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NEW APPROACH FOR THE MORPHOLOGICAL IDENTIFICATION OF DATE PALM (*PHOENIX DACTYLIFERA* L.) CULTIVARS FROM TUNISIA

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Abstract

Thirty vegetative date palm (*Phoenix dactylifera* L.) descriptors were screened. This selection was made on three cultivars with variable behaviour towards oasis stress: Deglet nour, Alig and Kintichi. The statistic tests showed an intra-cultivar stability of 6 characters: percentage of spinned midrib part, apical divergence angle, maximal pinnae width at the top leaf, percentage of solitary spine, spine length at the middle and maximal spine angle. These characters are steady; their measures are not affected by the change of cropping conditions. So they can be used for cultivars identification in any oasis and outside the fruiting period. Using these parameters, the genetic variation among 26 Tunisian date palm cultivars was studied. The graphic distribution identified by principal component analysis showed cluster according to fruit characteristics. We have distinguished a grouping of later maturity date palm varieties which opposed to those with earlier maturity. The Pearson coefficient showed significant correlation between the harvesting period and the percentage of spinned midrib part which can be used as an indicator of the cultivar maturity period. In addition, cultivars with the same fruit consistency are grouped together. These results are useful in the selection, collections and genes mapping.

Introduction

In Tunisia, date palm (*Phoenix dactylifera* L., 2n=36) is the most important fruit tree in the southern part of the country, where it has been cultivated for a long time, with an estimate of about 250 current varieties (Rhouma, 1994; 2005). More than 4 millions of date palm trees are spread on 32000 ha of oases. Almost 55% of this area is recently created where the major commercially desirable date varieties, Deglet nour, is extensively cultivated. However, the acreage of other date accessions are regressing and sometimes disappearing in spite of their important nutritional and economic value.

This situation is aggravated by the threat of destructive diseases such as the vascular fusariosis disease commonly named as Bayoud caused by the fungus *Fusarium oxysporum f.sp Albedinis* (Louvet & Toutain, 1973; Baaziz, 2000). It is estimated that Bayoud disease has killed more than 13 million trees in less than one century in Morocco and Algeria (Oihabi, 2000a, b) which has increased the genetic erosion. In Tunisia, Bayoud disease has not been reported. However, some other diseases are found, including non-identified diseases also those associated with the presence of some fungi, insects and nematode species.

Considering that the Phoenix plays an important role in increasing employment and capital income storage, the date palm biodiversity requires a more comprehensive knowledge in order to solve these problems. So, it is necessary to elaborate a strategy of preservation to protect production, adaptation and resistance. A program of the exhaustive resource inventory is a highly valuable stage phase in crop improvement strategies. A number of methods are currently available for analysis of genetic diversity in germplasm accessions, breeding lines and populations. These methods have relied mainly on the availability of three genetic markers: morphological, biochemical and molecular.

As to the phenotypic markers, the most common characters used to identify different cultivars in date palm are the morphology of leaves, spines and fruit characters. Such morphological features are sensitive to environmental factors (Sedra *et al*., 1993; 1996) and they can be observed only in mature trees. This was proposed in Tunisia by many reports which have described the importance of morphological traits in identifying the Tunisian date palm cultivars (Ben Salah, 1993; Ben Salah & Hellali, 2004; Rhouma, 1994; 2005). In spite of these descriptions, it remains very difficult to identify cultivars, especially outside fruiting period. In fact, owing to the great adaptive flexibility of this genus, many farmers cannot recognize cultivars outside their oasis (Munier, 1973).

The aim of the present work is to investigate the genetic diversity of Tunisian date palm cultivars using some selected descriptors in order to obtain an accurate description and knowledge of these genetic resources with the vegetative morphometric characters and to understand the distinctive performance of these cultivars.

Materiel and Methods

a. Descriptors selection: Most of the descriptors used in the setting of this work are adopted by the IPGRI (Anon., 2005) and quoted by other researchers (Peyron & Gay, 1988; Ben Salah, 1993, Ben Salah & Hellali, 2004; Sedra, 2001; Rhouma, 1994; 2005). Four descriptors were reported for the first time. A total of 30 descriptors were considered as shown in Table 1.

