Characterization and Assessment of Genetic Diversity of Wild Date Palm (*Phoenix dactylifera* L.) in Jordan*

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ABSTRACT

This study was conducted to survey and characterize wild populations of date palm in Jordan. Phenotypic parameters either vegetative, leaf morphology, physical and chemical properties of fruits, and genetic diversity within and among populations were also, analyzed. Ecogeographical survey showed that date palm trees are naturally growing in different locations across Jordan ranging in elevation from 390 m below sea level to 525 m above sea level with different levels of salinity. Eleven sites distributed all over the country were selected and the total number of populations studied was twenty four with three female trees randomly selected from each population. Results showed wide variations in morphological characters and in genetic diversity when using 12 Simple Sequence Repeat (SSR) markers. Differences were detected in almost all phenotypic traits. The results of the study indicated a high degree of independence among the geographical origin and morphological data; Genetic variation analysis showed clustering of trees collected from the same population and closely related geographical location. The cultivated date palm Medjool was closely related with populations from Wadi Alhazeem and Al Bokharieh location that form a distinct cluster separated from other studied wild date palm populations with similarity up to 72 %.

Keywords: *Phoenix dactylifera* L.; wild date palm; genetic diversity; biodiversity; Salinity; SSR markers.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a diploid (2n=36) angiosperm that belongs to monocots and considered as dioecious perennial tree. Date palm is the most important, an ancient cultivated species in the Middle East (Elshibli, 2009), which was cultivated in Iraq and Egypt before 3000 BC (Al– Ekidy, 2000; Abd El-Wahab and Wahdan 2007). Therefore, it is highly recommended to establish different protectorates in the date palm center of origin and consider them as hot spots for *in situ* conservation of date palm genetic resources.

In Jordan, the harvested area has been increased more than five folds in the last two decades and the production was increased from 281 tons in 1990 to 11213 tons in 2011 (FAO Statistics, 2011). The palm has played a significant role in humans' life especially in hot, dry and semi-dry regions since it provides food, shelter and serves as an ornamental tree in many cases. It is relatively salt and drought tolerant and has a

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significant impact in combating desertification (Ibrahim, 2010).

The cultivated date palms are usually propagated using asexual methods, which are known to promote and accelerate the genetic uniformity of newly released varieties and result in the erosion of genetic diversity of the species in certain geographical regions (Jaradat, 2011). Such conditions will eliminate the genetic variation within a species, which is needed to ensure its present and future adaptability to a particular environment and as a consequence of continued evolution to maintain genetic improvement in domestication and plant breeding programs (Elshibli, 2009). Wild date palm accessions are considered the most important genetic resource to any breeder to incorporate desirable traits into a new variety or cultivar and enable them to survive under specific environmental condition (Jaradat, 2011). For instance, characterization of date palm salt tolerant accessions of wild date palm grown naturally in Egypt identified promising accession with high levels of salinity tolerance and adaptability to harsh environments.

Morphological characterization of date palm has traditionally provided signatures of varietal genotype and purity. The criteria related to the phenotypic parameters are useful for cultivar characterization, diversity analysis and phylogenic relationship exploration among date palm ecotypes (Jaradat and Zaid, 2004). From the point of phenotypic attributes of date palm, there are no specific criteria to distinguish closely related wild date palms due to the environment effect and the existence of the metaxenia phenomena in date palm (Hammadi et al. 2009). Therefore, morphological evaluation of date palm requires a large set of morphological parameters to analyze. Descriptor for date palm trees was published by Rizk and El Sharabasy (2007). However, Jubrael, et al. (2005) showed the complexity of the identification and characterization of date palm varieties because it relies on a small number of phenotypic traits, mainly of leaves and fruits, which are greatly influenced by the environment. They emphasized the need of molecular markers to identify, characterize, and estimate the genetic diversity of this crop. An integrated approach is needed to incorporate morphological and genetic studies and to improve the knowledge on date palm taxonomy and diversity. In date palm, a number of molecular techniques are available for the characterization of variation at the DNA level including RAPD, AFLP, SNP and SSR (Eissa, et al., 2009).

In Jordan, studies on genetic variation of wild date palm in general are lacking. Therefore, it is important to survey and detect genetic variation among and within the wild type populations of date palm in Jordan.

MATERIALS AND METHODS

Plant material and collection sites

A field survey was conducted during 2012 to evaluate the wild date palm populations in 11 locations distributed across Jordan (Fig.1). Geographical site information for each population like altitude, longitude and latitude were determined by GPS using Garmin instrument (Garmin, USA) (Table 1). Weather data for each location were obtained from the Jordanian Meteorological Department for the year 2012 from the nearest weather station of the collection site. Weather data included average daily maximum and minimum temperature, relative humidity, wind velocity (knot) and average annual precipitation.

To determine soil physical and chemical properties, three representative soil samples were collected from each population at 45 cm depth. Soil moisture percentage was determined following the method described by Craze, (1990). Soil salinity was estimated

according to A.O.A.C, (1995) using a flame photometer for Na, titration procedure with $AgNO_3$ for Cl and $CaCO_3$ using a Calcimeter method. The organic matter content was calculated using potassium dichromate titration method (Magdoff, et al., 1996), while soil pH according to the method described by Eckert and Thomas, (2009) by using a pH meter. Soil electric conductivity (EC) was determined using a conductivity meter.

The number of populations in each collection site was based on tree density and distribution and varied from one to four per each location with a total number of 24 populations for all sites. From each population, three fruit trees were randomly selected and considered as a separate population with a total number of 72 collected trees for all locations. The collected 72 wild date palm trees was then subjected to a comprehensive phenotypic characterization that included fruit quality parameters, the analysis of physical and chemical properties of the soil for each collection and finally the genotyping of the collected plant samples to determine the inter- and intra-population genetic diversity using SSR markers.

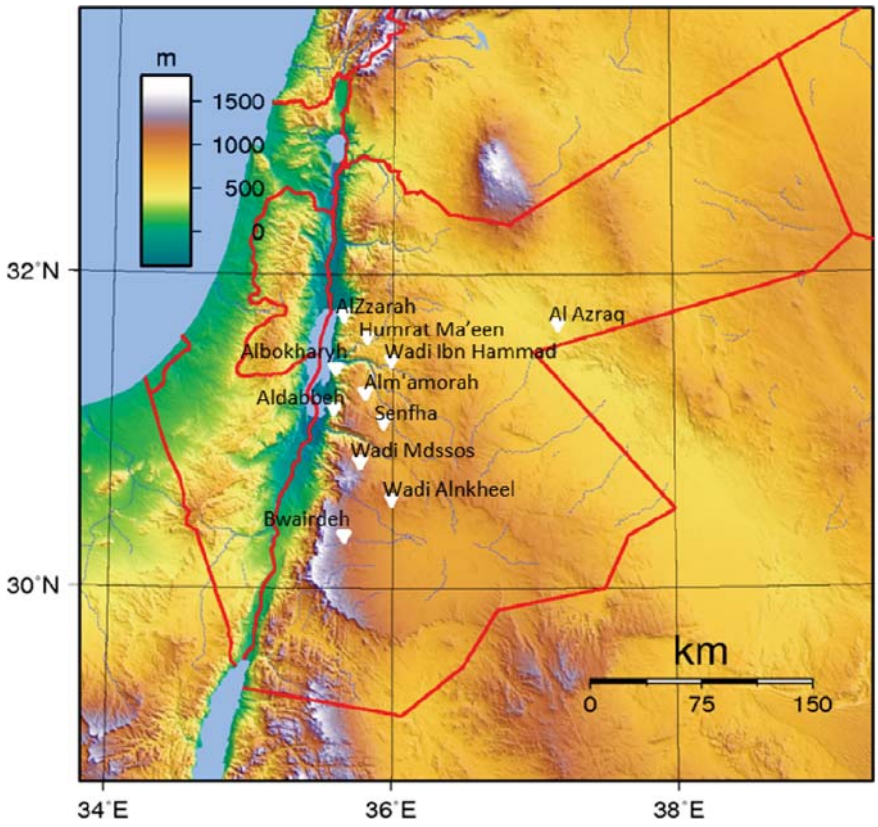

Fig.1: Jordan map showing the locations of the wild date palm populations.

