J. Amer. Soc. Hort. Sci. 136(4):282–287. 2011.

Genetic Diversity of Iraqi Date Palms Revealed By Microsatellite Polymorphism

Hussam S.M. Khierallah[1]
University of Baghdad, College of Agriculture, Date Palm Research Unit, Abu-Ghraib, Baghdad, Iraq

Saleh M. Bader
State Board of Agricultural Researches, Ministry of Agriculture, Baghdad, Iraq

Michael Baum
The International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria

Alladin Hamwieh
The International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria

Additional index words. *Phoenix dactylifera*, SSR markers, molecular characterization

Abstract. **Genetic diversity in 30 date palm (*Phoenix dactylifera* L.) cultivars in Iraq representing 24 female and six male cultivars was investigated using 22 microsatellite [simple sequence repeat (SSR)] primers. The tested SSR markers showed a high level of polymorphism. A total of 188 alleles were detected at the 22 loci ranging from three to 21 with an average of 8.54 alleles per locus. The average of heterozygosity for all cultivars was 0.503; genetic distance among cultivars varied from 0.171 to 0.938 indicating diverse relationships. The cultivar Ghanami Akhder was highly divergent from 'Ghnami Ahmer', whereas 'Jamal Al-Dean' was very closely related to 'Qitaz'. Unweighted pair group method arithmetic average ordered date palm cultivars into two main clusters. Principal coordinate analysis exhibited the similar clusters of cultivars as in the dendrogram.**

Date palm (2n = 2x = 36) is considered of great socioeconomic importance in the Arabian region. The tree has been, and is still, at the center of comprehensive agricultural development. It is believed to have originated from Mesopotamia (Wrigley, 1995). The numbers of known date palm cultivars that are distributed all over the world are ≈5000 of which ≈600 are found in Iraq. Iraq is one of the largest date-producing countries in the world. 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, large numbers of date palm trees were 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. Development of suitable DNA molecular markers for this crop may allow researchers to estimate genetic diversity, which will ultimately lead to the genetic conservation of date palm. The success of particular genetic conservation or breeding programs is dependent on understanding the amount and distribution of the genetic variation already present in the genetic pool (Jubrael et al., 2005). Morphological traits have been used to describe such genetic variation in date palm cultivars (Barreveld, 1993), which are mainly related to the fruit and influenced by the environment (Askari and Al-Khalifah, 2003; Sedra et al., 1993, 1998). In addition, biochemical studies, including isozyme and activity analyses of peroxidases, have been used to characterize date palms in Morocco and Tunisia (Baaziz, 1988; Baaziz and Saaidi, 1988; Bendiab et al., 1998; Majourhat et al., 2002; Ould Salem Mohamed et al., 2001). As such, analysis does not reflect precisely that polymorphisms occurred (Al-Jibouri and Adham, 1990).

DNA marker analysis in date palm is recently in the development stage. Askari and Al-Khalifah (2003) analyzed the genetic diversity among 13 different cultivars using random amplified polymorphic DNA (RAPD) markers. Similar reports are published for North African cultivars (Hussein et al., 2002; Sedra et al., 1998). Amplified fragment length polymorphism (AFLP) markers (Vos et al., 1995) were also been used for genetic fingerprinting and mapping for this crop. Jubrael et al., (2005) and Khierallah et al. (2011) used AFLP fingerprinting to characterize a number of Iraqi date palm cultivars. Microsatellites, also known as SSR, are ideal DNA markers for population studies and genetic mapping as a result of their abundance, high level of polymorphism, dispersion throughout diverse genomes, ease to assay by polymerase chain reaction (PCR), and ease to disseminate among laboratories (Udupa and Baum, 2001). Microsatellite markers were used for investigation of genetic diversity in date palm (Billotte et al., 2004). Zehdi et al. (2004) applied these markers to characterize Tunisian cultivars, whereas Al-Ruqaishi et al. (2008) used these primers to screen and analyze the genetic diversity among clonal genotypes of Omani cultivars. Recently, many researchers in date palm-producing countries have used SSR markers to genotype cultivars. Elshibli and Korpelainen (2008) investigated genetic diversity in Sudan germplasm representing 37 female and 23 male accessions using 16 SSR primers. In Qatar, Ahmed and Al-Qaradawi (2009) used 10 primers to analyze genetic

Received for publication 27 Jan. 2011. Accepted for publication 24 June 2011. This project was supported by a grant from Tottori University and funds from BMZ (Bundesministerium fur Wirtschaftliche Zusammenarbeit und Entwicklung), Germany, and the State Board of the Agriculture Research, Ministry of Agriculture, Iraq.
We acknowledge the technical support of A. Sabbagh (International Center for Agricultural Research in the Dry Areas, Aleppo, Syria).
[1]Corresponding author. E-mail: khierallah70@yahoo.com.

diversity among 15 cultivars. Akkak et al. (2009) developed 17 additional microsatellite markers. Hamwieh et al. (2010) developed 1000 SSR markers in date palm, mining genome sequencing data for this vital crop. The aim of this study is intended to characterize the genotype for 30 Iraqi important date palm cultivars using SSR markers.