Our aim is to select some reliable descriptors that can be used for the cultivars identification in any localities. For this purpose a screening was done in order to keep the descriptors that did not have an environment effect on their measures. The selection was based on three cultivars: Deglet Nour, Alig and Kintichi in order to verify the hypothesis of descriptor stability differences between cultivars with variable behaviour. Indeed, according to farmers the Deglet Nour variety is considered as the most sensitive to abiotic constraints, while the Kintichi and Alig ones are the most resistant. As Rhouma *et al*., (2006) noted, some cultivars have various degrees of sensibility towards stress. For each cultivar, the descriptors were applied on 90 adult leaves of aged palm trees at the rate of nine replications in each locality. For each cultivar, ten localities, each with a varied variable cropping environment (cultural practices, soil and irrigation conditions) belonging to Nefzaoua and Jerid oases are investigated. Their distribution is shown in Fig. 1.

b. Description of cultivars: The selected descriptors were applied on 26 cultivars of the continental oases chosen for their importance to farmers. Hlwa, Hissa, Loghrabi, Rotbayet elmansoura and Rotbayet yagouta cultivar were investigated for the first time; the others were cited by previous works (Masselot, 1901; Kearney, 1906; Rhouma, 1994; 2005). Table 2 summarizes some characteristics of the studied cultivars.

c. Data analyses: Data analyses were performed using SPSS program (12.0) which allowed us to make the intra-cultivar variance analysis. The stable selected descriptor measurements of the cultivars were subjected to a principal component analysis (PCA) to determine the characteristics with greater contributions to the total variability.

Table 1. List of measured descriptors.

	Characters	Code	Used by			
			I	P	R	S
Leaf	Length (cm) (figure2)	PM1	X	X	X	X
	Midrib length (cm) (figure2)	PM2				X
	Pinnated part length (cm) (figure2)	PM3	X	X	X	X
	Pinnated midrib part length (cm)	PM4			X	
	Spined part length (cm) (figure2)	PM8	X	X	X	X
	% of spinned midrib part	PM9				
	% of spinned leaf part	PM10				X
	Leaf width at the first spine (cm)	PM11	X	X		
	Maximal width (cm)	PM12	X			
	Midrib width (on first pinnae) (cm)	PM13	X			
	Apical divergence angle (°)	PM16	X			
Pinnaes	Number	PN1	X	X	X	X
	Density /cm	PN2				X
	% of antrose pinnae	PN3	X			
	% of introse pinnae	PN4	X			
	% of retrose pinnae	PN5	X			
	Maximal pinnae width at the middle leaf (cm)	PN6	X	X	X	X
	Pinnae length at the middle leaf (cm)	PN7	X	X	X	X
	Pinnae length at the top leaf (cm) (figure2)	PN8	X	X	X	X
	Maximal pinnae width at the top leaf (cm)	PN9	X	X	X	X
	Index of spacing	PN10	X			
Spines	Number	EP1	X	X	X	X
	Density /cm	EP2				X
	% solitary spine	EP3	X			
	% of spine grouped by per	EP4	X			
	% of spine grouped by 3	EP5	X			
	Spine length at the middle (cm)	EP6	X	X	X	X
	Maximal spine width at the middle (cm)	EP7				
	Maximal angle (°)	EP8				
	Minimal angle (°)	EP9				

I : IPGRI (2005), P : Peyron (1988), R: Rhouma Abdelmajid (1994 et 2005) et S: Sedra (2001)

