Morphological Systematics of Date-Palm Diversity (*Phoenix*, Arecaceae) in Western Europe and Some Preliminary Molecular Results

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Abstract

In the Southeast of the Iberian Peninsula lies the largest traditional date-palm (*Phoenix dactylifera*) cultivation area, at the northern limit of this species in the western Mediterranean. Large palm groves extended traditionally around Elche, Orihuela, Albatera, and Abanilla with smaller groves scattered in wadis, ravines, or watered gardens from Almeria to Castellón. Around and within the city of Elche (Comunidad Valenciana) grow over 250,000 palm trees, belonging to different local cultivars. Most of them show morphological likeness to different north-African cultivars such as 'Medjool' or 'Deglet Nour'. However, the diversity is extremely high in terms of fruits, leaf shape and color, and stems, including minor local types. Furthermore, *Phoenix iberica* has been described as a wild species from the wadis near the sea. It has glaucous leaves, stout stems and small dates with thin flesh. It shows similarities to 'Medjool' or 'Barhee' cultivars in some vegetative characters, but its fruits are intermediate between those of *Phoenix theophrastii* and *P. sylvestris*. A group of cultivars well-known for its green leaves and small fruits, which normally ripen under the climate of SE Spain, has been described as *Phoenix chevalierii*. We are studying the diversity of date palms in Spain by nuclear microsatellite polymorphism, polymorphic ITS regions and chloroplast microsatellite patterns. Macro- and micro-morphological characters were studied using multivariate analysis techniques. Overall, these allow us to compare *Phoenix dactylifera* cultivars from Africa and the Near East, and related *Phoenix* species.

INTRODUCTION

The origins of the date-palm, *Phoenix dactylifera* L. and its relationships with wild species (e.g. *P. sylvestris* Roxb., *P. theophrastii* Greuter) are obscure. All *Phoenix* species are dioecious, with 2n = 36 (sometimes 32), and freely hybridize. North Africa (Zeven and De Wet, 1993), or Khuzistan, the Zagros Mountains, and the South of the Dead Sea basin (Zohary, 1983; Zohary and Hopf, 1994), or western India are postulated as centers of origin. However, in the western Mediterranean and North Africa, a wide range of date-palm wild relatives grows.

Date-palm seeds from the Cueva de los Tiestos (Archaeological Museum of Jumilla, Spain) are similar to those of *Phoenix iberica* D. Rivera, S. Ríos & Obón, while those from the cave of Peliciego might belong to *Phoenix dactylifera* (Rivera and Obón, 1993; Rivera et al., 1988). Pliny (Natural History 13, 26, 1st Century AD) mentions palms from the eastern coastal area of Spain with harsh fruits that did not ripen as sweet as those of Barbary (the coastal regions of what is now Morocco, Algeria, Tunisia, and Libya) (*ferunt in maritimis Hispaniae fructum, verum inmitem, dulcem in Africa*). It seems that the palm was also frequent in that time in Andalusia (Schulten, 1963). Figures of palms and palm trees are represented in Eastern Iberian-Levantine pottery (5th century BC). Date-palm introduction to Iberia is attributed to the Phoenicians or Greek merchants. The genetic diversity of palms in Spain is high as a result of the continuous multiplication by seed (Ferry, 1996). In Iraq and in the south of Egypt it is usually multiplied using basal offshoots. In the north of Egypt, approximately 2/3 of the palms are propagated from seed (Riyadh, 1996).

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Phoenix dactylifera is the type species of the genus *Phoenix*. Greuter and Jarvis designated a specimen of *Palma hortensis* of Kaempfer from Palestine as lectotype of the species (Jarvis et al., 1993). The dates represented by Kaempfer resemble those of the cultivar 'Barhee', which now is marketed from Israel, where it was reintroduced probably in 1924, from Egypt or Iraq (Abu-Qaoud, 1996).

Phoenix iberica was described from wadis and ravines of the Chicamo River basin. It has stiff glaucous leaves, stout stems and small dates with thin flesh. In vegetative characters, it resembles 'Medjool' or 'Barhee', but its fruits are intermediate between those of *Phoenix theophrastii* and *P. sylvestris*. However it is found also in cultivation in Elche and Fortuna (Spain).

The palm of Barbary, *Phoenix chevalierii* D. Rivera, S. Ríos & Obón is an Iberian-Moroccan Group of cultivars, with yellowish to dark green leaves (similar to *P. canariensis* Chabaud leaves), but not glaucous or waxy, with very long feathery leaflets, and less stiff acanthophylls compared with those of the date palm. The male flowers have smaller petals and much shorter anthers, and the fruits are smaller but of good quality. It is known in cultivation in the Atlantic zones of Morocco and in the Segura River basin. The Arabs of Marrakech distinguish the dates of this palm with the name of "*Abeló*", differentiating them from "*Temar*" or *Phoenix dactylifera* dates. Jewish populations, colonizing the flanks of the High Atlas probably introduced it (Chevalier, 1952a). The palm groves of Marrakech provide good, quite early-flowering pollinators (Haddouch, 1996). Haddouch (1996) calculates that there are more than four millions palms in the oases of Morocco belonging to over 200 local cultivars: circa 50% of them are in Ouarzazate. Nearly 15% of the production is dedicated to feeding livestock.

In the Cape Verde Islands grows *Phoenix atlantica* A. Chev., "*Tamareira*" in Portuguese, with wide trunks, short acanthophylls and small fruits (Chevalier, 1952a; Zeven and De Wet, 1993; Meunier, 1962). The genetic discontinuities revealed by microsatellite and minisatellite analysis support the recognition of *Phoenix atlantica* as a distinct species (Henderson et al., 2006). Microsatellite analysis separates *P. dactylifera, P. canariensis* and their putative hybrids in the Canary Islands (González et al., 2004; Gonzalez and Sosa, 2007).