Materials and Methods

Plant materials. Leaves were collected from 30 well-defined reference Iraqi date palm cultivars grown in date palm stations belong to the Ministry of Agriculture (Table 1). Twenty-two female cultivar samples were collected from Al-Mahaweel Date Palm Station, Hilla Governorate, 80 km south of Baghdad. The remaining eight cultivar samples were collected from Al-Za'afarania Date Palm Station, Baghdad (Fig. 1). Total genomic DNA was extracted from young and healthy leaves according to the procedure mentioned by Benito et al. (1993) with minor modifications. After purification, the resultant DNA was quantified on 1% agarose gel electrophoresis as described by Sambrook et al. (1989).

Microsatellite amplification. A total of 33 date palm-specific primer pairs were tested; 16 of them were developed by Billotte et al. (2004) and 17 were developed by Akkak et al. (2009) as indicated in Table 2. PCR was performed in a total mixture of 20 μL containing: 13.9 μL H_2O, 50 ng of total cellular DNA (2 μL) as template, 1 μL/PCR buffer (Roche, Mannheim, Germany), 1 μL of 0.2 mM of dNTP PCR mix (Roche), 0.1 μL (0.625 U) of *Taq* DNA polymerase (Roche) and1 μL of 0.2 mM of each primer using forward primer end labeled (6FAM, NED, or TET). Amplifications were performed in a thermocycler (Applied Biosystems, Carlsbad, CA) under the following conditions: a denaturation step of 5 min at 95 °C followed by 35 cycles of 30 s at 95 °C, 1 min at 52 °C and 1 min at 72 °C, and a final extension step at 72 °C for 7 min. At the testing stage, amplification products were separated by electrophoresis according to their molecular weight in 1.4% agarose gels and then detected by staining with ethidium bromide as described by Sambrook et al. (1989). The DNA profiles were visualized on an ultraviolet transilluminator and documented by using a gel documentation system (Alpha Innotech Imaging Station; Cell Biosciences, Santa Clara, CA). For final analyses, 2 μL of amplified DNA (diluted to 1/10) and 8 μL of (Rox) DNA standard size (Applied Biosystems) were loaded into 96-well plates. Genotyping was carried out using an automatic DNA analyzer (Model 3100; Applied Biosystems). Amplifications were performed at least twice and only reproducible products were taken into account for further data analysis.

Table 1. Names, gender, and sources of collection of 30 date palm cultivars grown in Iraq.

No.	Cultivar	Gender	Source of collection[z]
1	Usta Umran	Female	Al-Mahaweel
2	Tebarzal	Female	Al-Mahaweel
3	Um Al-Dihen	Female	Al-Mahaweel
4	Guntar	Female	Al-Mahaweel
5	Khestawi	Female	Al-Mahaweel
6	Bream	Female	Al-Mahaweel
7	Ashrasi	Female	Al-Mahaweel
8	MaktomAsfar	Female	Al-Mahaweel
9	Buliani	Female	Al-Mahaweel
10	Leelwi	Female	Al-Mahaweel
11	ShwethiAhmer	Female	Al-Mahaweel
12	Jamal Al-Dean	Female	Al-Mahaweel
13	QulHusaini	Female	Al-Mahaweel
14	Helawi	Female	Al-Mahaweel
15	Qitaz	Female	Al-Mahaweel
16	Chipchab	Female	Al-Mahaweel
17	Zahdi	Female	Al-Mahaweel
18	ShwethiAsfar	Female	Al-Mahaweel
19	Khadrawi	Female	Al-Mahaweel
20	Baw Adem	Female	Al-Mahaweel
21	Deari	Female	Al-Mahaweel
22	Barhi	Female	Al-Mahaweel
23	Meer Haj	Female	Al-Za'afarania
24	Um Al-Blaliz	Female	Al-Za'afarania
25	GhanamiAkhder	Male	Al-Za'afarania
26	GhnamiAhmer	Male	Al-Za'afarania
27	Khekri	Male	Al-Za'afarania
28	Smeasmi	Male	Al-Za'afarania
29	Ghulami	Male	Al-Za'afarania
30	Greatli	Male	Al-Za'afarania

[z]Al-Mahaweel Date Palm Station, Hilla Governorate, Iraq; Al-Za'afarania Date Palm Station, Baghdad, Iraq.