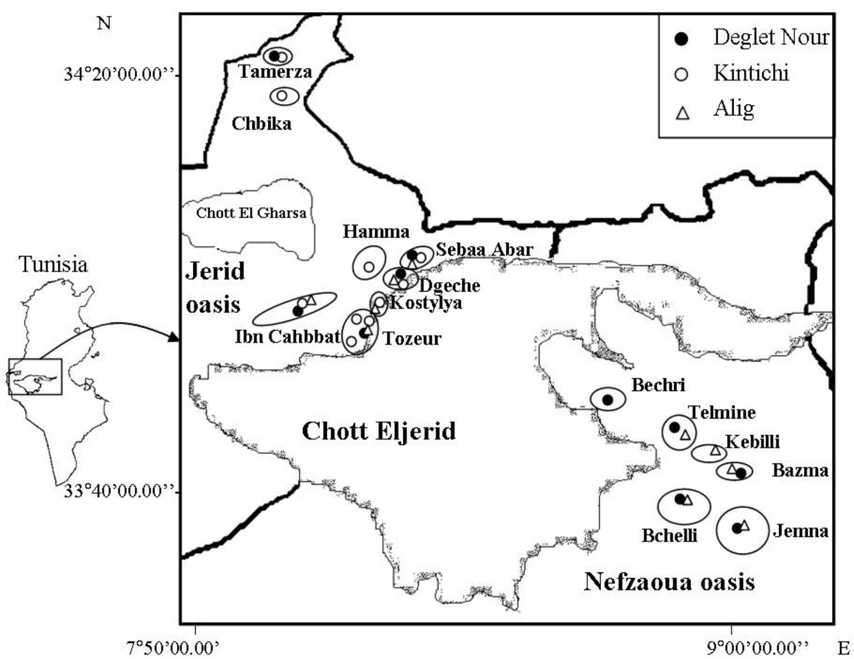

Fig. 1. Sites of collected date palm samples.

Table 2. Name, origin, and main characteristics of date-palm genotypes studied.

Noun	Geographical distribution[a]	Fruit characteristics		Harvesting (Rhouma 1995 and 2005)	
		Color	Consistency	Period	Day (±10)[b]
Alig	N, J	Dark brown	Semi-dry	Late	235
Ammary	N, J	Black	Soft	Early	150
Bejjou	N, J	Brown	Dry	Late	230
Bissr Helou	N, J	Pale brown	Dry	Season	195
Choddakh	N, J	Dark Amber	Semi-soft	Season	205
Choddakh Ben Jbir	N	Dark Amber	Semi-soft	Season	185
Dhahbi	J	Amber	Semi-soft	Late	215
Deglet Nour	N, J	Amber	Semi-soft	Late	225
Fermla	N	Brown	Semi-dry	Season	195
Fezzani	N, J	Amber	Semi-dry	Season	185
Gondi	N, J	Amber	Semi-soft	Season	195
Gosbi	N, J	Black	Soft	Early	155
Ghars souf	N, J	Dark brown	Soft	Season	200
Hissa	N, J	Honey	Soft	Early	155
Hlwa	N	Honey	Semi-dry	Late	215
Hamra	N, J	Amber	Semi-dry	Season	210
Horra	N, J	Amber	Dry	Season	210
Kintichi	J	Reddish	Dry	Late	215
Loghrabi	J	Dark brown	Semi-soft	Season	195
Om Leghlez	J	Amber	Soft	Early	180
Rtob Houdh	J, N	Amber	Soft	Season	205
Rtotbayet elmansoura	N	Brouwn	Soft	Season	185
Rotbayet yagouta	N	Dark amber	Soft	Early	175
Tronja	N, J	Dark brown	Semi-dry	Late	215
Tezerzayet Kahla	N, J	Black	Soft	Season	185
Tezerzayet Safra	J	Dark brown	Soft	Early	180

a- N: Oases of Nefzaoua, J: oases of Jerid. b-The day zero is the 1st of March (flowering month).

Results

a. Descriptors selection: Mean values, standard deviation of measure phenotypic characters applied on Deglet Nour, Alig and Kintichi cultivars as well as the variance analyses are given in Table 3. The comparison between localities unveils many stable descriptors in each variety. The Deglet Nour cultivar showed 10 stable descriptors: PM9, PM10, PM16, PN9, PN10, EP3, EP5, EP6, EP7 and EP8; 14 descriptors for the Alig cultivar: PM8, PM9, PM10, PM16, PN7, PN8, PN9, PN10, EP2, EP3, EP4, EP5, EP6 and EP8 and 10 descriptors for the Kintichi cultivar: PM9, PM10, PM12, PM16, PN6, PN9, PN10, EP3, EP6 and EP8. The Alig cultivar is found to be more resistant against oasis conditions. Contrary to both others cultivars, it is specified by the stability of its spined part length (PM8), Pinnae length at the top leaf (PN8), the spines density (EP2) and their groupings (EP4 and EP5). The Kintichi cultivar is particularly characterized by stability in the maximum width of the leaf (PM12) and in the maximal pinnae width at the middle leaf (PN6) whereas the Deglet Nour cultivar is distinguished by the stability of its maximal spine width at the middle (EP7).

