

Original Research Article

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## Study of Genetic Diversity of Iraqi Date Palms Using Some Morphological Markers

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### ABSTRACT

#### Keywords

*Phoenix Dactylifera*, Genetic Diversity, Morphological Markers, PCA.

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In order to study the genetic diversity for a number of cultivars and ecotypes for Iraqi date palms (*Phoenix dactylifera* L.). Forty Iraqi well known cultivars from five different regions in Iraq were chosen. These including cultivars grown in some date palm stations belonging to the Ministry of Agriculture. The study included 14 morphological markers comprise trunk, leaf and fruits traits. Results indicated that the average number of alleles for all cultivars was 4.2. Shoot tip shape had seven alleles while fruit shield had only two. Major allele frequency ranged from 0.3 for fruit shape to 0.825 for fruit texture. Gene diversity was also ranged from 0.296 for fruit texture suggesting lowest polymorphism information content (PIC) value (0.265) to 0.771 for shoot tip shape suggesting highest polymorphism information content (PIC) value (0.742). Results of simple correlation among studied phenotypic traits revealed that highest correlation value reached 0.543 between the trunk size and leaf (fronds) length, followed by the correlation between trunk size and leaf base (0.540), while the lowest correlation was 0.012 between fruit stalk color and leaf base. Cluster analysis based on the Euclidean distances using UPGMA split selected cultivars into two main groups independently of their geographic regions with 6.9 Euclidean distance. The scatter diagram plot of the multivariate Principal component analysis (PCA) of the first two (PC1 and PC2) based on morphological markers revealed 31.86% of the variation among cultivars with Eigen value of 4.67 while PC2 was 17.93% with Eigen value of 4.63. Results of PCA scatter diagram exhibit the same clusters as shown in the dendrogram.

### Introduction

The date palm (*Phoenix dactylifera* L.) is one of the oldest cultivated fruit trees and was well-known in Babylon, Iraq 4000 B.C. The palms were celebrated during that period for their strength and majesty (Khierallah *et al.*, 2015). Iraq remains one of the leading date producing countries in the world. Before 1991, it was the world's

largest producer (FAO 2004) and had the most extensive date forest in the world, (MacFarquhar, 2003). However, a serious decline over the past 30 years occurred due to numerous military conflicts since the 1980s. During recent wars, large numbers of date palm trees were destroyed. Water quality became crucial due to the harmful

effects of the wars which destroyed date palm orchards in various regions, but especially in Basrah governorate, Shatt Al-Arab region (Khierallah *et al.*, 2015). The numbers of known date palm cultivars that are distributed all over the world may be as high as 3000 out of which about 630 are found in Iraq. Wars and economic sanctions imposed on Iraq have negatively affected both the production and natural genetic diversity of the crop and inhibited the much needed impetus to rebuild the date palm industry. The collection and genetic characterization of the all Iraqi cultivars using different genetic markers is one of the important goals in date palm rehabilitation efforts. During the last few years, the General Board of Date Palm, Ministry of Agriculture has collected over 510 date palm cultivars and planted them in 30 date palm stations dispersed in the south and middle parts of the country. Such a number of genotypes reflect the much-needed efforts to get powerful, accurate and practical genetic markers that can be used in a high resolution mapping for these cultivars. The success of any plant genetic conservation or breeding program depends on understanding the amount and distribution of the genetic variation present in the genetic pool. Morphological traits have been used to describe genetic variation in date palm cultivars, which are mainly related to the fruit, leaf, trunk and other parts of the tree. An early survey of Iraqi cultivars using morphological description was done by Al-Baker (1962); he distinguished 531 cultivars and their origin, availability and fruit quality. Another morphological identification was done on 110 Iraqi cultivars by Al-Jboory (1971). In 1972, Al-Baker again described 627 Iraqi cultivars in his celebrated book in Arabic, *The Date Palm*, adding some fruit traits to the earlier description. Three decades later, Husien (2002) and Husien and Greab (2004) also

gave a brief description of 36 and 50 cultivars, respectively. Al-Saleh and Al-Ansary (2005) adopted 12 phenotypic traits to describe 110 cultivars, documented with color photos. Thirty vegetative date palm descriptors were screened by Hammadi *et al.*, (2009) in Tunisia. Al-Wusaibai *et al.*, (2014) studied 11 representative cultivars from eastern, central and western region of Saudi Arabia Kingdom. Recently, Twenty Pakistani date palm cultivars from nine different geographical regions were characterized to estimate the polymorphism and possible similarity level (Naqvi *et al.*, 2015). While Al-Asasfa *et al.* (2015) employed phenotypic traits to characterize and survey wild population of date palm in Jordan. The aim of this study is the use of morphological markers in assessing genetic diversity in some Iraqi date palm cultivars cultivated in five different regions.