Fig. 1. Sample collection sites of date palm in Iraq.

Simple sequence repeat genotyping. Electropherogram data were analyzed by using Foundation Data Collection software (Genetic Analyser Data Collection Version 2.0; Applied Biosystems). Allele size scoring was performed by Gene Mapper software (Version 3.7; Applied Biosystems). The Jaccard similarity matrix (Jaccard, 1908) was used for cluster analysis using the unweighted pair group method arithmetic average to study the genetic relationships among the cultivars. Jaccard similarity index, major allele frequency, heterozygosity, gene diversity, and polymorphism information content estimation were done using a software package (PowerMarker Version 1.31; Liu and Muse 2005). The phylogenetic diagram was drawn by PAST software [Version 1.91 (Hammer et al., 2001)] on the basis of Hamming similarity index with 100 bootstrap. Principal coordinate analysis (PCA) was performed according to Euclidean similarity index using the PAST software.

Results

The 33 primer pairs of Akkak et al. (2009) and Billotte et al. (2004) were tested for their ability to generate expected SSR

Table 2. Name, motif repeat, and sequence of 33 simple sequence repeat primers used for genotyping 30 Iraqi date palm cultivars.

No.	Primer name	Motif repeat	Primer sequences (5′-3′)		Reference
			Forward	Reverse	
1	mPdClR010	(GA)22	ACCCCGGACGTGAGGTG	CGTCGATCTCCTCCTTTGTCTC	Billotte et al., 2004
2	mPdClR015	(GA)21	AGCTGGCTCCTCCCTTCTTA	GCTCGGTTGGACTTGTTCT	
3	mPdClR016	(GA)14	AGCGGGAAATGAAAAGGTAT	ATGAAAACGTGCCAAATGTC	
4	mPdClR025	(GA)22	GCACGAGAAGGCTTATAGT	CCCCTCATTAGGATTCTAC	
5	mPdClR032	(GA)19	CAAATCTTTGCCGTGAG	GGTGTGGAGTAATCATGTAGTAG	
6	mPdClR035	(GA)15	ACAAACGGCGATGGGATTAC	CCGCAGCTCACCTCTTCTAT	
7	mPdClR044	(GA)19	ATGCGGACTACACTATTCTAC	GGTGATTGACTTTCTTTGAG	
8	mPdClR048	(GA)32	CGAGACCTACCTTCAACAAA	CCACCAACCAAATCAAACAC	
9	mPdClR050	(GA)15	CTGCCATTTCTTCTGAC	CACCATGCACAAAAATG	
10	mPdClR057	(GA)20	AAGCAGCAGCCCTTCCGTAG	GTTCTCACTCGCCCAAAAATAC	
11	mPdClR063	(GA)16	CTTTTATGTGGTCTGAGAGA	TCTCTGATCTTGGGTTCTGT	
12	mPdClR070	(GA)17	CAAGACCCAAGGCTAAC	GGAGGTGGCTTTGTAGTAT	
13	mPdClR078	(GA)13	TGGATTTCCATTGTGAG	CCCGAAGAGACGCTATT	
14	mPdClR085	(GA)29	GAGAGAGGGTGGTGTTATT	TTCATCCAGAACCACAGTA	
15	mPdClR090	(GA)26	GCAGTCAGTCCCTCATA	TGCTTGTAGCCCTTCAG	
16	mPdClR093	(GA)17	CCATTTATCATTCCCTCTCTTG	CTTGGTAGCTGCGTTTCTTG	
17	PDCAT1	(TC)21	CTGAAATCTCTGTTCAAATCCA	GTTTGGATCTATTTGTGAGT TATTTTCTTT	Akkak et al., 2009
18	PDCAT2	TCGCTG(TC)3(TC)3T(TC) 3T (TC)3T(TC)4TTCT GTCCCG(TC) 16T(TC)	GGCCTTCTCTTCCCTAATGGGA	GTTTCTTGCCCCTGTTCTTTCCCTC	
19	PDCAT3	(CA)8 - (GT)3(CA)4	CAAGGATAGGTGTGATGACCACC	GTTTGTCCTTTTAACTTCTT GCTGGAATT	
20	PDCAT4	(CA)8TT(CA) 4(GA)20	TAACGAGTCCACACAC	CTGGGTAAAGCTTATAAG	
21	PDCAT5	(AG)16	GGCCCGTCCTTGGATTAGAG	CTACGTTGTCCCGTCAATTGG	
22	PDCAT6	(CA)14(GA)23	AATCAGGGAAACCACAGCCA	GTTTAAAGCCTTCTCAAGAT AGCCTCAG	
23	PDCAT8	(TC)16	GCTTAAGTGGTTAGTTGCCAA	GTTTGGCAGAAGTATTGAAA AGTTGA	
24	PDCAT10	(TC)16	CACTGCTCCTGTTGCCCTGT	TGTAGAAGGGCAGAGGACGG	
25	PDCAT11	(TC)7(TC)20	TTAGTAGACTCCCCACCGTCCT	GTTTCATGGTGCTGGAGA ATGAA	
26	PDCAT12	(CT)19	CATCGTTGATTCCTAACCCCTC	GTTTAGATCTTGCATGGCAACGC	
27	PDCAT13	(GA)21GCA(GGA)GA (GGA)3	TGTTGCCATTCACATGCTGC	GTTTGGACTAGTCCCTCCCTCCC	
28	PDCAT14	(TC)19(TC)16	TGCTGCAAATCTAGGTCACGA	GTTTACCCCTCGGCCAAATGTAA	
29	PDCAT15	(GA)13-(GA)8(GA)6	ACAGAGAGGTGGAGTTTTCGGATT	TCTTCCTTTCAAACCAGCAAGCT	
30	PDCAT17	(GA)21	CAGCGGAGGGTGGGCCTC	GTTTCTCCATCTCCCTTTTTCT TCTGCTACTC	
31	PDCAT18	(CT)13G(CT)8CG(CT)3 CG(CT)3	CCTAAACCTGAATGAATCAAAGCA	ACTAACATAAGGACAGTGCTATGT GATTG	
32	PDCAT20	(GA)29	TTTCAGACACATCAAGTAACGATGA	GTTTACGTCCACCCCAAGTTACGA	
33	PDCAT21	(GA)5T(GA)2TA(GA)2GC(GA)5(GT)7	GTGTTTGAAGATTGATTT TGTGTTATGAG	GTTTCGAACTATAGGCATGC ACAATAGTATATTG	