Table 3. Descriptive statistics of Deglet Nour, Alig and Kintichi cultivars (M: mean value, SD: Standard deviation, P: *P*-value)

Cultivar Characters	Deglet nour			Alig			Kintichi		
	M	SD	P	M	SD	P	M	SD	P
PM1	433.82	35.83	0.00*	418.25	50.09	0.00*	375.69	53.31	0.00*
PM2	399.89	34.28	0.00*	394.08	50.43	0.00*	352.17	52.94	0.00*
PM3	302.68	28.29	0.00*	307.12	44.73	0.00*	300.50	42.43	0.00*
PM4	268.74	26.42	0.00*	282.95	44.76	0.00*	276.97	41.84	0.00*
PM8	131.11	12.52	0.01*	111.13	21.98	0.12	75.19	16.00	0.01*
PM9	32.84 a	2.21	0.07	28.30 b	4.64	0.15	21.31 c	3.03	0.90
PM10	30.31 a	2.12	0.05	26.64 b	4.40	0.19	19.96 c	2.90	0.82
PM11	7.13	0.59	0.00*	7.69	0.90	0.00*	6.76	0.68	0.01*
PM12	86.31	8.01	0.00*	66.54	8.67	0.02*	78.13	6.80	0.10
PM13	2.81	0.43	0.00*	3.22	0.38	0.01*	3.56 a	0.50	0.00*
PM16	79.39 a	6.47	0.11	73.06 b	9.08	0.23	79.06 a	5.45	0.09
PN1	171.64	8.78	0.00*	223.46	79.02	0.00*	187.94	18.57	0.00*
PN2	0.64	0.05	0.00*	0.79	0.24	0.00*	0.69	0.07	0.03*
PN3	26.66	4.11	0.00*	28.31	2.94	0.00*	32.50	2.98	0.01*
PN4	44.20	6.84	0.00*	39.14	5.37	0.01*	39.77	4.15	0.00*
PN5	29.15	3.45	0.00*	32.56	3.32	0.03*	27.73	2.26	0.03*
PN6	2.81	0.35	0.02*	3.78	0.39	0.00*	3.79	0.31	0.28
PN7	56.84	4.20	0.00*	46.03	4.59	0.23	50.63	4.32	0.00*
PN8	33.93	3.34	0.03*	24.17	3.86	0.41	23.52	3.62	0.01*
PN9	1.94 a	0.37	0.90	1.46 c	0.28	0.12	1.73 b	0.31	0.36
PN10	0.42 a	0.02	0.60	0.40 a	0.04	0.91	0.40 a	0.04	0.85
EP1	49.87	3.55	0.00*	41.27	5.49	0.00*	26.02	2.75	0.01*
EP2	0.38	0.03	0.00*	0.37	0.05	0.07	0.36	0.08	0.00*
EP3	40.38 b	5.89	0.21	46.90a	10.59	0.48	37.16 c	5.79	0.16
EP4	38.88	6.91	0.00*	36.20	7.86	0.88	60	7.16	0.04*
EP5	20.73 a	3.96	0.06	17.03 b	7.87	0.49	3.68	4.26	0.00*
EP6	12.56 b	0.98	0.14	15.21 a	2.39	0.16	9.09 c	1.16	0.07
EP7	0.45	0.07	0.10	0.49	0.10	0.00*	0.42	0.09	0.04*
EP8	43.78 b	5.50	0.12	48.94 a	5.02	0.57	46.03 b	4.77	0.15
EP9	10.56	1.19	0.01*	16.06	5.03	0.02*	17.42	4.13	0.00*

Significant ($p<0.05$)

On the whole, 8 common stable descriptors are showed for the three cultivars: PM9, PM10, PM16, PN9, PN10, EP3, EP6 and EP8. Both PM9 and PM10 descriptors characterise the spine part, PM9 character seems more stable than PM10 (Table 3). Therefore a date palm cultivar should be described by the percentage of the spinned midrib part (PM9) rather than that of the spinned leaf part (PM10). The S-N-K test showed an inter-cultivar stability of the parameter PN10 also called spacing index (Table 3), and hence it should be ruled out from the selected descriptors. The rest (six common stable descriptors) proved very discriminating; they show a highly significant difference between cultivars.

b. Description of cultivars: The average values of the 6 selected characters applied on the 26 cultivars are reported in the Table 4. The correlations between these variables are presented in the Table 5. Positive correlations were shown between PN9 (Pinnae length at the top) and PM16 (Apical divergence angle), between PN9 and EP8 (maximal angle) and between PM16 and EP8. The more the pinnae are thick the more the pinnae and spines are discarded from the midrib. However, a negative correlation was shown between PM9 (the percentage of spinned midrib part) and EP3 (the percentage of solitary spines) the shorter the spinned midrib part is, the more solitary spines we have.

