Determination of genetic diversity of Iraqi date palm (*Phoenix dactylifera* L.) by using ISSR technique

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Abstract

This study provided that all molecular markers inter simple equence repeat (ISSR) succeded with the sixty five Iraqi date palm (*Phoenix dactylifera* L.) cultivars, which collected from Hilla city in Iraq, to determine fingerprinting, polymorphic value, and genetic relationships among varieties of date palm cultivars, and also with the same type of cultivars. This study was used ten of ISSR primers from different sources and showed the average percentage of polymorphism per ISSR-primer was %98.9, primer efficiency was %0.13 and discrimination value was %0.09. This study revealed that high similarity percentage was about %67 to %100 between cultivars belonged to the same variety. Genetic similarty with twenty Iraqi date palm cultivars, using UBC-835 primer as a sample in this study revealed the highest distance was 0.5345 between Breem and each of varieties (Sabb Drrh, Khadrawi, Fom Alraman, shwrthi and Ghanami Ahmer), that means no similarity with these date palm cultivars. On the other hands, the lowest genetic distance was (zero) between Tebrazal and each of (Maktom and Najdi). Also between Sabb Drrah and each of (Khadrawi, Fom Alrman, Shwethi, and Ghanami Ahmer). In addition to that between Hamrawi and each of (Kestawi and Chipchab). There are no gentic distance with Brban and Sukkary. Also between Khadrawi and each of (Fom Alrman, Shwethi and Ghanami Ahmer). Additionaly, no genetic distance between Khadrawi and Chipchab, Maktom and Najdi. Also, no genetic distance between Fom Alraman and each of Shwethi, and Ghanami Ahmer). At the end no genetic distance between Shwethi, and Ghanami Ahmer. No distance between these varieties was observed.

Introduction

Date palm (*Phoenix dactylifera* L.*)* is the major fruit crop of arid climate regions. It (2n=2x=36) is considered of great socioeconomic importance in the rabian region (Wrigley, 1995). The number of known date palm cultivars that are distributed all over the world are 5000 of which 600 are found in Iraq. Before 1991, Iraq was the largest producer of dates in the world (Food and Agriculture Organization of the United Nations, 2008) and had the largest date forest in the world (MacFarquhar, 2003). However, during the Gulf and Iran- Iraq wars, Iraqi number of date palm trees was destroyed. Wars and sanctions imposed on Iraq have negatively affected both the production and natural genetic diversity of the crop in Iraq and inhibited the much- needed impetus to rebuild the date palm industry (Jubrael *et al.*, 2005).

The unique characteristics of date palm can be truly called "tree of life" and is considered as one of the most ancient plant. The rich fruit plays an important role in the nutrition of human population, and also several products are made that generate employment and thus influence socioeconomic aspect of people. Therefore, it is widely acknowledged sustainability value in social, economic and ecological terms. Moreover, this crop has great potential as a source of renewable energy, by producing bio-fuel since its fruits high in

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carbohydrates 44-88% total sugars (sudhersan *et al.*, 2009). In spite of the date palm is one of the oldest cultivated fruit trees, but there are a few genetic resources for improving the productivity and development of the dioecious date palm (Muthew *et al.*, 2014).

DNA-based markers and its traits in date palm progeny segregation could be used for selection instead of morphological traits. DNA fingerprinting, also known as DNA typing or genetic fingerprinting, uses for identifying individuals by the particular of their DNA. There are many molecular markers applied to identify date palm cultivars, for understanding and analyze the genetic relationships and genetic diversity among date palm varieties,. These technique has been applied for cultivar genotyping (Trifi *et al*., 1998 and 2000; Ben-Abdallah *et al*., 2000;) and for analyses of phylogenetic relationships and genetic varieties (Al-Khalifah and Askari, 2003 Al-Moshileh *et al*., 2004; El-Tarras *et al*., 2007). Inter Simple Sequence Repeats (ISSRs) technique uses amplification of DNA segments present at an amplifiable distance between two identical microsatellite repeats oriented in opposite directions (Khanam *et al*., 2012). The main aim of the present study is to investigate the suitability of ISSR, markers to distinguish some date palm cultivars, and to detect genetic diversity in natural field populations.

Materials and Methods

Plant Materials

Fifty five date palm females' cultivars and nine males as date superior pollinators. Collected from Hillah city in Iraq Fig. (1). The young whit leaves collected, up the palm nearby heart of the tree from both genders, which represented number of cultivars per species from different locals. Table (1) illustrated these details.

Table (1). Details of sixty five date palm cultivars were grown in Hillah city.