banding patterns in Iraqi date palms. A total of 22 primers showed polymorphic bands among the 30 cultivars (24 female and six male) successfully (Table 3).

The genetic diversity was widely varied among cultivars ranging from 0.168 to 0.923 at loci mPdCIR057 and PDCAT 18, respectively, with an average of 0.695. The 22 primers resulted in a total of 188 alleles and ranged from three to 21 alleles with an average of 8.545 alleles per locus. However, the major allele frequency ranged from 0.140 to 0.909 for PDCAT18 and mPdCIR057, respectively, with an average of 0.436. High levels of heterozygosity were detected in female cultivars with an average of 0.503 (Table 3). Genetic distance among cultivars varied from 0.171 to 0.938 indicating diverse relationships. The cultivar Ghanami Akhder is highly divergent from 'Ghanami Ahmer', whereas 'Jamal Al-Dean' was very closely related to 'Qitaz'. Genetic relationships among the 30 cultivars based on 22 microsatellite loci with bootstrapping values is shown in Figure 2. Cluster analysis revealed the presence of two major clusters denoted A and B. Cluster A consisted of three cultivars (Khekri, Barhi, and Smeasmi), whereas Cluster B consisted of three subclusters (denoted as B1, B2, and B3). Subcluster B1 consisted of 10 cultivars (Shwethi Asfar, Baw Adem, Deari, Ghulami, Zahdi, Meer Haj, Greatli, Usta Umran, Um Al-Blaliz, and Khestawi). Subcluster B2 consisted of four cultivars (Buliani, Maktom Asfar, Shwethi Ahmer, and Chipchab). Subcluster B3 consisted of 11 cultivars (Bream, Leelwi, Tabarzal, Ashrasi, Helawi, Um Al-Dihen, Guntar, Qul Husaini, Khadrawi, Jamal Al-Dean, and Qitaz). The two male cultivars, Ghanami Akhder and Ghnami Ahmer, were highly divergent from other cultivars and within each other and were not involved in any cluster (Fig. 2).