Table 4. Average values of selected variables of the cultivars

	PM9	PM16	PN9	EP3	EP6	EP8
Alig	28.30	73.06	1.46	46.90	15.21	48.94
Ammary	12.90	82.78	1.78	63.94	8.09	74.44
Bejjou	24.98	87.78	1.84	42.34	8.78	70.56
Bissr Helou	21.19	80.67	1.98	33.40	9.06	86.11
Choddakh	24.35	80.83	1.48	29.48	10.11	58.61
Choddakh Ben Jbir	20.33	80.83	1.30	37.18	7.90	54.17
Dhahbi	26.25	56.67	1.53	51.45	7.75	57.50
Deglet Nour	32.84	79.39	1.94	40.38	12.56	43.78
Fermla	25.66	70.83	1.23	21.10	7.72	48.33
Fezzani	20.18	77.08	1.70	34.85	9.00	43.33
Gondi	15.94	74.44	2.00	53.28	9.00	70.00
Gosbi	11.43	76.11	1.38	65.65	11.73	62.22
Ghars souf	22.30	61.67	1.44	63.37	11.17	57.50
Hissa	8.48	55.00	0.97	65.33	6.38	36.67
Hlwa	27.69	65.00	1.49	35.12	8.06	50.00
Hamra	23.04	63.33	1.23	43.99	12.44	44.44
Horra	28.29	85.00	2.23	32.12	8.90	53.33
Kintichi	21.31	79.06	1.73	37.16	9.09	46.03
Loghrabi	23.84	88.89	1.53	56.18	8.14	70.56
Om Leghlez	14.92	95.00	2.43	66.93	6.33	68.33
Rtob Houdh	16.09	80.00	1.80	67.14	6.61	57.78
Rtotbayet elmansoura	23.39	91.11	3.17	36.53	8.31	76.11
Rotbayet yagouta	18.65	47.78	1.06	55.45	7.94	45.56
Tronja	24.27	60.00	1.62	28.62	11.63	60.00
Tezerzayet Kahla	20.60	56.67	0.87	30.55	7.48	46.11
Tezerzayet Safra	16.23	58.89	1.20	50.34	9.60	53.89

Table 5. Correlation between selected variable.

	PM9	PM16	PN9	EP3	EP6	EP8
PM9	1	0.097	0.172	-0.643	0.414	-0.121
PM16		1	0.730	-0.022	-0.082	0.569
PN9			1	-0.036	-0.076	0.594
EP3				1	-0.143	0.141
EP6					1	-0.139
EP8						1

The scatter diagram of first three principal coordinates (PC1, PC2 and PC3) from PCA analysis of 26 Tunisian cultivars based on the 6 selected descriptors is shown in Fig. 3. This analysis showed an explanation of 83,81% of the total variability. This indicates a strong pattern of differentiation between cultivars. The first principal component which explains 38,17% of the total variability, received relatively high positive loadings from the variables PM16 (+0,88), PN9 (+0,89) and EP8 (+0,83). The second component, which explains an additional 31,26% of the total variation, received a high positive loading from the variable PM9 (+0,92), whereas the highest negative loading came from the variable EP3 (-0,80). The third principal component explains 17.4% of the total variation and it is positively correlated with the EP6 parameter (+0,78).

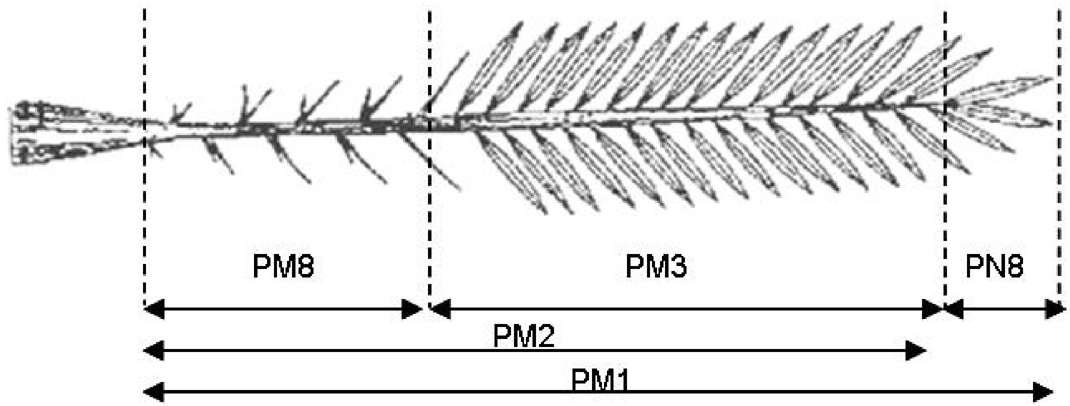

Fig. 2. The leaf characters descriptors.