## **Materials and Methods**

Forty Iraqi well known cultivars from five different regions in Iraq were chosen (Fig 1). Those were grown in some date palm stations belonging to the Ministry of Agriculture. The study included 14 morphological markers (Table 2).

Trunk size, shoot tip shape, leaves (fronds) traits (leaf length, leaf base, leaf bending) and fruit traits (fruit shape, fruit size, fruit texture, fruit maturation, fruit color, fruit test, fruit shield, fruit stalk color and fruit stalk length) were estimated visually and maintained three replications (palms) per cultivar. As regards the fruit characters observations were recorded in 15 fruits per palm. Fruits color and test were estimated in khalal stage. Phenotypic traits were then transferred into 1, 2, 3 ...etc symbol form in order to be ready for data analysis (Table 2).

## Data Analysis

Major allele frequency, gene diversity and polymorphism information content (PIC) and genetic distance among the 40 cultivars were estimated using software Power Marker V3.25 (Liu and Muse, 2005). For the genetic similarity analysis, Jaccard's coefficient of similarity (Jaccard, 1908) was used. A dendrogram was generated by cluster analysis using the unweighted pair group method of the arithmetic averages (UPGMA). Simple correlation values among phenotypes and Principal Coordinated Analysis (PCA) was also carried out to show multiple dimension of the distribution of the accessions in a scatter-plot with variance percentage and Eigen value for each component by PAST software version 1.62 (Hammer *et al.*, 2001).

## Markers Parameters and Correlation

Table 3 shows some of the parameters describing morphological markers used in the study. The average number of alleles for all cultivars was 4.2. Shoot tip shape had seven alleles while fruit shield had only two. Major allele frequency ranged from 0.300 for fruit shape to 0.825 for fruit texture. Gene diversity was also ranged from 0.296 for fruit texture suggesting lowest polymorphism information content (PIC) value (0.265) to 0.771 for shoot tip shape suggesting highest polymorphism information content (PIC) value (0.742).

Shoot tip shape as well as fruit shape seem to be a powerful markers to distinguish date palm cultivars. The present study results are in agreement with Al-Khalifa *et al.* (2012) and Al-Wusaibai *et al.* (2014) for fruit shape of the cultivars Khalas and Sukari respectively.

Results shown in Table (4) indicated to simple correlation among studied

phenotypic traits for the selected cultivars grown in five regions in Iraq. The highest correlation value reached 0.543 between the trunk size and leaf (fronds) length, followed by the correlation between trunk size and leaf base (0.540), while the lowest correlation was 0.012 between fruit stalk color and leaf base.

The study of the correlation between the phenotypic characteristics of the date palm has conducted by Naqvi *et al.* (2015) who found that there is a high correlation between the trunk size and the leaf length and base. As the trunk in the date palm is coated by the leaf base, it was reported that the size of the trunk is due to several factors, including the leaf base (Karab) cells enlargement as well as meristematic cells division and enlargement of the mantle meristem region of the palm shoot apex (Ibrahim, 2008).

## Cluster Analysis

Results shown in Figure 2 indicates to the cluster analysis of the cultivars grown in five environmental areas using the UPGMA method for Euclidean distance depending on phenotypic traits.