Data were analyzed using PCA based on 22 SSR loci representing 57% of the total variation. The results of the first two PC1 and PC2 axes exhibited the similar clusters of cultivars as in the dendrogram (Fig. 3).

Table 3. Major allele frequency, number of genotypes showed polymorphic bands, number of alleles generated, heterozygosity, gene diversity, and polymorphism information content (PIC) estimated by 22 simple sequence repeat markers in 30 Iraqi date palm cultivars.

Marker	Major allele frequency	Genotypes (no.)	Alleles (no.)	Heterozygosity	Gene diversity	PIC
mPdClR010	0.204	19	14	0.852	0.890	0.880
mPdClR016	0.500	8	5	0.529	0.654	0.602
mPdClR025	0.224	15	8	0.690	0.822	0.798
mPdClR032	0.333	13	5	0.815	0.772	0.736
mPdClR035	0.481	10	7	0.519	0.695	0.659
mPdClR050	0.370	17	12	0.926	0.813	0.797
mPdClR057	0.909	3	3	0.136	0.168	0.160
PdClR070	0.533	3	3	0.000	0.604	0.536
mPdClR078	0.320	16	15	0.760	0.832	0.816
mPdClR085	0.313	8	7	0.250	0.779	0.746
mPdClR090	0.391	10	9	0.261	0.781	0.757
mPdClR093	0.741	6	6	0.296	0.434	0.415
PDCAT4	0.407	13	7	0.556	0.738	0.701
PDCAT 5	0.276	13	7	0.862	0.816	0.791
PDCAT 6	0.231	21	21	0.654	0.903	0.897
PDCAT 11	0.600	7	7	0.100	0.598	0.566
PDCAT 12	0.850	5	4	0.100	0.269	0.256
PDCAT 14	0.220	19	10	0.960	0.865	0.850
PDCAT 15	0.440	8	6	0.480	0.732	0.699
PDCAT 17	0.385	11	9	0.500	0.774	0.746
PDCAT 18	0.140	19	19	0.560	0.923	0.918
PDCAT 21	0.731	6	4	0.269	0.431	0.394
Mean	0.436	11.364	8.545	0.503	0.695	0.669

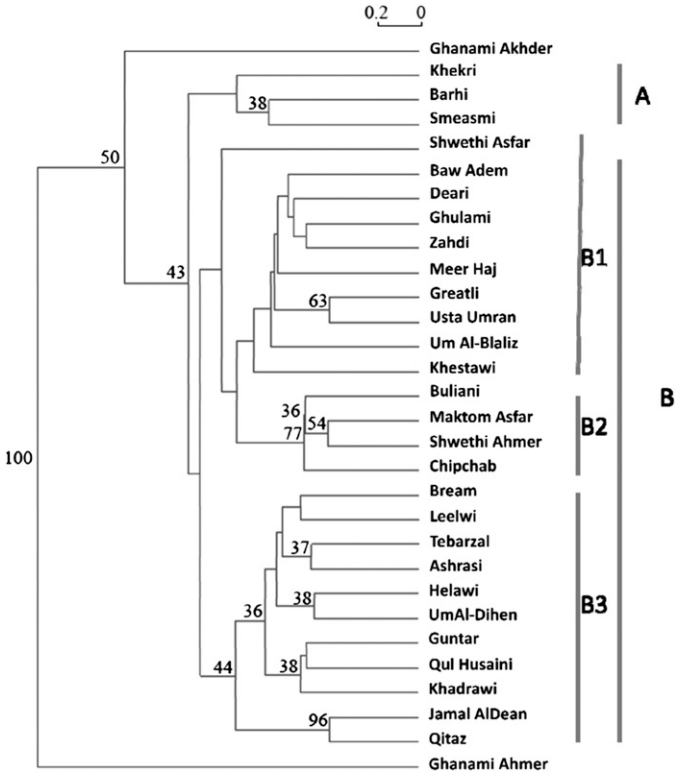

Fig. 2. Genetic relationships among 30 Iraqi date palm cultivars based on 22 microsatellite loci with 100 bootstrap.