No.	Cultivar	No. of Cultivar	Code	Gender
1	Bream	3	A	Female
2	Tebarzal	3	B	Female
3	Sabb Drrah	3	C	Female
4	Hamrawi	3	D	Female
5	Berban	3	E	Female
6	Ashrasi	3	F	Female
7	Zahdi	3	G	Female
8	Sultana	3	H	Female
9	Khadrawi	3	I	Female
10	Sukkary	3	J	Female
11	Khestawi	3	K	Female
12	Usta Umran	3	L	Female

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13	Guntar	3	M	Female
14	Maktom	3	N	Female
15	Nersi	3	O	Female
16	Maddany	3	P	Female
17	Barhi	2	Q	Female
18	Chipchab	2	R	Female
19	Najdi	2	S	Female
20	Fom Alrman	1	T	Female
21	Shwethi	1	U	Female
22	Greatli	3	V	Male
23	Ghanami Ahmer	3	W	Male
24	Smeasmi	3	X	Male

DNA Extraction

Leaves of date palm cultivars for all specious used in this study, which were collected from hillah city. Leaves (200mg) were grounded to a powder using liquid nitrogen and placed in the microfuge tubes then DNA were extraction by using Mini Kit (Geneaid Biotech. Ltd; Taiwan company), for yield purifying total DNA including genomic DNA, chloroplast and mitochondrial DNA, from plant tissue, according to manufacturer manual.

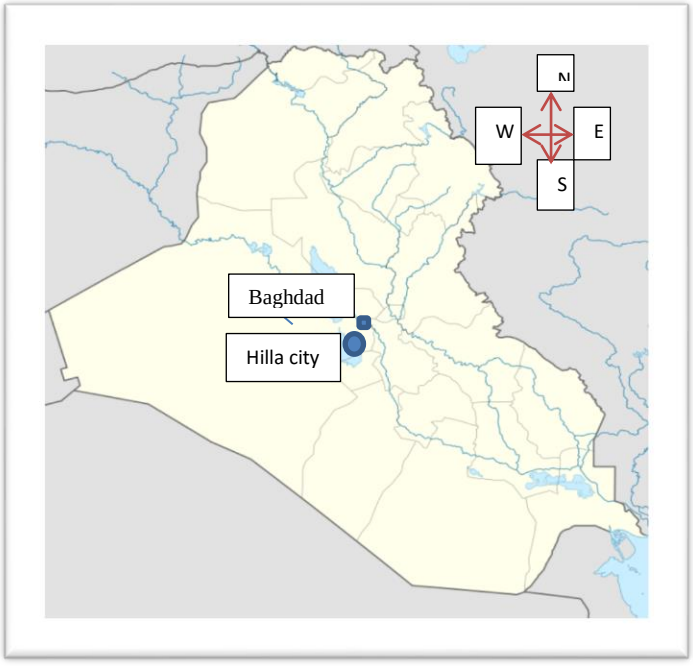

Fig. 1. Sample collection sites of date palm in Hillah city, Iraq.

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Primers

Ten ISSR primers are listed in tables(2) mentioned their names and nucleotide sequences of each primer and annealing temperatures.

Tabel(2). List of (ISSR) primers names and nucleotid sequences and annealing temperature.

No.	Primer name	Sequence (5′-3′)	Annealing temp.	Reference
1	ISSR 06	GAG AGA GAG AGA GAGAC	52 C˚	Monire marsafari,2013
2	UBC824	TCT CTC TCT CTC TCT CG	52 C˚	
3	UBC823	TCT CTC TCT CTC TCT CC	52 C˚	
4	UBC826	ACA CAC ACA CAC ACA C	52 C˚	
5	UBC835	AGA GAG AGA GAG AGA CYA	55 C˚	
6	UBC840	GAG AGA GAG AGA GAGAYT	52 C˚	
7	UBC841	GAG AGA GAG AGA GAGAYC	55 C˚	
8	UBC842	GAG AGA GAG AGA GAGAYG	55 C˚	
9	UBC888	ĤBDB CAC ACA CAC ACA CA	52 C˚	
10	UBC889	DBD ACA CAC ACA CAC AC	52 C˚	

*T, G or C=(B), T or C =(Y), T,G or A =(D)

DNA Molecular Size of Markers

Amplicon size was estimated using 100-bp DNA stander (ladder), corporation viogene. USA. Used for ISSR analysis, which description a convenient for sizing linear double- stranded DNA fragments from 100-bp to 3-Kbp. They ready- to- use DNA ladder involved of 12 double- stranded, blunt-end fragments with sizes of 3000, 2000, 1500, 1000, 800, 700, 600, 500, 400, 300, 200, and 100 base pairs.

Reaction Mixture (Master Mix)

AccuPower- PCR PreMix. Bioneer Corporation USA is the convenient to perform DNA amplification. Which have description, 0.2 ml thin- wall 8-strip tubes with attached cap/96 tubes, each tube contents the component as a fallowing in table (3).

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Table(3). PCR reaction mixture components.

Component Size (20µl reaction)	Reaction
Taq. DNA polymerase	1U
Each: dNTP (dATP, dCTP, dGTP, dTTA)	250 mM
Tries-HCl (pH9.0)	10mM
KCl	30mM
MgCl2	1.5mM
Stabilizer and tracking dye	5 µM

Agarose Gel Electrophoresis Analysis

Gel electrophoresis methods were done according to Sambrook and Russel (2001).

Agarose was made in 1% by dissolving 1g of agarose in 10ml of 10x TBE buffer and the volume was completed to 100 of distilled water.