Table 1. Location name, population number and geographic site information.

No.	Name / Province	Population number	Altitude (m)	Longitude	Latitude
1.	Bwairdeh / Aqaba	1	25	35.19463	30.33180
		2	46	35.20031	30.33057
		3	15	35.19217	30.33328
2.	Wadi Al Nkheel / Ma'an	4	363	35.31247	30.37106
3.	Wadi Mdssos / Ma'an	5	75	35.23598	30.40101
		6	70	35.23571	30.40119
4.	AlBokharieh / Karak	7	-385	35.27089	31.02508
5.	Al Dabbeh / Karak	8	-389	35.23575	30.54543
6.	Al M'amorah / Tafilah	9	-342	35.28425	30.54233
7.	Senfha / Tafilah	10	-56	35.29154	30.52580
		11	-75	35.29188	30.53222
8.	Al Zzarah / Balga	12	-360	35.35038	31.41457
		13	-360	35.33563	31.40233
		14	-370	35.33319	31.34452
		15	-257	35.33355	31.33385
9.	Humrat Ma'een / Madaba	16	-51	35.35087	31.39362
		17	45	35.35226	31.39342
		18	110	35.36073	31.38244
		19	150	35.36288	31.40137
10	Wadi Alhazeem / Zarqa	20	518	37.15108	31.35470
		21	525	37.14592	31.36223
11.	Wadi bn Hammad / Karak	22	172	35.38445	31.18081
		23	70	35.37547	31.18095
		24	35	35.37346	31.18070

Phenotypic analysis

The date palm tree height and trunk girth were measured at one meter above soil level (Metwaly, et al., 2009). Young and fully expanded fresh leaves from the middle part of the leaf in the tree of wild date palm of three replicates from each tree were sampled to study leaf length, midrib length, pinnae part length, spine part length, percentage of spine at midrib part and on whole leaf and petiole width. In addition, number of spines on the To analyze the physical and chemical properties of wild date palm fruits, fifteen fruits from each tree at "Rutab" stage were collected during the period that extended from late August until mid-September. These properties included: fruit, flesh and seed weights determined by weighing three fruits from each tree on a digital balance and the average was calculated. Fruit dimensions (length and diameter) of individual fruit were measured by a vernier caliper. Fruit color, was visually judged, depending on color chart as described by Eissa, et al. (2009). Moisture content was determined

according to A.O.A.C, (1995) on fresh weight basis. Consistency of fruit was determined according to moisture content percentage with dry, semi dry and soft fruit texture measurements. Fruit shape was calculated by dividing fruit length over fruit diameter. Fruit pH and acidity was determined as described in A.O.A.C, (1995). Total soluble solids (TSS): was determined as a percentage in the fruit juice using a digital refractometer 64 X. Fruit content of mineral elements were determined as for K by using a flame photometer method (Herrmann and Alkemade, 1963), Mg, Mn and Fe by using an Atomic absorption method (Dean and Ma, 2008).

Molecular analysis

Genetic diversity of wild date palm trees in Jordan was examined by analyzing 72 sampled accessions by molecular technique to detect differences within and among these accessions (inter- and intrapopulational genetic diversity). The Medjool cultivar was used as a control. The analysis was carried out using 12 SSR markers (DP 151, DP 157, DP 159, DP 160, DP 168, DP 169, DP 170, DP 171, DP 172, DP 175, DP 176 and DP 177) described previously in Elmeer, et al. (2011).

The extraction of genomic DNA was done using young, healthy and fresh leaf samples collected separately from individual trees for each wild date palm population. All samples were stored in an ice box in the collection site before transfer to laboratory and storage in a deep freezer at -20 C. Plant tissues were ground in liquid nitrogen using mortar and pestle to a fine powder that was used for gDNA extraction using the QIAGEN DNeasy Plant Mini Kit following manufacturer's instructions.

The PCR amplification was carried out using GeneAmp PCR System 9700 (Applied Biosystems, USA) using a touchdown program. The SSR DNA fragments were amplified using PCR in a 25 µL reaction mixture containing 5 µL of gDNA (100 ng) as a template, 2.5 µL of dNTPs (100 µM), 5 µL of 5× PCR buffer, 0.5 µM of each primer and 0.25 µL of 5 U/µL GoTaq DNA polymerase (Promega, Madison, Wisconsin). The PCR conditions were 94 °C for 5 min, followed by 20 cycles of 95 °C for 30 sec, 65 °C for 30 sec with a drop of 0.5 °C per cycle and 72 °C for 30 sec followed by 20 cycles of 94 °C for 30 sec, 55 °C for 1 sec, and 72 °C for 30 sec, and a final 10 min extension at 72 °C. The amplified PCR products were separated using a 6.5% polyacrylamide gel in a LI-COR 4300 DNA analyzer (LI-COR, Inc. Lincoln, Nebraska, USA) following manufacturer's instructions.

Data analysis

For the phenotypic and soil characteristics data, analysis of variance (ANOVA) was carried out using codes of the GenStat software (Payne, 2013). For pairwise comparison of the phenotypic data and soil characteristics data of collected populations, Bonferroni test was used with an overall level of significance at 5%. Means of phenotypic parameters (qualitative and quantitative) were used as markers to cluster the population. The Euclidean distance was converted to cluster analysis to use all quantitative traits and simple matching for the qualitative variables to form similarity matrix by using Gower (1971) equation with an equal weight for each variable. The clusters were formed using average link option (Unweighted Pair Group Method with Arithmetic Mean) which is also known as UPGMA. The dendrogram figure and the amalgamations of the populations at various levels of similarity were examined and the clusters of populations were saved at 85%, 80% and 75% similarity levels.

To analyze the fruit qualitative traits (color, consistency, maturity period and shape) in the populations, Contingency Chi-square test was used to assess the association between population and fruit

quality parameters, where F-value is a factor vector of the quality parameter.

For genetic diversity assessment, each SSR-PCR bands were transformed into a binary matrix where the presence of reproducible polymorphic DNA band at particularly position on the gels was scored as 1, while 0 denotes its absence. The Power Marker software V3.0 (Liu and Muse 2005) was used to compute the marker data to detect the percentage of heterozygosity and the polymorphism information content among the genotypes on the basis of the allele's size. The collected marker data was used to generate genetic distance matrix using the NTSys PC program (version 2.02). Genetic distance was measured using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) using the NTSys PC program (version 2.02) based on Dice similarity coefficient (1945). The coefficient similarity was employed to draw the precise relationships among the wild date palm trees. The resulted tree files were submitted to the Tree View (Win32; 1.5.2) software to draw a dendogrogam. The population structure analysis was performed using the structure software version 2.0.

RESULTS AND DISCUSSION

The survey showed that wild date palm trees are naturally growing in remote areas away from human disturbance and in different locations across Jordan. The wild date palm trees were found at different elevations ranging between 390 m below sea level to 525 m above sea level with longitude range of 35.19217' in the south east to 37.15108' in the west side and latitudes ranging from 30.33057' to 31.40137' (Table 1). The wild date palm populations were growing in different habitats ranging from desert, mountains, coastal areas and valleys (Figure 2). This indicates the ability of the wild trees to adapt harsh environmental conditions in their natural habitat. In addition, the wild date palm trees might be tolerant to different abiotic stresses including drought and salinity. This assumption showed ability of the wild date palm trees to survive under high concentration of Na, Cl, and $CaCO_3$ or low organic matter and moisture content in the soil (Table 2). For instance, soil analysis in population number 3 at Bwairdeh collection site showed high concentrations of Na and Cl (542 and 747.5 meq/ L, respectively), while the highest EC value was scored at population number 4 at Wadi Al Nkheel, indicating the ability of wild date palm population to grow there and to tolerate high salinity levels. In Wadi Madssos and Al Bokharyeh locations, wild date palm trees were found growing on soils with high levels of $CaCO_3$ (Table 2). For organic matter content, the lowest percentage (0.17 %) was found in Al M'amorah location while, the lowest moisture percentage was recorded at Wadi Alhazeem.