Discussion

It is well known that Iraq is one of the richest countries in the diversity of date palm cultivars with an estimated number of 600 cultivars. Discrimination between cultivars is based on phenotypic differences, which are often influenced by the environment. In this study, microsatellite genotyping was used to examine the genetic diversity and genetic relationships among 30 cultivars grown widely in Iraq. Although the frequency of microsatellite varies among species, they are abundant, dispersed throughout the genome, and show higher levels of polymorphism than other genetic markers. These features, coupled with their ease of detection, had suggested

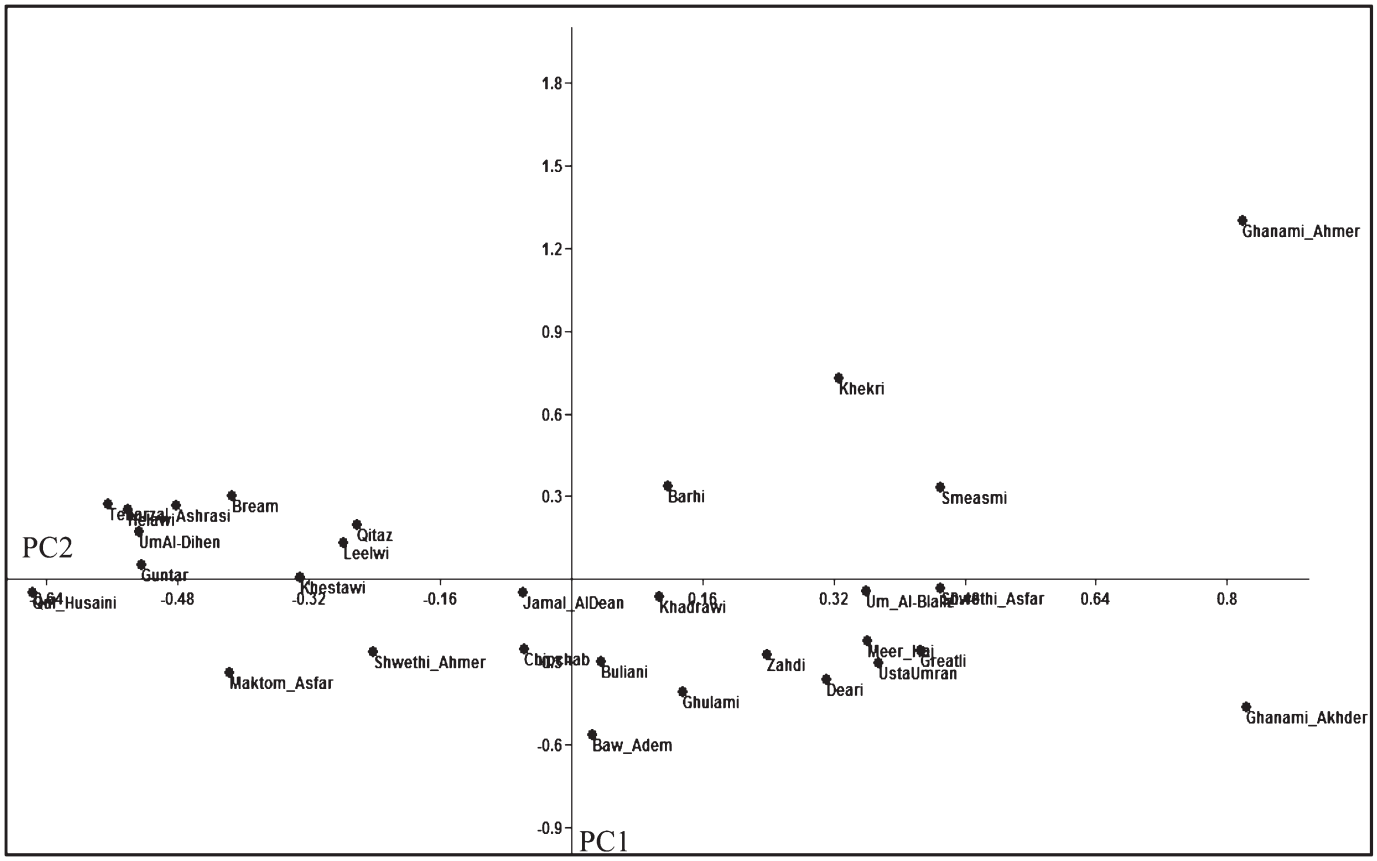

Fig. 3. Principal coordinate analysis (PCA) of 30 Iraqi date palm cultivars based on 22 microsatellite loci.

being useful molecular markers. Their potential for automation and inheritance in a codominant manner are additional advantages when compared with other types of molecular tools (Holton, 2001). The microsatellites examined in this study were highly polymorphic possessing a great number of alleles (188 alleles) distributed in 30 date palm cultivars. The number of detected alleles per locus in this study (8.54) was higher than 7.6 alleles per locus that scored by Zehdi et al. (2004) in 46 date palm cultivars cultivated in Tunisia and those studied by Ahmed and Al-Qaradawi (2009) representing 15 cultivars grown in Qatar. The mean heterozygosity value detected in the Iraqi cultivars was 0.503, indicating the presence of higher genetic diversity. Date palm growers believe that 'Ghanami Akhder' and 'Ghnami Ahmer' are two clones belonging to the same cultivar. The present investigation revealed high divergence between those two cultivars, thus suggesting they are independent cultivars and confirming the results of Al-Khateeb and Jubrae (2006) who used RAPD markers for the same purpose.