PCR Amplification

The amplification have been used the experimental protocol of AccuPower® PCR PreMix, as following: 2µl template DNA and 3µ primer (10 pmole/1µl),was added to the AccuPower® PCR PreMix tube. Sterilized deionized distilled water was added to AccuPower® PCR PreMix tubes to yield the final volume of 20µl. All samples were amplified individually by using PCR apparatus and corresponding annealing temperatures mentioned in table (2) with ISSR primers.

The amplification of ISSR- primers have been sued the same experimental protocol corresponding annealing temperatures mentioned in table (2) with ISSR primers. Initial denaturation was 94Cº for 4 mints followed by 35 cycles 65 sample at 94Cº for 1 mint, 45 second at annealing temperature and 1.5 mints at 72cº, and a final extension step at 72Cº for 8 mints. PCR products were separated by electrophoresis on 1.5% Agarose gels. Gel was visualized and imaged by U.V transilluminator (Sambrook *et al.*, 1989). Amplicons size products were estimated using 100-bp DNA ladder 100- 3000bp.

Genetic Relationships

Genetic diversity in the genome DNA, which can yield from application DNA-markers for determination genetic diversity among varieties (Nei and Li 1979). As a following:

1. The results revealed in profile, converting as a data in table for chacterization such as 1 for present bands and 0 for absent firer (3.4). Genetic relationships between selective cultivars which converted to characterization data to estimated similarity value by(SIMQUL) similarity for Qualitative Data, by formula Nei and Li:
   Similarity = 2n xy / nx + ny
2. Determination genetic distance between cultivars by using formula:

Genetic distance = 1- (2nxy / nx + ny) × 100

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Whereas nxy : Number of bands in x and y

nx : Number of all bands in x

ny: Number of all bands in y

Results and Discussion

ISSR-Primers results analysis

Ten of ISSR primers were used. It showed that the high number of amplified were 138 amplicons with UBC-824 primer figure(2). The lowest number were 49 amplicons with UBC-841figer(3), and the highest number of polymorphic were 9 bands, which represented UBC-824 primer and the lowest was 4 bands with the UBC-841 primer. In the other hand the highest percentage of polymorphism was %100 with the (ISSR-06, UBC-823, UBC-824, UBC-826, UBC-835, UBC-840, UBC-841, UBC-842, and UBC-889) primers, and the lowest percentage of polymorphism were %89 with UBC-888 primer. The highest primer efficiency was 0.57 with ISSR06 primer