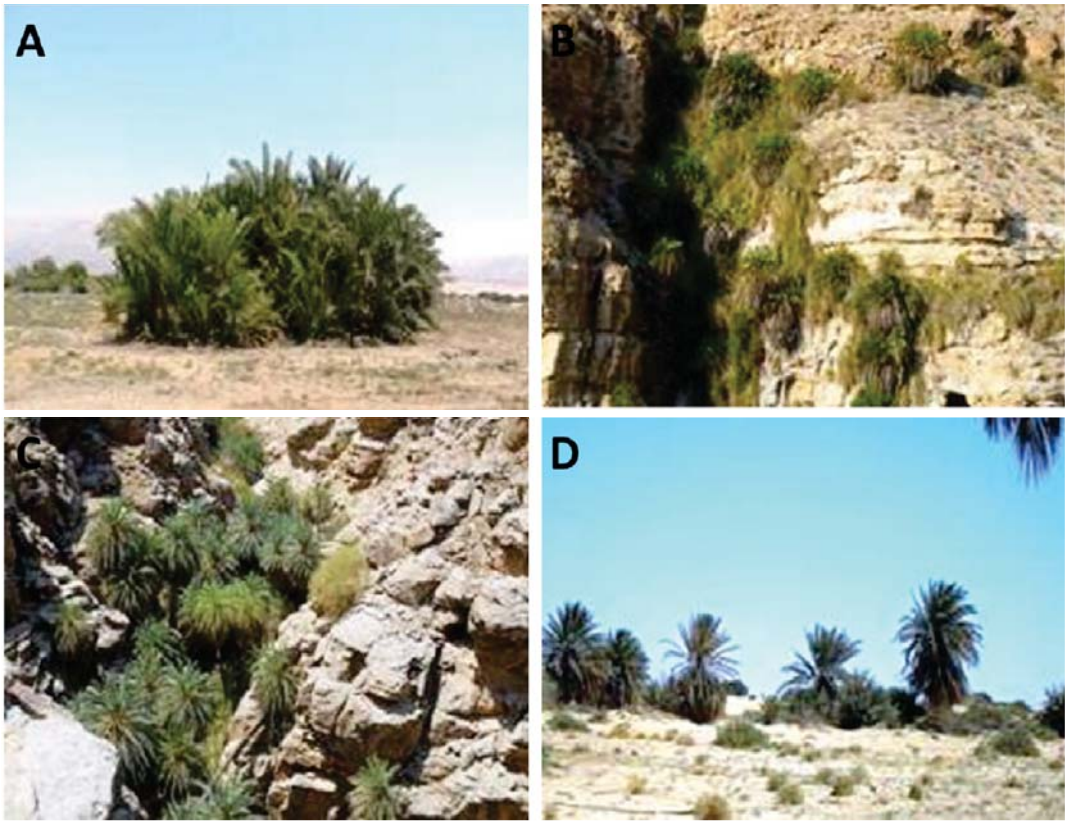

Fig.2: Jordanian wild date palm in the different habitat; A: Coastal, B: Mountainous, C: Valleys and D: Desert.

Table 2. Soil characteristics of the locations for each wild date palm population across Jordan.

Location	Moisture content %	Na (meq/ L)	Cl (meq/ L)	CaCO3 %	Organic matter %	pH	Ec (ds / m)
Bwairdeh P1	10.67 def	127.10 g	249.20 n	19.57 fg	3.47 gh	7.63 ab	66.83 n
Bwairdeh P2	16.67 gh	216.60 i	697.50 o	18.30 f	1.07 de	7.70 abc	102.03 p
Bwairdeh P3	31.67 i	542.40 m	747.50 p	15.30 e	1.80 f	7.77 a-e	121.60 q
Wadi Al Nkheel P4	13.00 efg	22.00 b	14.20 bc	10.07 d	0.20 a	7.97 d-g	3.67 a-d
Wadi Mdssos P5	4.67 abc	462.70 kl	151.70 j	34.47 jk	0.57 a-d	7.77 a-e	50.47 k
Wadi Mdssos P6	5.33 abc	74.40 de	97.50 h	37.87 l	0.53 a-d	7.93 c-g	16.30 h
Al Bokharyeh P7	12.33 d-g	90.40 f	100.80 h	36.53 kl	3.33 gh	7.60 a	25.00 i
Al Dabbeh P8	50.67 j	80.90 ef	175.80 k	30.27 i	4.10 i	7.77 a-e	54.30 l
Al M'amorah P9	4.33 abc	2.80 a	4.20 a	6.57 c	0.17 a	7.77 a-e	2.88 ab
Senfha P10	2.33 a	8.20 a	42.50 ef	4.17 b	0.47 abc	7.83 a-e	2.14 a
Senfha P11	3.67 ab	474.70 l	194.20 l	3.63 ab	2.03 f	7.77 a-e	70.37 o
Al Zzarah P12	10.33 def	29.50 bc	48.30 f	7.03 c	0.30 ab	7.73 a-d	6.57 e
Al Zzarah P13	12.00 def	22.50 b	47.50 f	6.53 c	0.03 a	7.77 a-e	5.01 b-e

Location	Moisture content %	Na (meq/ L)	Cl (meq/ L)	CaCO3 %	Organic matter %	pH	Ec (ds / m)
Al Zzarah P14	4.67 abc	152.50 h	75.80 g	13.80 e	4.98 j	7.87 b-f	25.70 i
Al Zzarah P15	3.67 ab	258.90 j	170.80 k	3.67 ab	6.90 k	7.77 a-e	38.30 j
Humrat Ma'een P16	5.33 abc	37.80 c	19.20 cd	6.57 c	0.80 bcd	7.93 c-g	5.27 cde
Humrat Ma'een P17	18.00 h	122.00 g	119.20 i	13.53 e	3.73 hi	7.73 a-d	26.20 i
Humrat Ma'een P18	8.00 bcd	67.40 d	37.50 e	29.33 i	0.57 a-d	7.80 a-e	8.97 f
Humrat Ma'een P19	11.00 def	452.10 k	229.20 m	1.70 a	0.80 b-d	8.17 g	62.40 m
Wadi Alhazeem P20	2.33 a	6.90 a	20.80 cd	21.27 gh	2.90 g	7.60 a	3.33 a-d
Wadi Alhazeem P21	1.33 a	7.50 a	8.30 ab	14.70 e	0.57 a-d	8.00 efg	3.07 abc
Wadi Ibn Hammad P22	10.00 def	27.10 bc	22.50 cd	33.00 j	1.03 cd	7.77 a-e	5.57 de
Wadi Ibn Hammad P23	8.67 cde	31.60 bc	27.50 d	22.30 h	1.63 ef	7.80 a-e	12.43 g
Wadi Ibn Hammad P24	13.67 fgh	3.70 a	2.70 a	3.40 ab	0.37 ab	8.03 fg	4.70 b-e

* Means within the same column followed by the same letter were not significantly different as determined by Bonferroni test at 0.05 probability level.

Results of the current study showed high levels and significant variations among wild type genotypes for most of the studied phenotypic traits (Table 3 and 4). For tree height; the highest plants (9.8 m) were found in population number 14 at Al Zzarah location, while trees in Wadi Al Nkheel produced the highest trunk girth (2.0 m) (Table 3). For the leaf morphology, the highest values of measured parameters were in Al M'amorah location, while the Bwairdeh location had the highest values of spine parameters. The highest physical fruit characteristics means values regarding weight, length and diameter (10.6 g, 4.0 cm and 2 cm, respectively) were recorded at population number 21 at Wadi Alhazeem location (Table 4). Besides, wild date palm fruit samples from population number 4 at Wadi Al Nkheel gave the highest TSS values compared with other locations. For mineral composition, fruits collected from population number 18 at Humrat Ma'een location and number 13 at Al Zzarah had the highest K and Fe content (2.6% and 31.3 ppm, respectively).