Jubrael et al. (2005) and Khierallah et al. (2011) suggested a common genetic basis among date palm genotypes despite the differences in fruit characters and tree morphology. Other authors reported similar results in Tunisian date palm (Sedra et al., 1998; Zehdi et al., 2004). The long history of date palm domestication with an unknown origin (Wrigley, 1995) and the nature of date palm culture may have played an important role in the composition of date palm genomes (Elshibli and Korpelainen, 2008). New cultivars may appear as a result of the continuous selection carried out by farmers after sexual reproduction. Exchange of propagules, which are a mixture of vegetative and seed-propagated materials, is conducted between farmers. All of these processes together may result in a mixed genome in date palm within the country (Elshibli and Korpelainen, 2008).

Results of the present study using SSR analysis showed a high level of polymorphism among Iraqi cultivars. However, the cultivar nomenclature and classification in Iraq still remains to be based on fruit characters, including morphological, physical, and chemical traits.

In conclusion, Iraqi date palm germplasm requires further investigation and adoption of proper markers that may assist in identifying the economically and agronomically important cultivars.

Literature Cited

Ahmed, T. and A. Al-Qaradawi. 2009. Molecular phylogeny of Qatari date palm genotypes using simple sequence repeat marker. Biotechnology 8:126–131.

Akkak, A., V. Scariot, T. Dorello, P. Boccacci, C. Beltramo, and R. Botta. 2009. Development and evaluation of microsatellite markers in (*Phoenix dactylifera* L.) and their transferability to other *Phoenix* species. Biol. Plant. 53:164–166.

Al-Jibouri, A.A.M. and K.M. Adham. 1990. Biochemical classification of date palm male cultivars. J. Hort. Sci. 65:725–729.

Al-Khateeb, T.A. and J.M. Jubrael. 2006. The use of RAPD markers for sex and male cultivars identification in (*Phoenix dactylifera* L.) in Iraq. Acta Hort. 736:162–170.

Al-Ruqaishi, I.A., M. Davey, P. Alderson, and S. Mayes. 2008. Genetic relationships and genotype tracing in date palm (*Phoenix*