Two main groups of cultivars was determined independently of their geographic regions with 6.9 Euclidean distance value, the first group was divided into two subgroups with 6.3 Euclidean distance first included: Behrab, Soltani, Saei, Khastawi Euphrates Hamrawi cultivars and the second included Ashrasi and Khiara cultivars with Euclidean distance of 4.85, while the second main group divided into two subgroups with Euclidean distance of 5.7 Barhi was the first while the second divided into two subgroups with Euclidean distance 5.1. The first group included: Khatibi, Khadhrawi Basra

**Table.1** Names and Geographic Regions of Some Iraqi Date Palm Cultivars

No.	Cultivars	Geographic region	No.	Cultivars	Geographic region
1	Bream	South	21	Meer Haj	East
2	Khadhrawi Basrah	“	22	Meer Ali	“
3	Deari	“	23	ShelaniAhmer	“
4	Barhee	“	24	QulHusieni	“
5	Hasawi	“	25	UstaOmran	Center
6	Sayer	“	26	Ashrasi	“
7	Gentar	“	27	Dubaini	“
8	Um Al-Dehn	“	28	Tebarzal	“
9	Dakwani	West	29	Barban	“
10	Tabarzal	“	30	Qeetaz	“
11	Khiarah	“	31	Zahdi Baghdad	“
12	Khateebi	“	32	Khadhrawi Baghdad	“
13	Qayari	“	33	Um Al-Blaleez	Euphrates
14	Hamrawi	“	34	Siltani	“
15	Saaee	“	35	Ibraheemi	“
16	Zahdi West	“	36	Ouainat Aube	“
17	Khadhrawi Mandili	East	37	Khastawi FM	“
18	Maktoom Asfar	“	38	Zahdi FM	“
19	Behrab	“	39	Khadhrawi FM	“
20	Qrenfeli	“	40	Khastawi Shthatha	“

**Table.2** Characterization Table of Iraqi Dale Palm Cultivars Using Some Morphological Markers

No.	Phenotype	Marker	Symbol	No.	Phenotype	Marker	Symbol
1	Trunk size	thin	1	8	Fruit texture	soft	1
		thin-medium	2			semi dry	2
		medium	3			dry	3
		medium-thick	4				
		thick	5				
2	Leaf length	short	1	9	Fruit maturation	early	1
		short- medium	2			early-medium	2
		medium	3			medium	3
		medium- long	4			medium-late	4
		long	5			late	5
3	Leaf base	narrow	1	10	Fruit (khalal) color	yellow	1
		narrow -medium	2			yellow-red	2
		medium	3			red	3
		medium- wide	4				
		wide	5				
4	Shoot tip Shape	pyramidal small	1	11	Fruit (khalal) test	sweet	1
		pyramidal medium	2			medium	2
		pyramidal wide	3			bitter	3
		spherical small	4				
		spherical medium	5				
		spherical large	6				
		interminable	7				
5	Leaf bending	little erect	1	12	Fruit shield	adherent	1
		medium erect	2			separated	2
		erect	3	13	Fruit stalk color	green	1
		Erect in sides	4			yellow-green	2
		level					
6	Fruit shape	oval	1		yellow	3	
		interminable	2		orang	4	
		inverse	3				
		cylindrical	4				
		taper	5				
7	Fruit size	small-medium	1	14	Fruit stalk length	short	1
		medium	2			medium	2
		medium-large	3			long	3
		large	4				

**Table.3** Number of Alleles, Major Allele Frequency, Gene Diversity and Polymorphism Information Content for Some Morphological Markers Used to Assess Genetic Diversity of Iraqi Date Palm Cultivars

No.	Morphological Markers	No. of Alleles	Major Allele Frequency	Gene diversity	Polymorphism information content (PIC)
1	Trunk size	5	0.564	0.629	0.593
2	Leaf length	5	0.564	0.609	0.560
3	Leaf base	5	0.744	0.419	0.389
4	Shoot tip shape	7	0.385	0.771	0.742
5	Leaf bending	5	0.400	0.738	0.698
6	Fruit shape	5	0.300	0.754	0.710
7	Fruit size	4	0.675	0.504	0.466
8	Fruit texture	3	0.825	0.296	0.265
9	Fruit maturation	5	0.450	0.705	0.662
10	Khalal color	3	0.450	0.635	0.559
11	Khalal test	3	0.725	0.436	0.395
12	Fruit shield	2	0.745	0.381	0.309
13	Fruit stalk color	4	0.525	0.636	0.584
14	Fruit stalk length	3	0.564	0.579	0.509
	Average	4.2	0.565	0.578	0.532