Variations in the phenotypic parameters are strongly supported by projection of the accessions in UPGMA cluster analysis. A dendogram based on Dice similarity coefficient (1945) using UPGMA was used for cluster analysis of phenotypic data (Figure 3). The cluster divided populations into four groups under coefficient 80 %. Group 1 composed of 2 populations of Senfha locations (population 10 and 11). Group 2 included 10 populations distributed in six locations; populations number 2 and 3 at Bwairdeh, population number 5 at Wadi Mdssos, populations 14 and 15 at Al Zzarah, populations 22, 23 and 24 at Wadi Ibn Hammad location. Group 3 contained 2 populations at Al Zzarah (population number 12 and 13). Group 4 included 10 populations distributed at eight locations; population number 1 at Bwairdeh, population number four at Wadi Al Nkheel, population number 6 at Wadi Mdssos, population 7 at Al Bokharyeh, population 8 at Al Dabbeh, population 9 at Al M'amorah , populations number 16, 17 and 19 at Humrat Ma'een and finally population number 20 at Alazraq location.

Means of some of the vegetative properties such leaf morphology and physiochemical characteristics of fruits were comparable indicating a morphological variation between populations. Metwaly, et al. (2009) reported phenotypic variation with seeded date palm trees in

Egypt. In this study, leaf morphological traits like leaf and midrib lengths (349.20 and 294.70 cm) were lower in values compared with data obtained by Eissa, et al. (2009) and Metwaly, et al. (2009). In addition, most of the physical and chemical properties of collected fruits showed significant differences within and among populations (Table 4) and locations (data not shown) and this is in general agree with the results obtained by Osman (2008). However, the average means of fruit characteristics in this study were less than data obtained by Eissa, et al. (2009) and Metwaly, et al. (2009). The variations in fruit parameters such as consistency, maturity date, color and shape were in agreement with results obtained by Eissa, et al. (2009) and Metwaly, et al. (2009). However, these parameters are known to be affected by the pollen grains original sources, a phenomenon known as metaxenia (Swingle, 1928). For fruit pH, moisture content, TSS and nutrient analysis (K, Mg, Mn and Fe), the results of this study were similar to these obtained by Forouzan, et al. (2012), but disagree with the data obtained by El-Sohaimy and Hafez, (2010).

Results indicate independence of the morphological data and geographical locations. Such assumption is supported by the findings of Hammadi, et al. (2009), who evaluated similar accessions in different geographical locations and found phenotypic differences. Such phenotypic variations for the tested traits are expected in wild date palm populations (Jaradat, 2011) knowing that the majority of date palm studies described above were dealing with genetically uniform plant material such as cultivars (Salem, et al, 2008). The variations in the phenotypic data is also expected to occur because of the genetic differences between unrelated trees collected from different locations (Vij, et al., 2005).

Table 3. Morphological traits related to tree height and girth, leaf, spines and pinnae of the collected wild date palm trees from different locations across Jordan.

	Tree		Leaf							Spines				Pinnae			
	Height (m)	Trunk girth (m)	Length (cm)	Midrib length (cm)	Pinnae length (cm)	Spine length (cm)	Spine at midrib (%)	Spine at leaf (%)	Petiole width (cm)	No.	Density /cm	Length (cm)	Width (cm)	No.	Density /cm	Length at mid (cm)	Length at top (cm)
Bwairdeh P1	5.23 ab	1.70 abc	273.00 h	249.20 g	221.60 e	27.67 d	11.08 abc	10.07 a-f	3.12 d	22.56 lm	0.82 ij	16.22 ij	0.94 ab	126.40 ef	0.57 a	49.33 fg	23.78 a
Bwairdeh P2	5.87 a-d	1.63 abc	256.00 f	227.70 e	203.10 c	24.56 c	10.79 abc	9.59 a-e	2.78 bc	23.00 lmn	0.94 k	17.11 jk	0.91 ab	118.40 cd	0.58 ab	46.22 e	28.33 cde
Bwairdeh P3	5.53 abd	1.47 abc	216.10 a	189.00 a	170.10 a	18.89 a	10.00 a	8.74 ab	2.39 a	25.00 no	1.32 n	19.22 l	0.92 ab	110.90 a	0.65 f-i	29.44 a	27.11 bcd
Wadi Al Nkheel P4	9.03 cde	2.03 c	308.80 p	277.00 mn	229.90 ij	47.11 i	17.02 fg	15.26 i-l	3.78 gh	10.22 de	0.22 d	9.22 cd	0.83 ab	158.40 kl	0.69 jk	62.11 lm	31.78 fgh
Wadi Mdssos P5	6.90 a-e	1.43 abc	221.80 c	198.00 c	177.40 b	20.56 ab	10.41 ab	9.27 abc	2.53 ab	23.89 mno	1.16 m	18.67 kl	0.96 ab	112.70 ab	0.63 d-g	34.00 b	23.78 a
Wadi Mdssos P6	7.87 a-e	1.97 bc	287.10 k	256.90 h	225.20 fg	31.67 e	12.34 a-e	11.01 a-h	2.58 ab	17.00 hi	0.54 g	13.33 fgh	0.77 a	140.40 gh	0.62 cde	54.22 h	30.22 efg