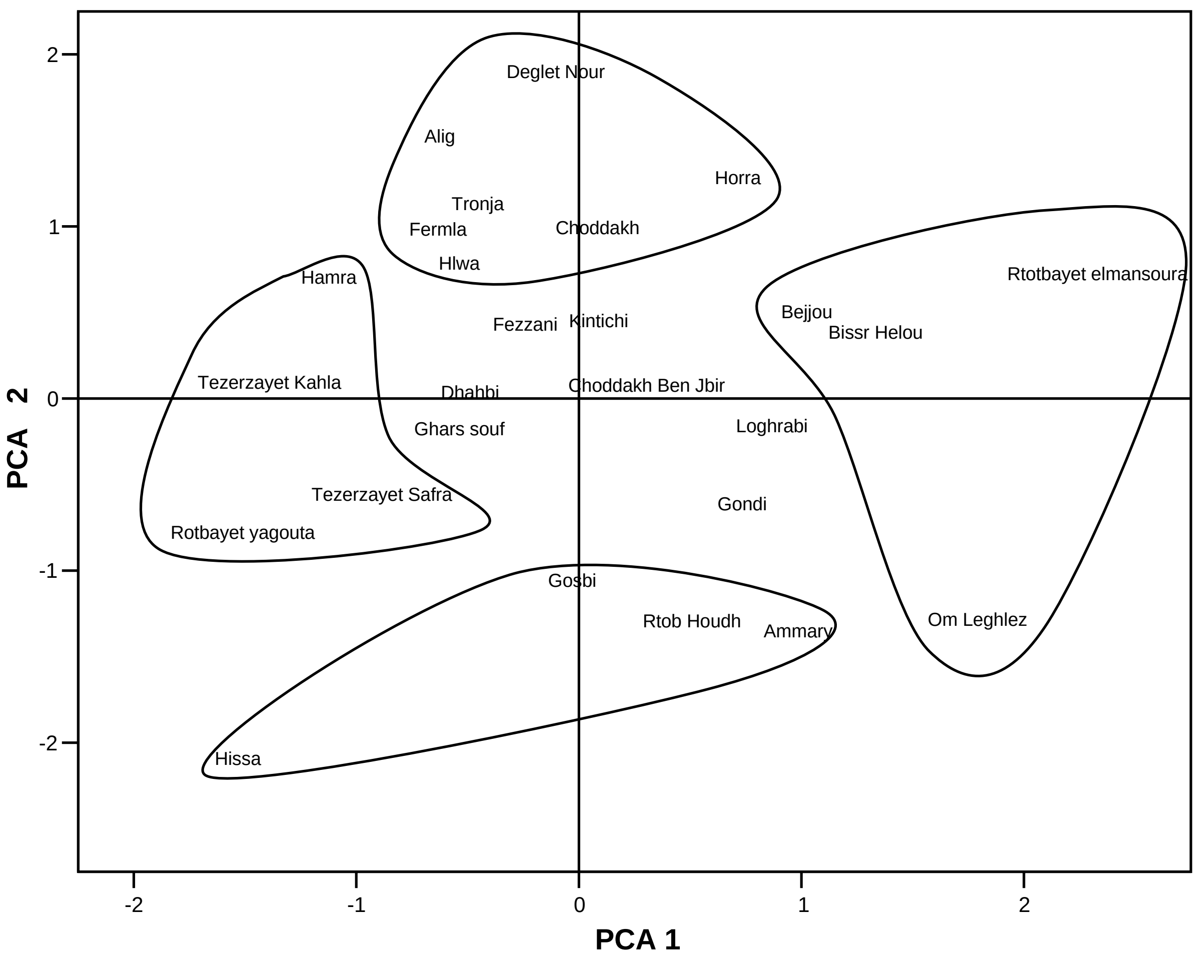

Fig. 3. Representation of date palm cultivars on the plane 1-2 of principal component analysis.