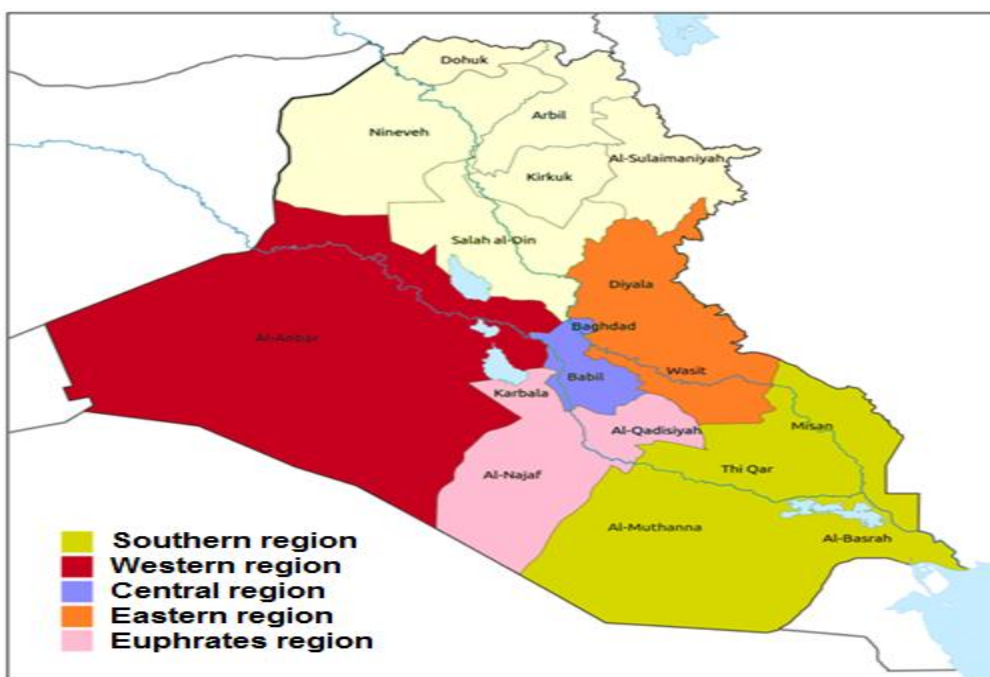
**Table.4** Simple Correlation Values Among Some Phenotypes for 40 Date Palm Cultivars Grown in Five Regions of Iraq

	Trunk size	Leaf length	Leaf base	Shoot tip shape	Leaf bending	Fruit shape	Fruit size	Fruit texture	Fruit maturation	Khalal color	Khalal test	Fruit shield	Fruit stalk color	Fruit stalk length
Trunk size	1.000													
Leaf length	0.543	1.000												
Leaf base	0.540	0.522	1.000											
Shoot tip shape	0.389	0.245	0.334	1.000										
Leaf bending	0.183	0.138	-0.068	0.042	1.000									
Fruit shape	0.229	0.305	0.101	0.161	-0.130	1.000								
Fruit size	0.154	0.135	0.302	-0.002	0.232	0.397	1.000							
Fruit texture	-0.094	-0.107	-0.125	-0.147	-0.155	0.282	0.080	1.000						
Fruit maturation	0.162	0.385	0.390	0.204	-0.365	0.161	0.192	0.200	1.000					
Khalal color	-0.146	-0.092	-0.396	0.038	0.189	0.119	0.089	-0.135	-0.180	1.000				
Khalal test	-0.140	-0.192	-0.134	0.211	0.025	0.031	-0.158	0.094	-0.083	0.275	1.000			
Fruit shield	0.119	0.127	0.105	0.082	-0.284	0.156	0.061	-0.133	0.121	-0.058	0.092	1.000		
Fruit stalk color	0.034	-0.053	0.012	-0.072	-0.043	0.285	0.044	0.407	-0.095	-0.099	0.112	0.059	1.000	
Fruit stalk length	0.042	0.420	0.290	0.164	-0.092	0.285	0.268	0.088	0.219	-0.200	-0.227	0.116	0.051	1.000