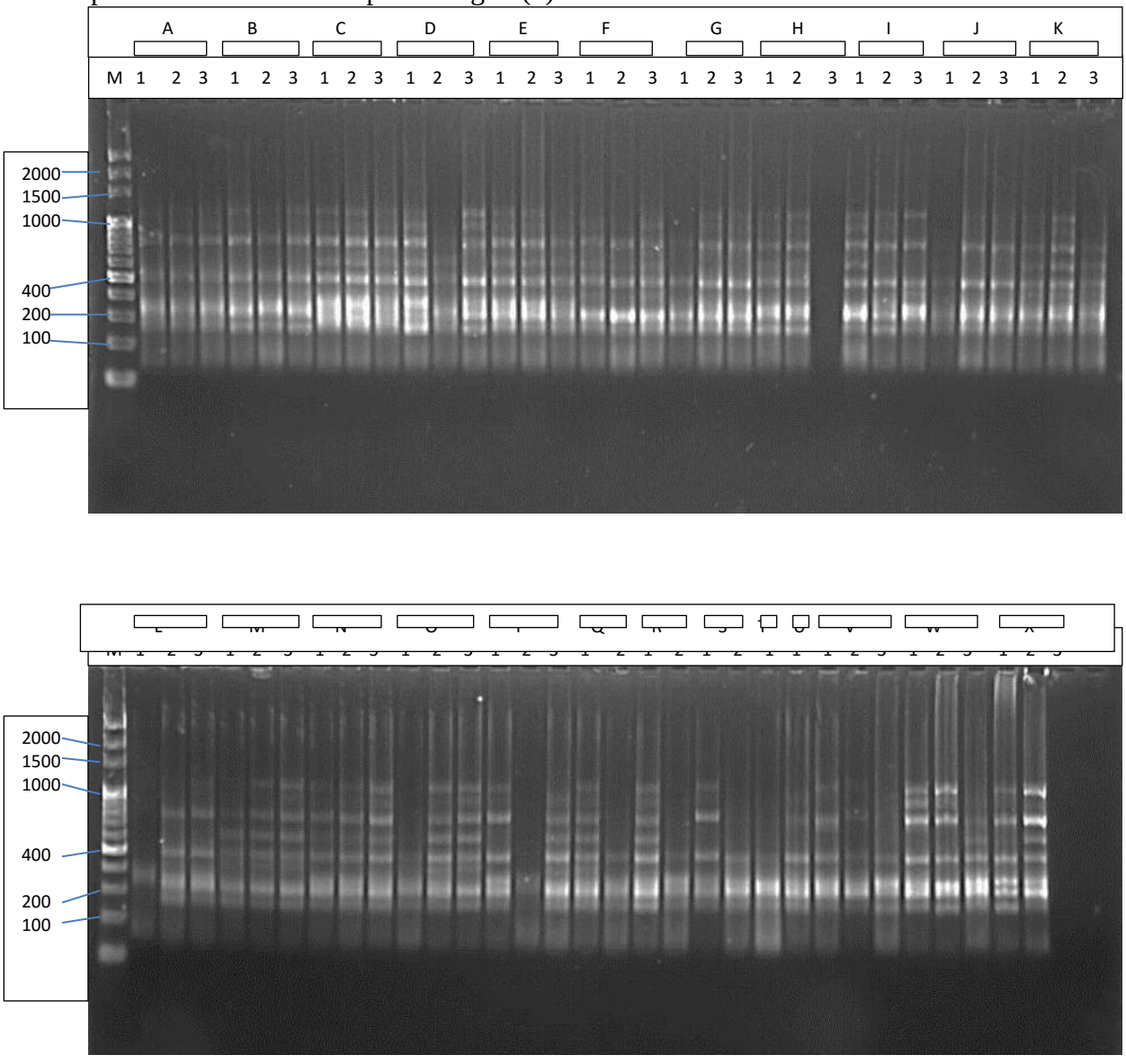

Figure 2: The amplification obtained with primer UBC-824

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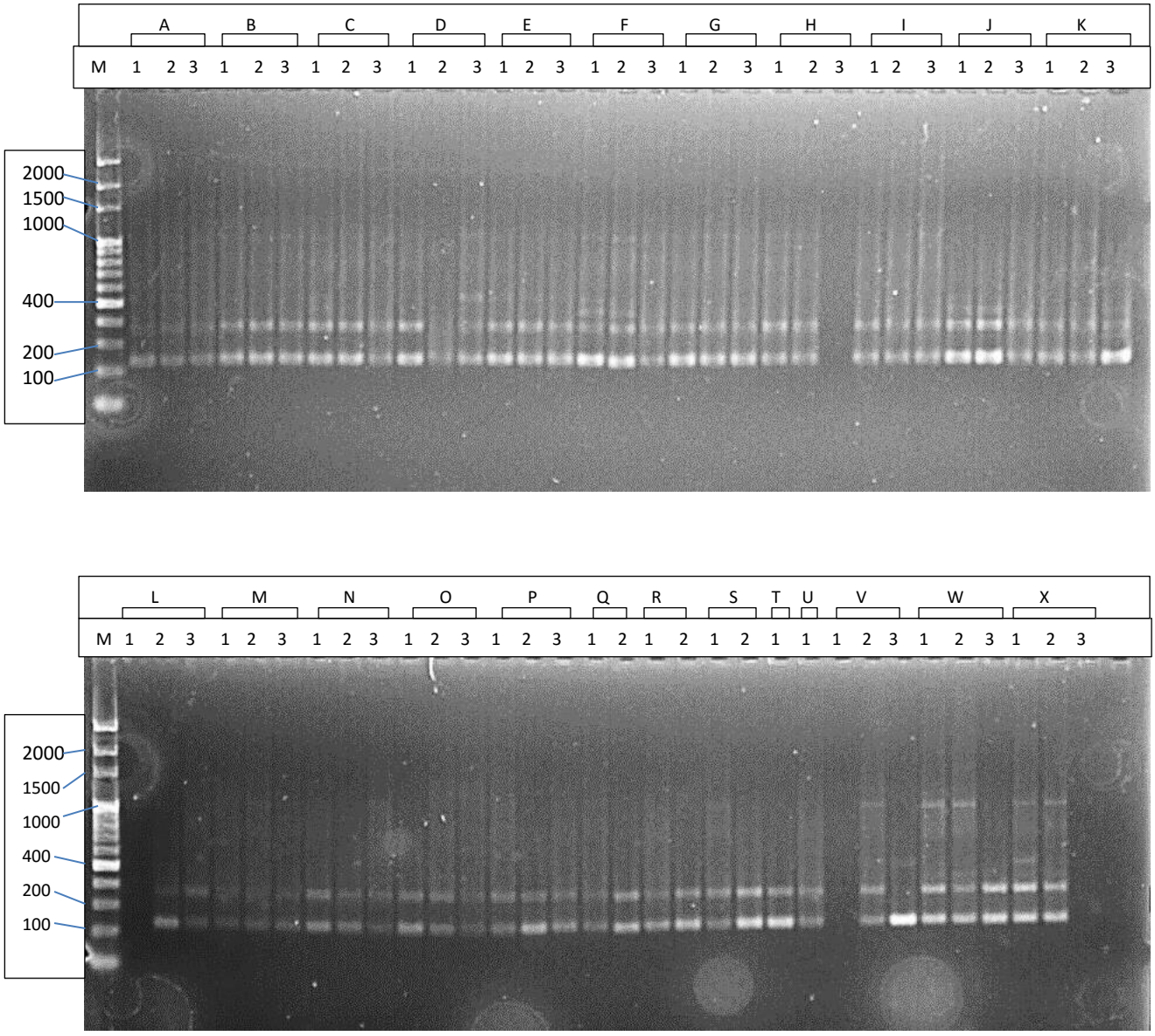

Figure 3: The amplification obtained with primer UBC-841

Table(4), the fragment size range (bp), No. of mainbands, No. of amplified bands, no. of monomorphic bands, No. of polymorphic bands, No. of unique bands, polymorphism, primer efficiency and discrimination value of each ISSR primer.