Major groups can be easily identified. The PCA results (Fig. 3) showed that the first axis opposes Rotbayet elmansoura, Besser Helou, Bejjou and Om Leghlez cultivar to Rotbayet yagouta, Tezerzayet kahla, Tezerzyet safra and Hamra one by PM16, EP8 and PN9 markers. The first cultivars are characterized by a high apical divergence angle, thick pinnae at the top leaf and a high maximal angle of spines but the others are characterized by a weak apical divergence angle, thin pinnae at the top leaf and a low maximal angle of spines. The second component analysis oppose Alig, Deglet Nour, Tronja, Fermla, Hlwa, Choddakh and Horra cultivar to Rtob houdh, Ammary, Gosbi and Hissa one by following attributes: PM9 and EP3. The first cultivars group has positive correlation with the second component analysis, so they are characterized by high percentages of spinned midrib part and weak percentages of solitary spines. The others cultivars have weak percentages of spinned midrib part and high percentages of solitary spines. The third axis did not clearly show a grouping of cultivars according to EP6 parameter.

This distribution showed that the geographical origin has not defined any quantitative morphological structure since no clearly geographical groups are showed. Indeed, we can distinguish some cluster of our cultivars according to their fruit characteristics. In fact, PCA result show grouping of cultivar based on harvesting period (Fig. 4a). So we can distinguish a cluster composed by Ammary, Hissa, Gosbi, Rotbyet yagota and Tezerzyet safra cultivar which have early maturities. These cultivars are opposed with the later maturity cultivars such as Deglet nour, Alig, Horra, Tronja, hlwa, Bejou, Kintichi cultivars. The Pearson coefficient showed significant correlation between the day of maturation with PM9 parameter (+0,834) and EP3 parameter (-0,524). It seems that the spinned midrib part can be used as an indicator of maturity: the higher its percentage is, the later maturity occurs. In addition, we can discern some other cultivar cluster according to their fruit consistency (Fig. 4b). The plan 1-2 show that the soft fruit cultivars, semi-dry fruit cultivars and the dry fruit cultivars are clearly separated but no clear correlation was identified with six selected parameters.

Discussion

Overall analysis of selected leaves characteristics shows a high diversity between cultivars. Certainly this phenotypic variability reflects a genetic diversity while the environment effect was eliminated. This diversity is due to genetic recombination during the sexual reproduction being given that each cultivar derives from a unique descended individual of seed, cloned thereafter by vegetative multiplication (Elhoumaizi *et al*., 2002).

The morphological studies of date palm have always been considered difficult to undertake because they require a large set of phenotypic data and because they are varied due to the environment effect (Munier, 1973). The present study has sifted morphological characters that are not controlled by edaphic or climatic factors. Several previously published data have indicated some morphological characters but the major has an adaptive response to the environment. Most of the previous work suggested important characters for the discrimination between cultivars by citing the length and the width of the palm, the length of pinnaes and their thickness, number of spines, spines angle and groups of spines. Only width pinnae of the summit may be withheld from these characters in the classification of cultivars. The invention of apical divergence angle (Nixon, 1950) is very useful; it showed a measure of insurance and distinction between cultivars. This has also been used by other researchers (Elbekr, 1972; Rizk & ElSharabasy, 2007). The spinned leaf part is often used in phenotypic studies (Peyron & Gay, 1988; Ben Salah, 1993; Rhouma, 1994; 2005; IPGRI, 2005). Our study showed a clear stability in the percentage of the spinned midrib part, it is more appropriate in the identification of cultivars.

The results of the present study indicates a high degree of independence between the geographical origin and morphological data. This is due to cultivars interchange between oases. This was also observed in other species cultivated in oases such as olive (Ouazzani *et al*., 1995) and pomegranate trees.