Al Bokharyeh P7	4.90 a	1.87	281.70	256.10	226.00	30.11	11.76	10.69	3.66	19.44 jk	0.65 h	13.44	0.97	136.20	0.60	52.44	25.56
		bc	j	h	fgh	e	a-d	a-g	fg			gh	ab	g	bc	h	abc
Al Dabbeh P8	9.33	1.13 a	283.60	256.00	223.90	32.11	12.54	11.32	3.26	18.11 ij	0.56 g	13.22	0.84	137.80	0.62	53.44	27.56
	de		j	h	ef	e	a-e	a-h	de			fgh	ab	g	cd	h	b-e
Al M'amorah P9	5.77	1.73	349.20	294.70	236.00 l	58.67	19.91 g	16.81 l	4.77 j	3.44 a	0.06 a	4.78 a	0.77 a	175.10	0.74	69.11	54.56 k
	a-d	abc	t	p		k								n	m	o	
Senfha P10	6.67	1.63	313.00	278.20	229.10	49.11	15.12	13.36	3.91	8.33 cd	0.17	7.44	0.91	161.60	0.71	64.00	34.78
	a-e	abc	q	mn	hij	i	ef	g-j	gh		cd	bc	ab	lm	kl	mn	hi
Senfha P11	7.53	1.67	219.30	188.90	169.80	19.11	10.11 a	8.71	2.54	25.22 o	1.32 n	19.33 l	0.84	112.90	0.67 ij	31.44 a	30.44
	a-e	abc	b	a	a	a		ab	ab				ab	ab			efg
Al Zzarah P12	7.33	1.77	302.00	272.90	233.90	39.00	14.30	12.91	3.46	12.22 ef	0.31 e	10.56	0.77	152.40	0.65	59.33	29.11
	a-e	bc	n	kl	kl	g	def	f-j	ef			de	ab	ij	f-i	jk	d-g
Al Zzarah P13	8.60	1.83	309.30	277.30	229.90	47.44	17.11	15.33	3.92	10.22	0.22 d	9.00	0.84	158.40	0.69	62.22	32.00
	b-e	bc	p	mn	ij	i	fg	jkl	gh	de		cd	b	kl	jk	lm	gh
Al Zzarah P14	9.80 e	1.73	267.10	243.70 f	218.10	25.56	10.48	9.56	3.00	22.78	0.89	17.11 jk	0.81	122.90	0.56 a	47.56	23.44 a
		abc	g		d	c	ab	a-d	cd	lm	jk		ab	de		ef	
Al Zzarah P15	9.70 e	1.77	249.20	224.10	201.80	22.33	9.97 a	8.96	2.58	23.00	1.03 l	17.67	0.88	116.90	0.58	44.11	25.11
		bc	e	d	c	b		ab	ab	lmn		jkl	ab	bc	ab	d	ab
Humrat Ma'een	6.67	1.47	297.20	268.30 j	232.20	36.11	13.47	12.15	3.27	14.22 fg	0.39 f	11.33 ef	0.98	148.20 i	0.64	58.22	28.89
P16	a-e	abc	m		jk	f	b-e	d-h	de				ab		d-h	ij	def
Humrat Ma'een	6.80	1.50	276.00	248.10	220.60	27.89	11.23	10.10	3.02	21.22 kl	0.76 i	14.22 hi	0.89	128.70 f	0.58	50.22	27.89
P17	a-e	abc	i	g	de	d	a-d	a-f	cd				ab		ab	g	bcd
Humrat Ma'een	7.47	1.63	292.20	264.20 i	227.90	36.33	13.74	12.43	3.17	16.00	0.44 f	12.11	0.80	143.30	0.63	56.56 i	28.00
P18	a-e	abc	l		ghi	f	cde	e-i	d	gh		efg	ab	h	def		bcd
Humrat Ma'een	6.60	1.37	228.80	193.40	174.10	19.33	9.99 a	8.45 a	2.61	24.22	1.26 n	18.22	0.94	114.70	0.66	38.44 c	35.33 i
P19	a-e	ab	d	b	b	a			ab	mno		jkl	ab	abc	ghi		
Wadi Alhazeem	5.87	1.57	316.90	279.90	227.30	52.56	18.77 g	16.59 l	4.24 i	6.33	0.12	6.33	0.97	163.30	0.72	65.00	37.00 i
P20	a-d	abc	r	no	ghi	j				bc	abc	ab	ab	m	lm	n	
Wadi Alhazeem	5.57	1.53	316.30	280.10	228.60	51.56	18.41 g	16.30	4.01	7.22 c	0.14	7.44	0.84	161.80	0.71	65.11	36.22 i
P21	abc	abc	r	no	ghi	j		kl	hi		bcd	bc	ab	lm	kl	n	
Wadi Ibn	7.33	1.63	305.40	275.40	233.60	41.89	15.20	13.71	3.69	15.44	0.37	12.22	0.86	154.70	0.66	60.44	30.00
Hammad P22	a-e	abc	o	lm	kl	h	ef	h-k	fg	gh	ef	efgh	ab	jk	hi	kl	def
Wadi Ibn	7.60	1.67	298.80	270.00	234.10	35.89	13.30	12.01	3.24	13.33 f	0.37	11.33 ef	0.78	150.90	0.64	58.33	28.78
Hammad P23	a-e	abc	m	jk	kl	f	b-e	c-h	de		ef		ab	ij	e-i	ij	def
Wadi Ibn	5.83	1.37	326.30	281.60	228.10	53.44	18.97 g	16.38	4.59 j	4.44	0.08	5.44	0.97	165.10	0.72	67.33	44.78 j
Hammad P24	a-d	ab	s	o	ghi	j		kl		ab	ab	ab	ab	m	lm	o	

* Means within the same column followed by the same letter were not significantly different as determined by Bonferroni test at 0.05 probability level.

Table 4. Morphological traits related to fruits physical and chemical properties of the collected wild date palm trees from different locations across Jordan.

	Weight (g)	Flesh weight (g)	Seed weight (g)	Length (cm)	Diameter (cm)	Color	Consistency	Maturity date	Shape	pH	Moisture %	TSS %	K %	Mg %	Mn (ppm)	Fe (ppm)
Bwairdeh P1	3.23 g	1.97 f	1.27 ghi	2.20 de	1.30 b-e	Orange	Semi Dry	Early Sep.	Ovate	5.87 e	40.67 c	25.20 f	0.47 a	0.08 b	7.63 c	13.00 b
Bwairdeh P2	2.27 ef	1.23 cde	1.03 d-g	1.97 cd	1.17 b-e	Orange	Soft	Early Sep.	Ovate	5.47 bc	74.33 hi	22.93 de	0.87 abc	0.45 m	29.33 l	14.13 bcd
Bwairdeh P3	2.47 f	1.30 de	1.17 f-i	2.37 e	1.37 de	Orange	Semi Dry	Early Sep.	Ovate	5.87 e	48.33 cd	16.07 a	1.17bcd e	0.23 jk	23.30 j	14.67 cd
Wadi Al Nkheel P4	1.17 b	0.63 abc	0.53 b	1.57 ab	0.97 abc	Yellow	Soft	Early Sep.	Ovate	5.37 b	70.00 ghi	29.07 k	1.77 fgh	0.44 m	10.00 de	13.87 bc
Wadi Mdssos P5	2.30 ef	1.57 ef	0.73 bcd	1.97 cd	1.33 cde	Orange	Soft	Early Sep.	Ovate	5.37 b	65.67 gh	17.97 b	1.47 def	0.02 a	10.40 ef	10.33 a
Wadi Mdssos P6	1.77 cde	1.03 bcd	0.73 bcd	1.77 ac	0.97 abc	Orange	Semi Dry	Early Sep.	Ovate	5.47 bc	49.67 cd	27.33 hij	0.47 a	0.16 def	5.23 b	21.20 hi
Al Bokharyeh P7	5.20 h	4.50 g	0.70 bc	2.97 f	1.50 ef	Yellow	Dry	Late Aug.	Cylindrical	5.37 b	69.33 ghi	26.47 gh	1.07 bcd	0.09 b	9.53 d	21.33 hi
Al Dabbeh P8	1.50 bcd	0.63 abc	0.87 c-f	1.47 a	0.93 ab	Orange	Dry	Late Aug.	Ovate	5.03 a	25.00 b	26.87 ghi	1.67 e-h	0.14 de	4.70 b	10.00 a
Al M'amorah P9	1.43 bcd	0.67 abc	0.77 b-e	1.47 a	0.93 ab	Orange	Dry	Late Aug.	Ovate	5.73 de	13.00 a	28.93 k	1.67 e-h	0.20 g-j	10.93 f	22.07 ij
Senfha P10	0.50 a	0.33 a	0.17 a	1.57 ab	0.63 a	Yellow	Semi Dry	Late Aug.	Ovate	5.37 b	50.67 cde	28.43 jk	1.67e-h	0.27 kl	9.53 d	20.70 ghi
Senfha P11	1.83 cde	0.97 be	0.87 c-f	2.00 cd	1.00 a-d	Yellow	Soft	Late Aug.	Ovate	5.57 bcd	69.00 ghi	16.73 ab	2.33 ij	0.31 l	3.73 a	22.27 ij
Al Zzarah P12	1.33 bcd	0.60 ab	0.73 bcd	1.57 ab	1.07 bcd	Orange	Soft	Early Sep.	Ovate	5.57 bcd	71.33 ghi	28.37 jk	1.97 f-i	0.13 cd	8.47 c	18.13 ef
Al Zzarah P13	1.90 def	0.83 a-d	1.07 e-h	1.60 ab	1.03 bcd	Orange	Soft	Early Sep.	Ovate	5.70 cde	67.33 ghi	28.60 k	1.97 f-i	0.20 g-j	12.33 g	31.30 l
Al Zzarah P14	1.77 cde	0.93 a-d	0.83 b-e	1.57 ab	1.03 bcd	Orange	Semi Dry	Early Sep.	Ovate	5.67 cde	52.67 def	23.83 e	1.60d-g	0.08 b	8.00 c	19.80 gh
Al Zzarah P15	1.90 def	0.83 a-d	1.07 e-h	1.77 abc	1.17 b-e	Orange	Soft	Early Sep.	Ovate	5.63 cde	75.00 hi	21.83 d	1.83 f-i	0.06 ab	17.70 i	19.30 fg
Humrat Ma'een P16	1.77 cde	0.80 a-d	0.97 c-f	1.77 abc	1.10 bcd	Orange	Soft	Early Sep.	Ovate	5.67 cde	78.33 i	27.93 ijk	2.03 ghi	0.30 l	17.93 i	17.57 e