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N.	Primer	Fragment Size rang (bp)	No. of main bands	No. of amplified bands	No. of monomorphic bands	No. of polymorphic bands	No. of unique	polymorphism (%)	primer efficiency	discriminator value(%)
1	ISSR-06	120-800	8	114	0	8	0	%100	0.57	0.11
2	UBC-824	120-1000	9	138	0	9	0	%100	0.07	0.12
3	UBC-823	120-400	6	76	0	6	0	%100	0.08	0.08
4	UBC-826	150-800	8	89	0	8	0	%100	0.09	0.11
5	UBC-835	200-1500	7	93	0	7	0	%100	0.1	0.12
6	UBC-840	200-500	6	100	0	6	0	%100	0.06	0.08
7	UBC-841	200-400	4	49	0	4	0	%100	0.08	0.06
8	UBC-842	200-500	6	106	0	6	0	%100	0.06	0.08
9	UBC-888	300-1500	9	101	0	8	1	%89	0.08	0.11
10	UBC-889	250-1000	8	127	0	8	0	%100	0.06	0.11
Total No. of bands			71	993	0	72	1	-	-	-
Average bands per primer			7.1	9.93	0	7.2	0.1	-	-	-
Average per primer %			-	-		--	-	98.9	0.13	0.09

and the lowest was 0.06 with (UBC-842, and UBC-889) primer. Whereas the highest percentage of discriminatory value was %0.12 with (UBC-824 and UBC-835) primers, and the lowest percentage was 0.06 with UBC-841 primer. However, the average percentage per ISSR-primer in this study was %98.9 for polymorphism, %0.13 for primer efficiency and %0.09 for discrimination. Meyer *et al*. (1993) suggested that microsatellite- primed PCR (MP-PCR) constructed some advantages of RAPD analysis (i.e., no need for sequence information) and microsatellite analysis (i.e., use of high-stringency annealing situation, needing to more reproducible banding patterns). Subjecting to Tikunov (2003) the repeating of nucleotides of ISSR is best than RAPDs because ISSR primers are longer (15 to 20bp) so need higher annealing temperature. ISSR has been effective because bands of the same size may not be homologous, but this situation is considered minimal at the intraspecific level, the homologous bands were about %90 of products (Schrader and Graves 2004). In this study we have suggested appropriate ISSR technology determine genetic diversity with Iraqi date palm cultivars and to

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enlarge the number of markers. Currently, date palm cultivars lead for selective by farmers based on their observed and locally adapted genotypes. Whereas only small part of date palm genome that consider mainly genes encoding these agronomic characterization is affected by this selective way and distinguish a narrow genetic variation among the cultivars.

Genetic Similarity of ISSR

Genetic variation in this study was illustrated table (5). The genetic distance with twenty Iraqi date palm cultivars, by using UBC-835 primer as a sample in this study. The results revealed the highest distance was 0.5345 between Breem and each of varieties (Sabb Drrh, Khadrawi, Fom Alraman, shwrthi and Ghanami Ahmer), that means no similarity with these date palm cultivars. On other hands the lowest distance was (zero) between Tebrazal and each of (Maktom and Najdi). Also between Sabb Drrah and each of (Khadrawi, Fom Alrman, Shwethi, and Ghanami Ahmer). In addition to, between Hamrawi and each of (Kestawi and Chipchab). There are no distance with Brban and Sukkary. Also between Khadrawi and each of (Fom Alrman, Shwethi and Ghanami Ahmer). No distance between Khadrawi and Chipchab, Maktom and Najdi. Also no distance between Fom Alraman and each of Shwethi, and Ghanami Ahmer). At last no distance between Shwethi, and Ghanami Ahmer. No distance between these varieties.

Table (5) Genetic similarities between twenty individuals of Iraqi date palm cultivars by ISSR.