dactylifera L.) in Oman based on microsatellite markers. Plant Genet. Resources 61:70–72.
Askari, E. and N.S. Al-Khalifah. 2003. Molecular phylogeny of date palm (*Phoenix dactylifera* L.) cultivars from Saudi Arabia by DNA fingerprinting. Theor. Appl. Genet. 107:1266–1270.
Baaziz, M. 1988. The activity and preliminary characterisation of peroxidases in leaves of cultivars of date palm (*Phoenix dactylifera* L.). New Phytol. 111:403–411.
Baaziz, M. and M. Saaidi. 1988. Preliminary identification of date palm cultivars by esterase isoenzymes and peroxidase activities. Can. J. Bot. 66:89–93.
Barreveld, W.H. 1993. Date palm products. FAO Agr. Serv. Bul. 101. Food and Agriculture Organization of the United Nations, Rome, Italy.
Bendiab, K., M. Baaziz, and K. Majourhat. 1998. Preliminary date palm cultivar composition of moroccan palm groves as revealed by leaf isozyme phenotypes. Biochem. Syst. Ecol. 26:71–82.
Benito, C., A.M. Figueiras, C. Zaragozal, F.J. Gallego, and A. Dela Penal. 1993. Rapid identification of *Trijticeaegeno* types from single seed using the polymerase chain reaction. J. Plant Mol. Biol. 21:181–183.
Billotte, N., N. Marseillac, P. Brottier, J.L. Noyer, J.P. Jacquemoud Collet, C. Moreau, T. Couvreur, M.H. Chevallier, J.C. Pintaud, and A.M. Risterucci. 2004. Nuclear microsatellite markers for the date palm (*Phoenix dactylifera* L.): Characterization and utility across the genus *Phoenix* and in other palm genera. Mol. Ecol. Notes 4:256–258.
Elshibli, S. and H. Korpelainen. 2008. Microsatellite markers reveal high genetic diversity in date palm (*Phoenix dactylifera* L.) germplasm from Sudan. Genetica 134:251–260.
Food and Agriculture Organization of the United Nations. 2008. Food and Agriculture Organization statistical databases (FAOSTAT). 12 Feb. 2011. <http://faostat.fao.org/site/339/default.aspx>.
Hammer, Ø., D.A.T. Harper, and P.D. Ryan. 2001. PAST: Palaeontological statistics software package for education and data analysis. Palaeontol. Electronica 4:1–9.
Hamwieh, A., J. Farah, S. Moussally, K. Al-Sham'aa, K. Almer, H. Khierallah, S. Udupa, S. Lababidi, J. Malek, M. Aaouineand, and M. Baum. 2010. Development of 1000 microsatellite markers across the date palm (*Phoenix dactylifera* L.) genome. Acta Hort. 882:269–277.
Holton, T.A. 2001. Plant gnotyping by analysis of microsatellites, p. 15–27. In: HenryR.J. (ed.). Plant genotyping: The DNA fingerprinting of plants.. CABI, Cambridge, UK.
Hussein, E.H., S.S. Adawy, D. El-Khishin, H. Moharam, and H. El-Itriby. 2002. Genetic variability studies and molecular fingerprinting of some Egyptian date palm (*Phoenix dactylifera* L.) cultivars. I. A preliminary study using RAPD markers. Arab J. Biotechnol. 5:217–224.
Jaccard, P. 1908. Nouvel le srescherchessur la distribution florale. Bulletin de la Socieìteì vaudoise des sciences naturelles 44:223–270.
Jubrael, J.M.S., S. Udupa, and M. Baum. 2005. Assessment of AFLP-based genetic relationships among date palm (*Phoenix dactylifera* L.) varieties of Iraq. J. Amer. Soc. Hort. Sci. 130:442–447.
Khierallah, H.S., S.M. Bader, M. Baum, and A. Hamwieh. 2011. Assessment of genetic diversity for some Iraqi date palms (*Phoenix dactylifera* L.) using AFLP markers. Afr. J. Biotechnol. (in press).
Liu, K. and S.V. Muse. 2005. PowerMarker: Integrated analysis environment for genetic marker data. Bioinformatics 21:2128–2129.
MacFarquhar, N. 2003. Forbidden fruit: Iraq dates hit by war and sanctions. 1 July 2010. <http://www.iht.com/ articles/83194.html>.
Majourhat, K., K. Bendiab, L. Medraoui, and M. Baaziz. 2002. Diversity of leaf peroxidases in date palm (*Phoenix dactylifera* L.) as in an example of marginal (seedling derived) palm groves. Sci. Hort. 95:31–38.
Ould Salem Mohamed, A., M. Trifi, H. Sakka, A. Rhouma, and M. Marrakchi. 2001. Genetic inheritance analysis of four enzymes in date palm (*Phoenix dactylifera* L.). Genet. Resources Crop Evol. 48:361–368.
Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular cloning: A laboratory manual. 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Sedra, M.H., H. El-Filali, and D. Frira. 1993. Observarions sur quelques caracteristiques phenotypiques et agronomiques du fruit des varietes et clones du palmier dattier selectionnes. Al-Awami 82:105–120.
Sedra, M.H., P. Lashermes, M. Trouslot, and S. Hamon. 1998. Identification and genetic diversity analysis of date palm (*Phoenix dactylifera* L.) varieties of Morocco using RAPD markers. Euphytica 103:75–82.
Udupa, S.M. and M. Baum. 2001. High mutation rate and mutational bias at (TAA)n microsatellite loci in chickpea (*Cicerarietinum* L.). Mol. Genet. Genomics 265:1097–1103.
Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP a new technique for DNA fingerprinting. Nucleic Acids Res. 23:4407–4414.
Wrigley, G. 1995. Date palm (*Phoenix dactylifera* L.), p. 399–403. In: Smartt, J. and N.W. Simmonds (eds.). The evolution of crop plants. Longman, London, UK.
Zehdi, S., M. Trifi, N. Billotte, M. Marakchi, and J.C. Pintaud. 2004. Genetic diversity of Tunisian date palms (*Phoenix dactylifera* L.) revealed by nuclear microsatellite polymorphism. Hereditas 141:278–287.