Humrat Ma'een P17	1.87 cde	0.87 a-d	1.00 c-f	1.70 abc	1.07 bcd	Orange	Soft	Early Sep.	Ovate	5.57 bcd	70.67 ghi	25.87 fg	2.17 hij	0.09 bc	9.87 de	26.20 k
Humrat Ma'een P18	1.83 cde	0.93 a-d	0.90 c-f	1.80 bc	1.07 bcd	Yellow	Soft	Early Sep.	Ovate	5.60 bcd	74.33 hi	27.97 ijk	2.63 j	0.14 de	15.47 h	29.93 l
Humrat Ma'een P19	1.53 bcd	0.70 a-d	0.83 b-e	1.57 ab	0.93 ab	Orange	Soft	Early Sep.	Ovate	5.57 bcd	64.00 fgh	20.07 c	1.97 f-i	0.18 e-h	8.30 c	30.50 l
Wadi Alhazeem P20	8.77 i	7.43 h	1.33 hi	2.90 f	1.87 fg	Yellow	Soft	Mid Sep.	Cylindrical	5.77 de	66.67 gh	28.93 k	1.07 bcd	0.18 f-i	14.93 h	23.13 j
Wadi Alhazeem P21	10.60 j	9.20 i	1.40 i	4.03 g	1.93 g	Yellow	Soft	Mid Sep.	Cylindrical	5.67 cde	65.67 gh	28.87 k	0.63 ab	0.16 d-g	10.00 de	10.53 a
Wadi Ibn Hammad P22	1.77 cde	0.77 a-d	1.00 c-f	1.73 abc	1.00 a-d	Orange	Soft	Early Sep.	Ovate	5.77 de	61.33 efg	28.93 k	1.93 f-i	0.22 ij	26.20 k	15.63 d
Wadi Ibn Hammad P23	1.53 bcd	0.67 abc	0.87 c-f	1.70 abc	1.03 bcd	Orange	Soft	Early Sep.	Ovate	5.63 cde	67.33 ghi	27.83 ijk	1.77 fgh	0.21 hij	9.50 d	25.00 k
Wadi Ibn Hammad P24	1.30 bcd	0.60 ab	0.70 bc	1.47 a	1.00 a-d	Orange	Soft	Early Sep.	Ovate	5.80 de	69.00 ghi	28.87 k	1.20 cde	0.13 cd	9.50 d	25.23 k

* Means within the same column followed by the same letter were not significantly different as determined by Bonferroni test at 0.05 probability level.

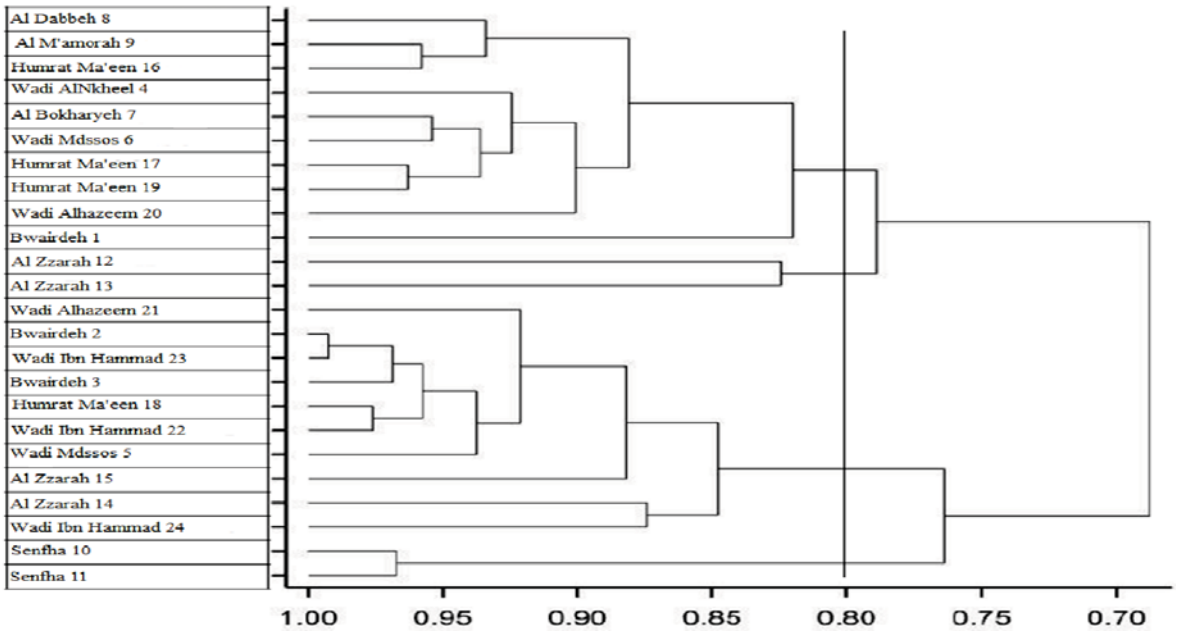

Fig 3: Cluster analysis and dendogram for 24 populations based on 33 phenotypic parameters.

In this study, most of the leaf morphology parameters were either positively or negatively affected by sodium concentration in the soil (Table 5). For instance, a positive correlation between high salinity and spines number and length were found, while negative correlations with leaf length and TSS in fruit were observed.

The results obtained with the phenotypic data indicate that the collected accessions from same location might possess high similarity at the genetic level. To validate this hypothesis, molecular analysis using 12 SSR markers previously tested on date palm (Elmeer, et al., 2011) were used to analyze genetic diversity of Jordanian wild date palm trees. The SSR analysis results indicated that eleven of 12 markers generate polymorphic banding patterns at the expected band size (Table 6). The SSR markers examined on the (72 accessions + Medjool) produced thirty four polymorphic alleles with an average mean of 3.09 alleles per locus (Table 6). However, the number of alleles varied between 2 in primers DP 159, DP168 and DP172 and 4 in primers DP 151, DP 157, DP171 and DP175 (Table 6). The 11 polymorphic primers produced SSR band sizes ranging from 140 bp (marker DP157) to 324 bp (marker 151). The mean of gene diversity for the tested SSR markers ranged from 0.00 for DP 160 to 0.36 for DP 157 (Table 6). The overall average major allele frequency was 0.82 ranging from 0.71 to 1.00. Similarly, the polymorphism information content PIC value, which is commonly used in genetics as measure of polymorphism for marker locus, was 0.22 ranging between 0.00 and 0.29. The observed major allele frequency, gene diversity and PIC values of the tested SSR markers in this study were found to be less than the finding of Elmeer, et al. (2011). This could be attributed to the unique mechanism responsible for generating SSR allelic diversity in plants and the source of genomic DNA used in this study (Elmeer, et al., 2011).

Table 5. Correlation among phenotypic parameters with soil characteristics and climatic data

Fruit weight	- 0.15	- 0.08	0.20
Flesh weight	- 0.17	- 0.12	0.21
Fruit length	0.01	0.08	0.25
Fruit diameter	0.04	0.09	0.27
Seed weight	0.11	0.27	0.04
TSS %	- 0.99 **	- 0.68 *	0.07
Leaf length	- 0.94 **	- 0.66 *	- 0.06
Midrib length	- 0.98 **	- 0.67 *	0.02
Pinnae part length	- 0.99 **	- 0.64 *	0.13
Spine part length	- 0.81 **	- 0.61 *	- 0.17
spines number	0.79 **	0.62 *	0.20
Spine length	0.83 **	0.63 *	0.16
Pinnae number	- 0.86 **	- 0.67 *	- 0.10
	Na	Cl	CaCO3

*: Significantly correlated at 0.05 level.

**: Significantly correlated at 0.001 levels.

(-): negative correlation.

Table 6. Genetic biodiversity information of Jordanian wild date palm using 12 SSR markers.