c	A	B	C	D	E	F	H	I	J	K	M	N	O	P	Q	R	S	T	U	V	W
A	0.0000																				
B	0.3343	0.0000																			
C	0.5345	0.2002	0.0000																		
D	0.3812	0.0469	0.1533	0.0000																	
E	0.3062	0.0281	0.2283	0.075	0.0000																
F	0.4125	0.0782	0.122	0.0313	0.1063	0.0000															
H	0.4687	0.1344	0.0658	0.0875	0.1625	0.0562	0.00														
I	0.5345	0.2027	0.000	0.1533	0.2283	0.1220	0.0658	0.0000													
J	0.3062	0.0281	0.2283	0.0750	0.000	0.1063	0.1625	0.2283	0.0000												
K	0.3812	0.0469	0.1533	0.000	0.0750	0.0313	0.0875	0.1533	0.0750	0.0000											
M	0.2687	0.0656	0.0659	0.1125	0.0375	0.1438	0.2000	0.2658	0.0375	0.1125	0.0000										
N	0.3343	0.000	0.2002	0.0469	0.0281	0.0782	0.1344	0.2002	0.0281	0.0469	0.0656	0.0000									
O	0.0906	0.2437	0.4439	0.2906	0.2156	0.3219	0.3781	0.4439	0.2156	0.2906	0.1718	0.2437	0.0000								
P	0.1593	0.1750	0.3752	0.2219	0.1469	0.2532	0,3094	0.3752	0.1469	0.2219	0.1094	0.1750	0.0687	0.0000							
Q	0.2531	0.0812	0.2814	0.1281	0.0531	0.1594	0.2156	0.2814	0.0531	0.1281	0.0156	0.0812	0.1625	0.0938	0.0000						
R	0.3812	0.0469	0.1533	0.000	0.0750	0.0313	0.0875	0.1533	0.0750	0.000	0.1125	0.0469	0.2437	0.2219	0.1281	0.0000					
S	0.3343	0.000	0.2002	0.0469	0.0291	0.0782	0.1344	0.2002	0.0281	0.0469	0.0656	0.000	0.2906	0.1750	0.0812	0.0469	0.0000				
T	0.5345	0.2002	0.000	0.1533	0.2283	0.1220	0.0658	0,000	0.2283	0.1533	0.2658	0.2002	0.4439	0.3752	0.2844	0.1533	0.2002	0.0000			
U	0.5345	0.2002	0.000	0.1533	0.2283	0.1220	0.0658	0.000	0.2283	0.1533	0.2658	0.2002	0.4439	0.3752	0.2844	0.1533	0.2002	0.000	0.0000		
V	0.0343	0.300	0.5002	0.3469	0.2719	0.3782	0.4344	0.5002	0.2719	0.3469	0.2342	0.3000	0.0563	0.1250	0.2188	0.3469	3000	0.5002	0.5002	0.0000	
W	0.5345	0.2002	0.000	0.1533	0.2283	0.1220	0.0658	0.000	0.2283	0.1533	0.2658	0.2002	0.4439	0.3752	0.2844	0.1533	0.2002	0.000	0.000	0.5002	0.000

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Mean present similarity with them. This results indicated to present closely related with population which have no distance with others, as well as useful believed that the source of germplasm belong nearby their source. In another study, the genetic distance between 4 varieties in Saudi Arabia was 0.66 to 0.85 (Abdulla and Gamal, 2010). There are many types of microsatellites have been used to assess the genetic diversity and the phylogeny among date palm cultivars (Salem *et al*.,2001; Lefebure *et al*.,2001; Devanand and Chao, 2003; Zehdi *et al*.,2004).

Phylogentic Tree

Relationships between varieties can be conducted using appropriate programs. Analyses clusters including the tested varieties are apparently related according to variation of date palm. For produced a genetic distance matrix using the formula of Nei and Li (Nei and Li., 1979), which gave the similarity between any two population on the basis of the number of generated bands. The matrix was then computed with the Neighbour program based on the unweighted pair group method with the arithmetic averaging (UPGMA), (Munshi.,and Osman.,2010). Whereas computed these product treefile in the installation program (NTSYS-PC) or any Tree view program can be used to draw phylogenetic diagrams (Trifi *et al*. 2000). In this study have been yield plots for ISSR primers such as illustrated in the figure (4).

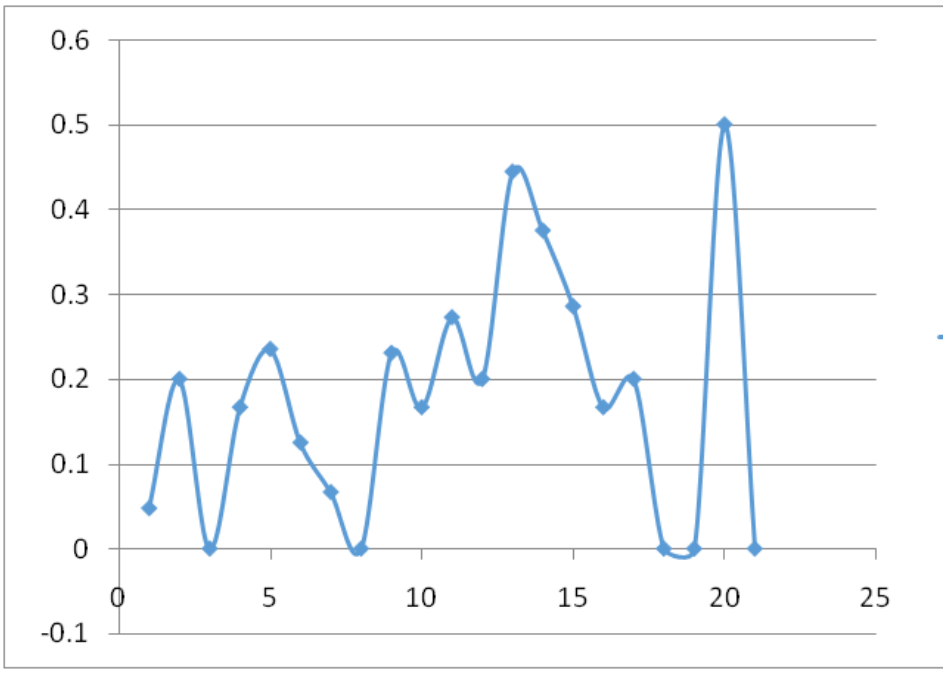

Figure (4) plot of twenty one genotypes locus which amplified with ISSR (UBC-835) primer.