**Table.5** Variance Percentage and Eigen Values for all Principle Component Analysis for Iraqi Date Palm Cultivars Depending On Morphological Markers

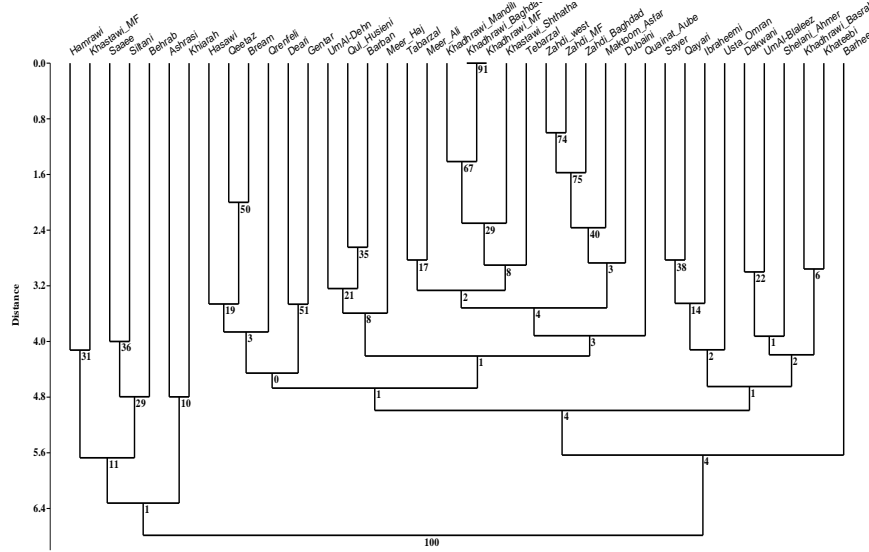
PC	% Variance	Eigen value
1	31.86	4.67
2	17.93	2.63
3	13.30	1.95
4	10.03	1.47
5	6.13	0.89
6	4.57	0.67
7	3.85	0.57
8	3.39	0.497
9	2.84	0.42
10	2.13	0.31
11	1.43	0.21
12	1.23	0.18
13	0.72	0.11
14	0.59	0.09