Marker	Major allele frequency	allele per locus	No. of observation	Availability	Gene Diversity	PIC
DP151. (267-324 bp)	0.81	4	72	0.99	0.26	0.22
DP157. (140-175 bp)	0.71	4	69	0.95	0.36	0.29
DP159. (140-150 bp)	0.79	2	70	0.96	0.33	0.27
DP160. (209-212 bp)	1.00	2	73	1.00	0.00	0.00
DP168. (220-226 bp)	0.89	2	72	0.99	0.21	0.19
DP169. (209-216 bp)	0.76	3	72	0.99	0.34	0.27
DP170. (221-226 bp)	0.78	3	72	0.99	0.32	0.26
DP171. (206-239 bp)	0.75	4	70	0.96	0.33	0.26
DP172. (223-228 bp)	0.86	2	70	0.96	0.24	0.21
DP175. (213-224 bp)	0.78	4	73	1.00	0.32	0.26
DP176. (227-237 bp)	0.94	3	72	0.99	0.11	0.10
DP177. (227-241 bp)	0.79	3	71	0.97	0.31	0.26
Mean	**0.82**	**3**	**71**	**0.98**	**0.26**	**0.22**

The molecular data were used to build a dendogram based on Dice coefficient similarity to measure genetic similarity between the 73 date palm accessions using UPGMA (Figure 4). In the contrary to the phenotypic data similarity analysis, the genetic diversity of wild date palm was completely related to the geographical distribution and location site, which may be attributed to the difficulty of transferable accessions from one region to another. The data shown in figure 4 showed that the genetic similarity among wild date palm accessions ranged from 0.60 to 1.00. In general, higher levels of genetic similarity were found between Jordanian wild date palm trees collected from the same population followed by populations from the same location with few exceptions. The existence of wide genetic diversity in wild date palm germplasm is possibly related to the adaptation to a broad range of environmental conditions and their high heterozygosity ability.

Interestingly, the collected wild date palm trees from AlAzraq location were closest to the Medjool cultivar, while Humrat Ma'een trees had the greatest genetic difference from it. At 0.6 similarity level, two groups can be clearly identified where group 1 contains the Medjool cultivar beside 9 trees from three populations (population 7 at Albokharyh location and populations 20 and 21 from Alazraq location). The second group can be further divided into four subgroups where the first subgroup contains the majority of trees collected from Wadi Madsoss and Bwairdeh locations, the second subgroup contains the majority of trees collected from Wadi Alnkheel, Senfha and Alm'amorah locations, the third group contains the majority of trees collected from Humrat Ma'een, Al Zzarah, Wadi Ibn Hammad and Al Dabbeh (Figure 4). The highest similarity coefficient value was observed among Ma'een populations and also at Al Zzarah populations (1.0).

For population structure analysis, the natural logarithm of the probability of the data was performed using the structure software. The proportional to the posterior probability of Delta K was found to be the highest at K = 4 (Figure 5A). These results suggest that the analyzed wild date palm in Jordan can be divided into four genetically distinct groups

(Figure 5B). Medjool cultivar was classified with group number two that contain Al Bokharyeh, and Wadi Alhazeem locations trees. The individuals in this group were more closely with coefficient of similarity reaching up to 0.72 supporting the data of the NTSys analysis (Figure 4). The Humrat Ma'een populations showed the greatest genetic difference from other groups with a coefficient of similarity less than 0.60. In general, the population structure analysis reflected the origin of collection sites for Jordanian wild date palm and indicates the existence of genetic relatedness between trees in the same location. This might be due to the fact that these accessions are adapted to their agroecological conditions and inbreeding events are taking place in each location, which might be due to pollination from common male parents (data not shown).

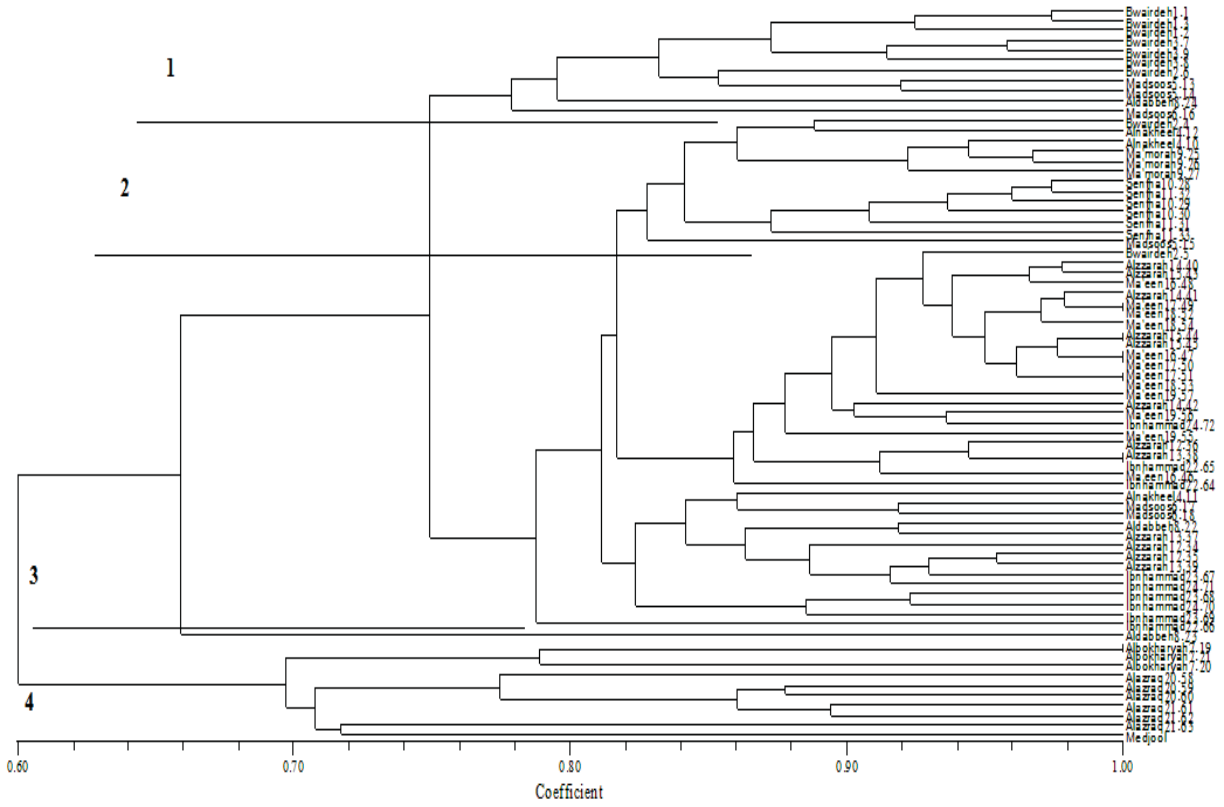

Fig.4: Dendogram showing the clustering of wild date palms based on molecular aspect according to NTSys software.