The results of these plots revealed genotypes locus in this study, all of these loci distanced the assessment distance between the genotypes, relationships, the similarity between varieties, and these information can be paved the way for constructed phylogenetic tree. However these plots included two axes, X and Y, whereas X represented the genetic distance, and Y represented all varieties which used in this study, by these positions can determined the similarity between the cultivars and cluster the genetic distance as a dandrogram. For one sample from one type of primers as illustrated in the figure (6).

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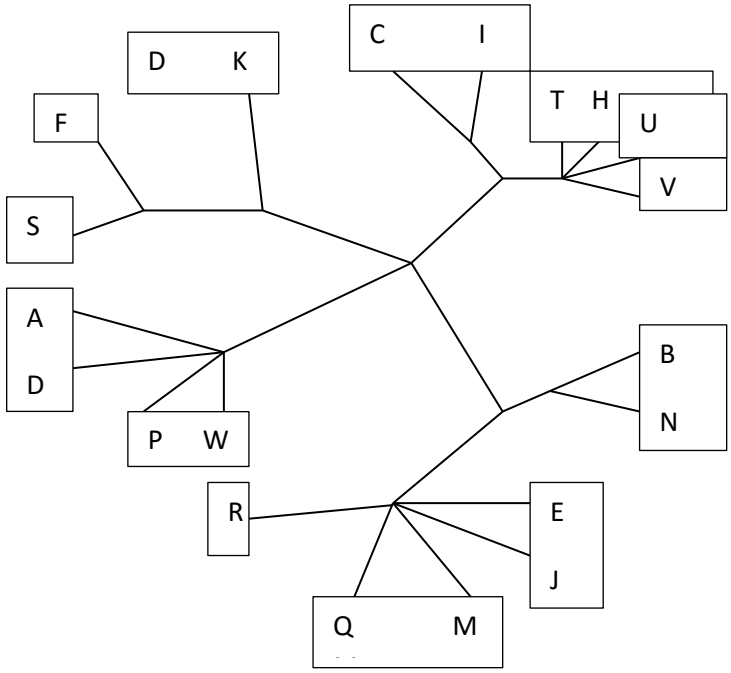

Figure (5) Genetic relationships among twenty one date palm cultivars estimated by ISSR (UBC-835) modified from Nei and Li's formula genetic distance matrix.

The results revealed genetic relationships among twenty one genotypes, divided in to three clusters, clustersI ranged of distance from 0.0123 to 0.0781represented each of cultivars (sabb Drrah, Khadrawi, Fom Alrman, and Shwethi). ClustersII ranged of distance from 0.1343 to 0.1656 represented each of cultivars (Hamrawi, Khestawi, Ashrasi, and Najdi). ClustersIII ranged of distance from 0.2125 to 0.2937 represented each of cultivars (Tebarzal, Maktom, Brban,Sukkary, Guntar, Barhi, and Chipchab).

References

AbdAlla, M.M.;, Abd El-Kawy, A.M. (2010). Karyotype analysis for date palm (*Phoenix dactylifera* L.) compared with tissue culture derived plants. New York Science Tourual., 3;165-170.

Al- Khalifah, N.S.; Askari, E. (2003). Molecular phylogeny of date palm(*phoenix dactylifera* L.) cultivars from Saudi Arabia by DNA fingerprinting. Theor Appl Genet., 107: 1266- 1270.

Al- Moshileh, A.M.; Motawei M.I.; Al-Wasel, A. Abdel- Latif, T. (2004). Identification of some date palm (*phoenix dactylifera* L.) cultivars in Saudi Arabia Using RAPD Fingerprints. Agril Marine Sci., 9:1- 3.

Ben- Abdallah, A.; Stiti, K.; Leoivre, P.; Jardin, P.D. (2000). Date palm (*phoenix dactylifera* L.) cultivar identification using Random Amplified polymorphic DNA (RAPD). Ahiers/ Agricultures. 9:103- 107.

El- Tarras, A.; Al- Tawatti, N.; Al- Malki, F. (2007). Genetic fingerprinting of some KSA cultivars using modern biotechnological techniques. Biotechnolgy., 6: 263- 267.

Devanand, P.S.; Chao, C.T. (2003). Genetic variation within 'Medjool' and 'Deglet Noor' date (*Phoenix dactylifera* L.) cultivars in California detected by fluorescent-AFLP markers. J. Hort. Sci. Biotech. 78:405-409.