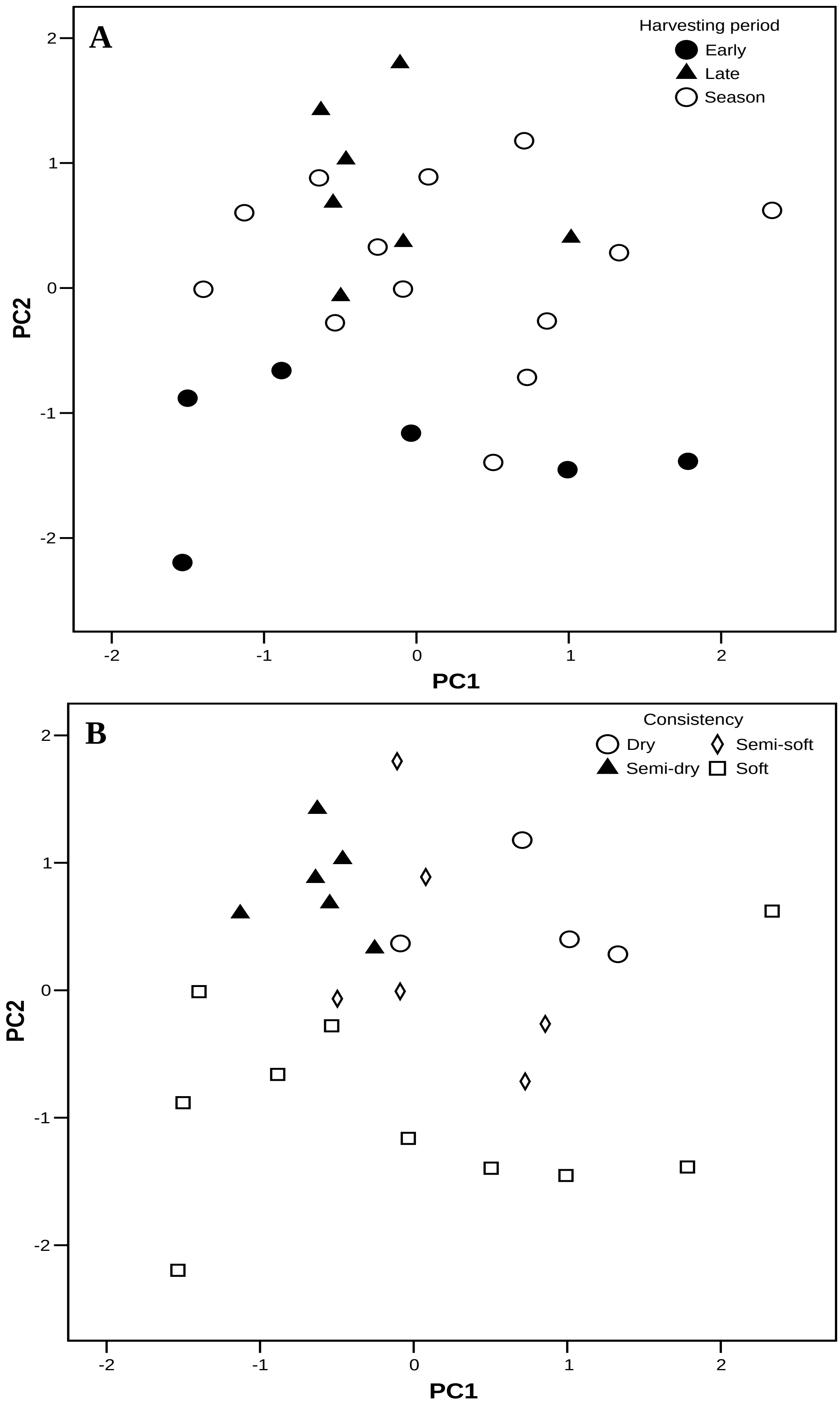

Fig. 4. Representation of fruit characteristics of the same cultivars on the plane 1-2 of the principal component analysis. (A: Harvesting period, B: Fruit consistency).

The majority of the phenotypic date palm studies are aimed at studying the spectrum genetic variation but they cannot allow definitive discrimination between cultivars, fruit quality and plant behaviour. However, the study of Elhoumaizi *et al*., (2002) highlighted a strong morphological resemblance between some date palm cultivar known for their resistance to Bayoud disease. Moreover, our study identified, for the first time, a stable date palm vegetative character that can be used as an indicator of fruit maturity and consistency. Indeed, for the Tunisian continental date palms, the percentage of the spinned midrib part is a good marker of the maturity period. Such information would certainly be useful especially for researchers, workers and for various programs such as cultivar selection and collection in these oases.

Date palm selection by peasants is often based on fruit characteristics. Hence, this study highlighted the relationship between vegetative and fruit characteristics which may be genetically related. Vegetative tools are so important in genotype evolution process; they are decisive in date palm cultivar selection and adaptation.

This method of taxonomy cannot resolve definitively the cultivar identification problem. In fact, many different populations may have the same aspect in spite of their different genotypes.

Future studies should consider the possible relations of other important phenotypic markers related to the tolerance towards oases stress. This should be backed up by others studies such as molecular ones to provide reliable tools for measuring genetic divergence.

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