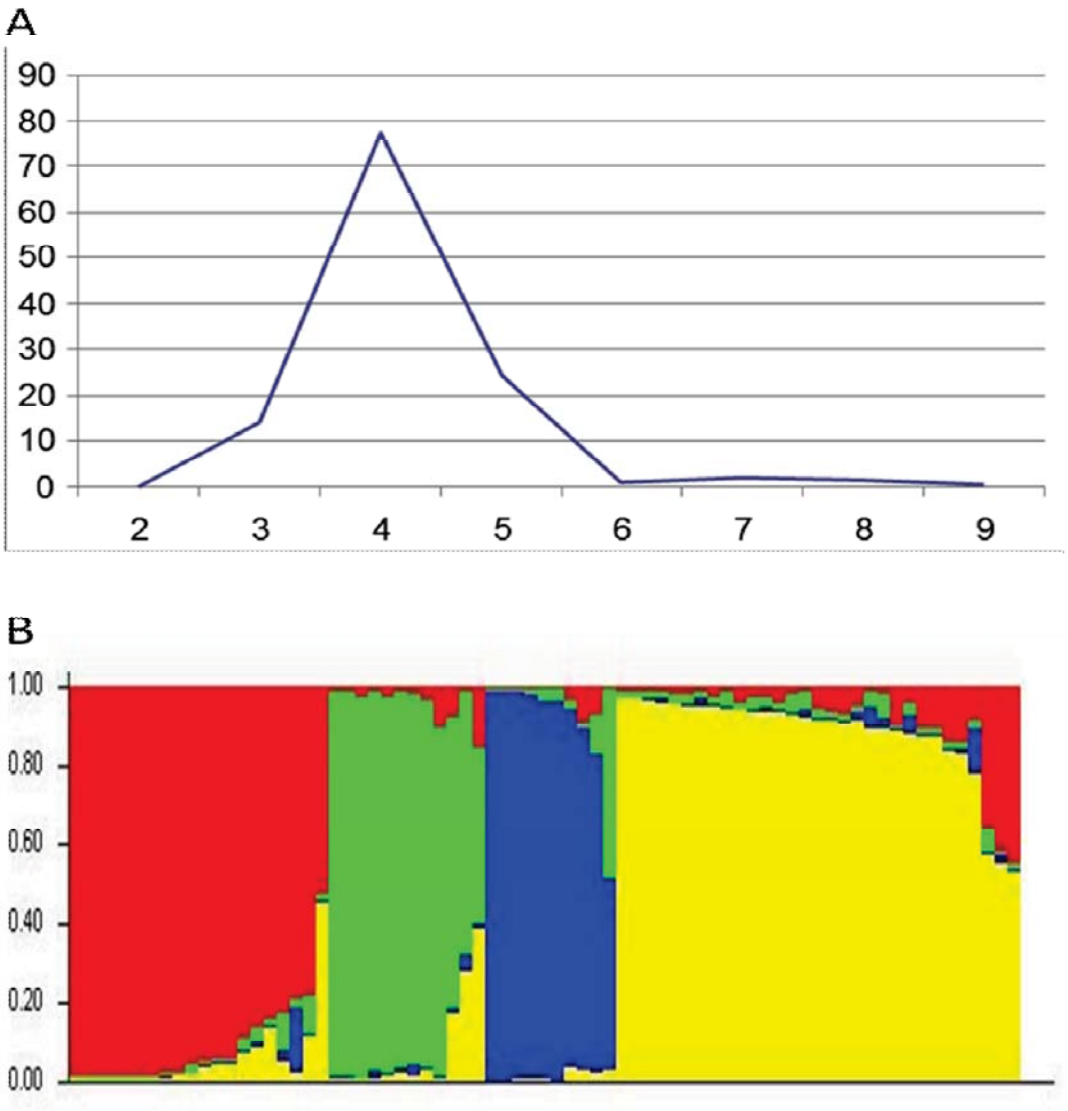

Fig. 5: A) Estimation of the most probable number of clusters (K), based on 20 independent runs and K ranging from 1 to 10 by using magnitude of ΔK for each K value. Population structure assignment at K = 4 for (72 wild date palm tree + 1 tree from the date palm cultivar Medjool). B) Division and the frequency distribution of 72 wild date palm accessions and Medjool cultivar resulted from the K = 4. Each accession is shown by vertical line that is partitioned into colored segments representing groups (Q1=red, Q2=green, Q3= blue and Q4= yellow).

Molecular markers clearly allow the comparison of genetic material of two individual plants or populations avoiding any environmental influence on genetic structure, which may help in estimating genetic erosion in natural populations of date palm. In this study, SSR molecular markers were used to study the genetic diversity and population structure of Jordanian wild date palm germplasm. The SSR analysis was applied successfully to detect the level of genetic similarity among and within Jordanian wild date palm genotypes. Such analyses are considered complement to the morphological and physiochemical characterization of wild date palm. This analysis exhibited high level of efficiency for detecting polymorphism among the wild date palm trees and it was used efficiently to estimate the genetic diversity pattern and its distribution in natural date palm populations in Jordan.

In conclusion, the phenotypic and molecular characterization of the Jordanian wild date palm populations will facilitate their genetic conservation and improvement efforts providing a unique DNA fingerprint for each individual tree. The High level of genetic diversity existed in the natural wild date palm populations in Jordan provides could be a good starting point to for their utilization in future breeding programs. Wild date palm should be improved by dynamic management that includes *in situ* conservation of date palm genetic resources to keep the level of genetic diversity as high as possible.

Conclusion

The survey showed that wild date palm in Jordan is distributed in different geographical regions and was growing under extreme environmental conditions experiencing high temperature, poor soils of low fertility, and high salinity. Its populations are varied in density, growth habit and habitats. Populations of these genetic resources are endangered due to cuttings, extreme disturbance, lack of care and attention and ignorance of its ecological and genetic importance. Our study revealed clear cut differences in phenology, phenotypic plasticity, growth development, density and abundance of wild date palm trees in different regions of the country. Molecular analyses revealed the existence of genetic differences among the populations studied. These results emphasize the importance of wild date palm in Jordan for future research and improvement breeding programs. It seems that natural populations although are characterized by low yield, less stature of trees and dense spines and thus difficulty in harvesting yield, but sure they tolerate/resist extremes of environmental conditions including lack of moisture, poor soil conditions and low fertility and maybe agricultural pests. Therefore, research should be directed toward the beneficial impact of wild date palm populations in Jordan as an important genetic resource that should be protected, conserved and utilized for improvement of grown cultivars in the region.

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توصيف وتقدير الاختلافات الوراثية لنخيل التمر البري (*Phoenix dactylifera* L.) في الأردن

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ملخــص

تم القيام بهذه الدراسة لمسح وتوصيف المجتمعات البرية لنخيل التمر في الأردن. إضافة الى دراسة الصفات الشكلية سواء للمجموع الخضري كشكل الأوراق أو الخواص الفيزيائية والكيميائية للثمار وكذلك تحليل الاختلافات الوراثية المحتملة داخل وبين مجتمعات النخيل البري. أظهرت نتائج المسوحات أن أشجار النخيل البري موجودة طبيعيا في مواقع مختلفة من الأردن وتنمو الأشجار على ارتفاعات تتراوح ما بين 390 مترا تحت مستوى سطح البحر وحتى 525 مترا فوق سطح البحر وعلى مستويات مختلفة من ملوحة التربة. تم في الدراسة اختيار أحد عشر موقعا موزعة على مناطق مختلفة في الأردن، وأعتمد عدد المجتمعات التي تمت دراستها على كثافة أشجار النخيل البري وتوزعها في كل موقع. بلغ عدد المجتمعات التي تناولتها الدراسة أربعة وعشرون مجتمعا أخذ من كل منها ثلاث أشجار مؤنثة تم اختيارها عشوائيا. أظهرت النتائج وجود اختلافات واسعة في الصفات الشكلية للنخيل البري ووجود أثني عشر دليلا (مؤشر) SSR داخل وبين المجتمعات. تم العثور على اختلافات في كافة الصفات الشكلية تقريبا. أشارت النتائج إلى وجود درجة عالية من الاستقلالية بين المناطق الجغرافية والبيانات الشكلية للنخيل البري وهذا يمكن أن يكون عائدا إلى اختلافات بين المواقع. كان لتركيز عنصر الصوديوم في التربة تأثير معنوي على بعض الصفات الشكلية إضافة إلى تأثيره على المواد الصلبة الكلية الذائبة TSS . أظهرت نتائج التحليل الوراثي وجود تكتل للأشجار التي جمعت من نفس المجتمع للنخيل البري والمواقع الجغرافية التي تتبعها والأكثر قربا لها. كان الصنف المزروع من النخيل (مدجول) هو الأكثر قربا وعلاقة مع مجتمعات النخيل البري في موقعي وادي الهزيم والبخارية حيث شكلت هذه المجتمعات تكتلات مميزة ومختلفة عن باقي مجتمعات النخيل البري التي تمت دراستها وبنسبة تماثل بينها تصل الى 72%.

الكلمات الدالة: نخيل التمر، نخيل بري اختلافات وراثية، تنوع حيوي، ملوحة، مؤشر SSR.

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