**Figure.1** Iraq Map Illustrating the Five Major Regions of Date Palm Cultivation

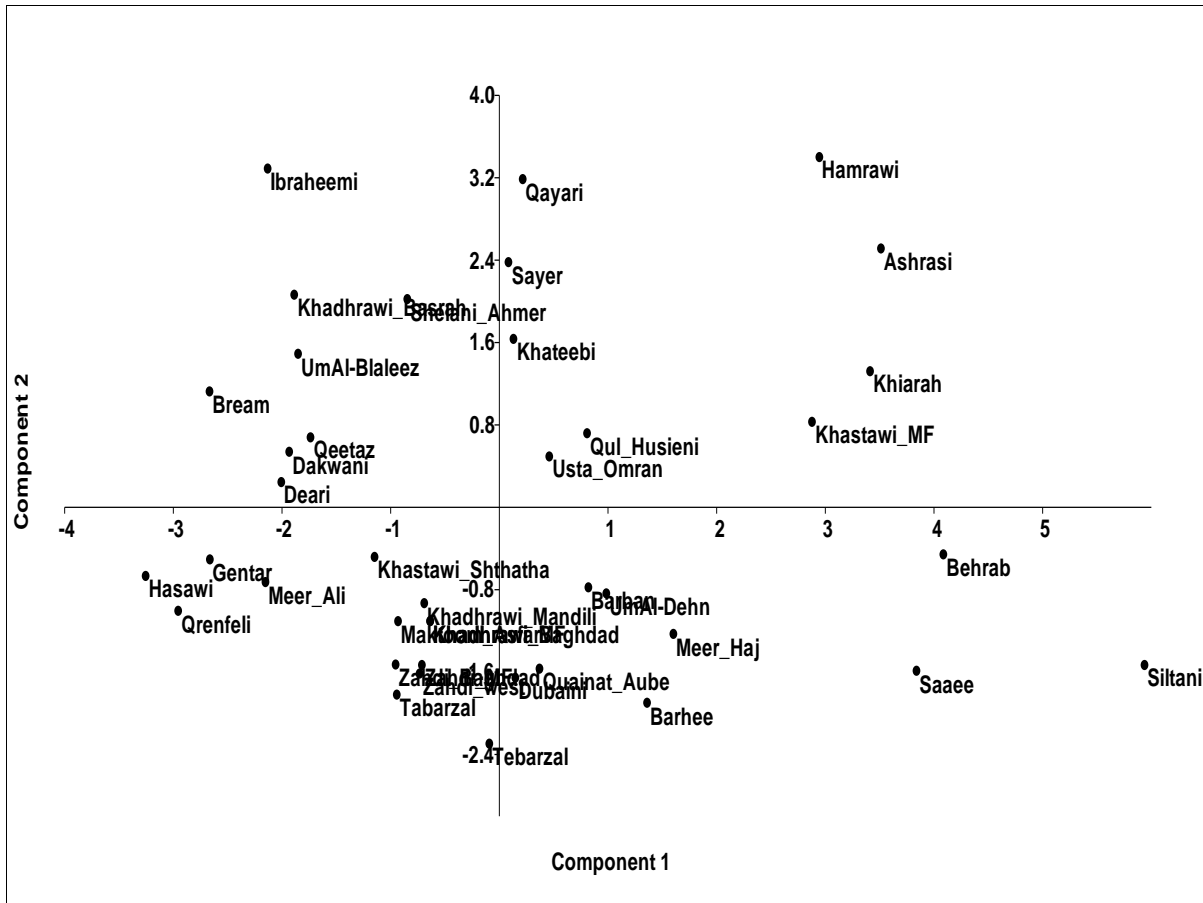




**Figure.2** Cluster Analysis for Iraqi Date Palm Cultivars Depending on Morphological Markers



**Figure.3** Scatter Diagram Plot of the First Two (Pc1 and Pc2) of Principle Component Analysis for Iraqi Date Palm Cultivars Depending on Morphological Markers



Shellaney Ahmer, Um Al-balaliz, Dakwani, Usta Imran, Ebrahimi, Qiari and Sayre. The second group included: Auiant Ayoub, Dubeni, Maktom Asfar, Zahdi Middle, Zahdi Euphrates, Zahdi West, Tebrzal, Khastawi shithatha Khadhrawi Euphrates, Khadhrawi Baghdad, Khadhrawi Mandeli, Meer Ali, Tabrzel, Meer Haj, Burben, Qul Huseini, Um Al-Dhen, Quntar, Deri, Qurnfuli, Bream, Qitaz.

### Principal Component Analysis

In order to confirm the results of cluster analysis describing genetic relationships among Iraqi date palm cultivars, data were analyzed by multivariate Principal Component Analysis (PCA) (Fig 3). The scatter diagram of the first two (PC1 and PC2) based on morphological markers revealed 31.86% of the variation among cultivars with Eigen value of 4.67 while PC2 was 17.93% with Eigen value of 4.63. The rest components from 3 to 14 with its Eigen value is shown in Table 5. Results of PCA scatter diagram exhibit the same two clusters as shown in the dendrogram.

Consistent results of the present study with the findings of Al-Doss *et al.* (2001) in their study for 21 vegetative traits can be used to distinguish between the 17 cultivars of date palm in Saudi Arabia using cluster analysis and PCA analysis, and with what Mohammed *et al.* (2002) found when they use PCA analysis to distinguish between 26 Moroccan date palm cultivars. Our findings are also agreed with Al-Najjar (2014) through PCA analysis of 38 phenotypic vegetative traits to distinguish between 40 Iraqi male cultivars which gave 19.20% variance in the first component. Determination of phenotypic characteristic of date palm cultivars is an important step genetically and taxonomically. Great importance in recent studies that currently

trending to identify the genes responsible for these traits, scientists and researchers working in universities and global research centers currently doing intensive efforts using genomics tools such as Genome Wide Association Studies (GWAS) to identify the genes responsible for the most important phenotypic traits of the date palm.

It can be concluded that there exists wide variability among Iraqi date palm cultivars studied and further molecular analysis will help to determine the relationship among these cultivars forming the basis to classify date palm cultivars in Iraq into clusters based on the above traits.

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