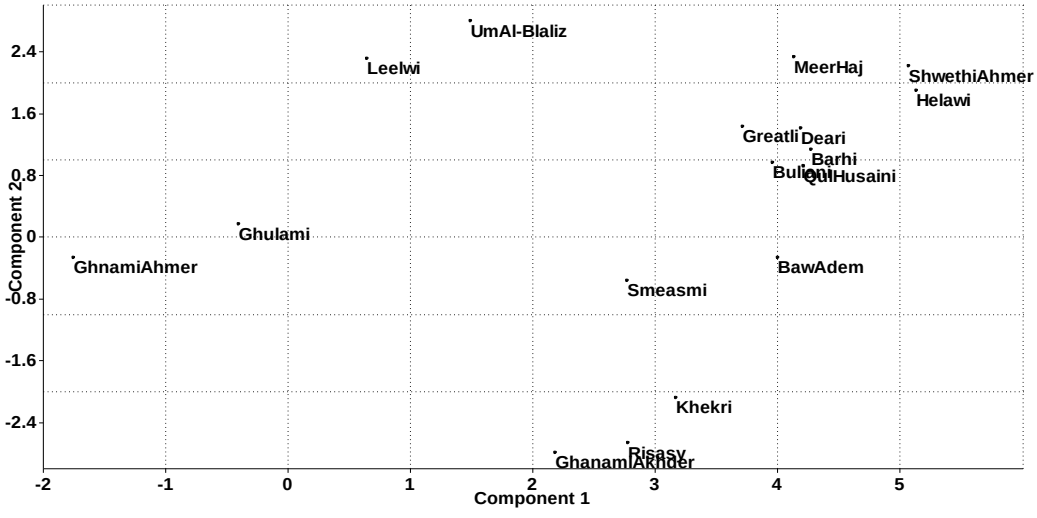


Figure-2.B. Principle component analysis of the 17 Iraqi date palm cultivars estimated by ISSR markers.

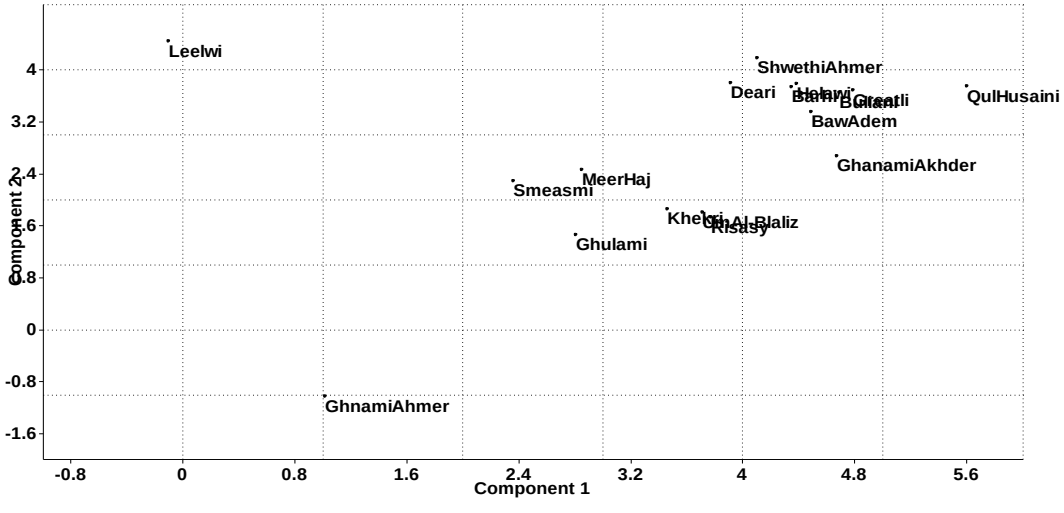
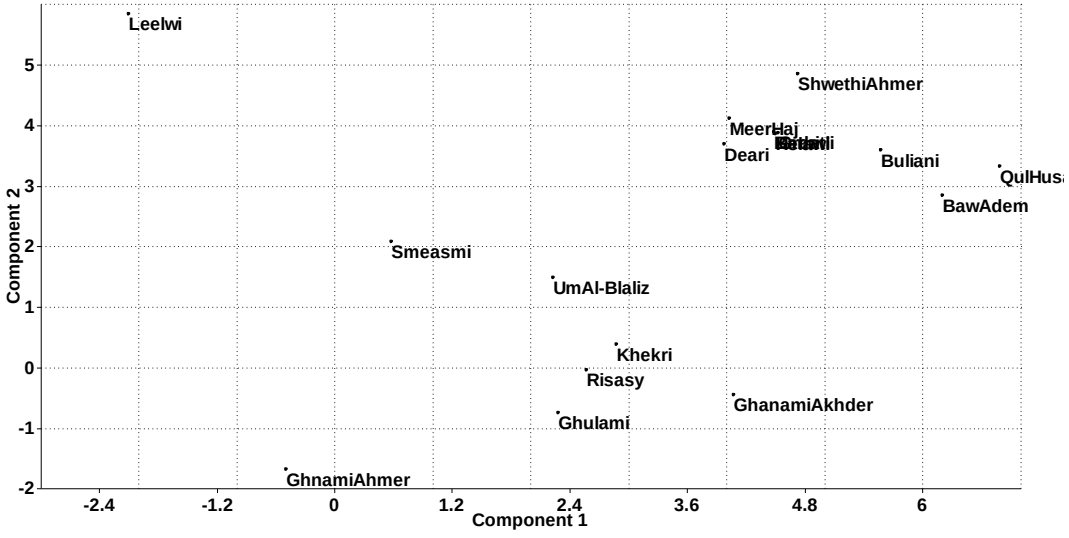


Figure-2.C. Principle component analysis of the 17 Iraqi date palm cultivars estimated by RAPD + ISSR markers.



Same cluster indicating the possibility of linkage between these markers and sex region in date palm.

In conclusion, both RAPD and ISSR markers were efficient in detecting genetic relationship among Iraqi date palm cultivars. ISSR markers exceeded RAPDs in their efficiency of assessing genetic diversity in Iraqi date palm cultivars.

4. ACKNOWLEDGEMENTS

Authors appreciated Prof. Kadhim M. Ibrahim for revising the manuscript.

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