Food and Agriculture Organization of the United Nations (2008). Food and Agriculture Organization statistical databases (FAO STAT). 12 Feb. 2011.

Jubrael, JMS; Udupa, S. M.; Baum, M. (2005). Assessment of AFLP-based genetic relationships among date palm (*Phoenix dactylifera* L.) varieties of Iraq. J Amer Soc Hort Sci., 130(3):442-447.

Khanam, Sakina; Arjun Sham; JeffreyL. Bennetzen and Mohammed A.M.(2012). Analysis of molecular marker-based characterization and genetic variation in date palm(*Phoenix dactylifera* L.). Australian Journal and Crop Science AJCS 6(8): 1236-1244.

Lang, N. T. and Hang, P.T.C. (2007). Omonrice, 15: 174-178.

Lefebure V., Goffinet B, Chauvet JC, Caromel B, Signoret P, Brand P, Palloix A. (2001). Evaluation of genetic distances between pepper inbred lines for cultivar protection purpoeses: comparison of AFLP, RAPD and phinotypic data. Theor. Appl. Genet. 102:741-750.

MacFarquhar, N. (2003). For bidden frut: Iraq dates hit by war and sanction. 1 July 2010. http://www. Iht. Com/articles/83194.htm.

Methew Lisa S.; Manuel spannagl, Ameena Al- Malki, Binu George, Maria F, Trres, Eman K, Al- Dous, Eman K, Al-Azwani, Emad Hussein, Sweety Mathew, Klaus FX, Mayer, Yasmin Ali Mohamoud, Karsten Suhre and Joel A Malek(2014). Afirst genetic map of date palm (*phoenix dactylifera* L.) reveal long-range genome structure conservation in the palms. BMC Genomics., 15:285.

Moghaieb, R.E.A.; Abdel- Hadi, A.A.; Ahmed, M.R.A.; Hassan, A.G.M. (2010) Genetic diversity and sex determination in date palms (*phoenix dactylifera* L.) based on DNA markers Arab J Biotechnol., 13:143-156.

Meyer W.; Mitchell T.G.; Freedman E.Z. and Vilgalys R. (1993). Hybridization probes for conventional DNA fingerprinting used as single primers in the polymerase chain reaction to distinguish strains of *Cryptococcus neoformans*. Journal of Clinical Microbiology 31: 2274–2280.

Munshi, A. and Osman, G. (2010). Investigation on molecular phylogeny **of** som date palm (*Phoenix dactylifera* L.) cultivars by protein, RAPD and ISSR markers in Saudi Arabia. Aust. J. Crop Sci., 4(1):23-28.

Nei, M. and Li WH., (1979). Mathematical models for studing genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sc. USA. 76: 5269-5273.

RUSSEL, R. (1931). Bunch management of date varieties other than Deglet Noor. Date Growers' Inst. Report 8: 3-5.

Salem, E.; Nebauer S. G., Mus M. and Segura J. (2001). Population genetic study in the Balearic endemic plant species *Digitalis minor* (scrophulariaccae) using RAPD markers. American Journal of Botany 88:1750- 1759

 ISSN 2072-3875

Sambrook, J. and Russell, D. W. (2001). Invitro application of DNA by the polymerase chian reaction, in molecular cloning: Chpter., 8: 691-733. A laboratory manul. 3 ed., Cold Spring Harbor Laboratory Press, New York.

Schrader, J.A. and Graves, W.R.(2004). Systemates of *Alnus maritima* (seaside alder) resolved by ISSR polymorphisms and morphological characters. Journal of the American Society for Horticultural Science Vol. 129: 231- 236.

Sedra, M.H.; Lashermes, P.; Trouslot, P.; Combes, M.C. (1998). Identification and genetic diversity analysis of date palm (*phoenix dactylifera* L.) varieties from Morocco using RAPD markers. Euphytica, 103:75-82.

Sudhersan, C. Y. ; Al-Shayji, Y. ,Jibi and Manuel s.(2009). Date palm crop improvement via TXD hybridization integrated with in vitro culture technique. Acta Hort., 829: 219- 224.

Trifi, M.; Bensliman, A.; Rhouma, A.; Rode, A.; Marrakchi, M. (1998). Molecular characterization of Tunisian date palm varieties. In: The 1 Int. Conf. on date palms. Hilton Hotel Al-Ain UAE, p 183-193.

Trifi M.; Rhouma, A.; Marrakchi, M. (2000). Phylogenetic relationships in Tunisian date- palm (*Phoenix dactylifera* L.) germplasm collection using DNA amplification fingerprinting. Agron., 20:665-671.

Wrigley, G. (1995). Date-palm (*Phoenix dactylifera* L.), The evolution of crop plants, in: Smartt J., Simmonds N.W. (Eds.), 2nd ed., Longman, Essex, UnitedKingdom, , pp. 399–403.

Zehdi, S.; Sakka, H.; Rhouma, A.; Salem, AOM.; Marrakchi M. and Trifi M.(2004). Analysis of Tunisian date palm germplasm using simple sequence repeat primers. Afr J Biotechnol; 3:215–219.