Phoenix theophrastii Greuter (Vái, E. Crete, Greece) has small fruits, robust short acanthophylls and stiff glaucous leaflets (Greuter, 1967). It was also found in Prevali (Crete), Datça and Gölköy (Turkey). Fossil remains of this species (acanthophyll, fruit and leaflet casts in palaeosol, dated 37000 BP) were recovered from Weichselian interstadial strata on the Thera-Therasia islands (Santorini group, Greece) (Friedrich, 1980). Kislev et al. (2004) found one *P. theophrastii* seed in waterlogged strata of Atlit-Yam (Israel) dated c. 8000 BP.

Barrow (1998), using the intergenic spacer region of 5S DNA units described a cluster, which comprised *P. dactylifera*, *P. theophrastii*, and *P. sylvestris* with low resolution. Thus, deeper morphological and molecular studies are required to determine whether these species are clearly distinct and what is the status of the other related species not previously analyzed (*P. chevalierii, P. iberica, P. atlantica*).

MATERIALS AND METHODS

Morphological Study

For seed morphology we studied 62 samples belonging to 12 *Phoenix* species, 10 *Phoenix dactylifera* cultivars and 12 feral/wild populations. The seeds were acquired from different repositories in Europe. Seeds of wild species also came from botanical gardens, or were collected from wild populations. For vegetative and floral characters, we studied living specimens grown in our experimental collections (UMH), or in La Concepción (Málaga), in the nurseries Huerto del Cura (Elche), Ajauque (Abanilla), and Palmasur (Mutxamiel), in the Station Phoenix (Elche), the National Germplasm Repository at Riversides or wild populations. The available data underline the need to use living material as well as dried herbarium specimens for clearly determining relationships. The characters analyzed were those used by Barrow (1998), Chevalier (1935,1952a, b), Rivera et al. (1997), Greuter

(1967) and some from the date palm descriptors (IPGRI, 2006). There were 45 binary qualitative characters and 37 quantitative characters.

Herbarium specimens are kept at the herbarium UMH (Universidad Miguel Hernández, Spain).

Only the 36 qualitative binary characters occurring in 20% to 80% of OTUs were used for the multivariate analysis. Categorical data (ecological and morphological) were transformed into a 0-1 matrix: the matrix had 35 rows (OTUs) and 36 columns (categorical variables). Different cultivars of the same species were treated as independent OTUs. From this matrix a Multiple Correspondence Analysis (MCA), a principal component analysis with categorical data (Benzecri, 1992) was performed.

The 37 quantitative characters were analyzed, using the standardized average values for each, through Principal Component Analysis (PCA), see Figure 2 and Correspondence Analysis (CA).

This analysis was performed with "R" software (Ihaka and Gentleman, 1997). We used subroutines based on SPAD (Lebart and Morineau, 1985) and routines implemented by Palazón and Calvo (1999).

The Hierarchical Cluster Analysis (HCA) was done with the OTUs of the quantitative matrices above, considering Ward's minimum variance algorithm (Ward, 1963; Lebart et al., 1984). This technique optimizes the result and assists the researcher in interpreting the single tree produced by the analysis instead of multiple trees.

Molecular Analysis

DNA extraction was performed with fresh material following Doyle and Doyle (1991) with some minor modifications. Recalcitrant samples were extracted using Qiagen DNeasy Plant Mini Kit.

Nuclear DNA analysis was carried out on the species in Table 1 (except *P. andamanensis, P. caespitosa* and *P. paludosa*) using 15 microsatellites and PCR reactions as described in Billotte et al. (2004), except for some PCR failing reactions for locus mPdCIR057, as well as the ribosomal ITS region, using primers ITS 5 and ITS4 (White et al., 1990).

ITS analysis was performed on the above species and successful amplification was obtained for all of them, including *P. acaulis* (Barrow, 1998), except for some *P. dactylifera* accessions, where only ITS1 region was amplified. In-depth analysis of the ITS region will be carried out in split reactions (ITS5 and ITS2; ITS3 and ITS4).

PCR amplifications were performed on a Perkin Elmer 2400, running 35 cycles of the following program: 45 sec at 95ºC, 45 sec at 52ºC and 1 min at 72ºC. PCR products were purified using QIAquick (Qiagen, California, USA) columns, following manufacturer's protocols and quantified in a 1% agarose gel stained with Ethidium bromide. PCR products were standardized to 20 ng/mcl.

Cycle sequencing reactions for the ITS region were performed using standard dideoxy cycle protocols for sequencing with dye terminators on ABI 310 automated sequencer (Applied Biosystems).

For microsatellites, forward primers were subsequently labeled with the fluorescent dyes, NED, HEX or 6-FAM (Applied Biosystems). Fluorescent dye selection was determined by the amplification product size of each primer pair, with primers amplifying products within the same size class being labeled with distinct dyes to enable subsequent characterization. Fragment analysis was performed on an ABI Prism 3130 and data resolved using Genotyper 2.5 (Abisystem).

RESULTS AND DISCUSSION

Morphological Study

The multivariate analysis discussed here is based on categorical variables (MCA / HCA, Figure 1 and MCA) and quantitative (PCA / HCA, Fig. 4 and CA / HCA). It illustrates close relationships between *Phoenix dactylifera, P. sylvestris, P. theophrastii, P.*

atlantica, P. canariensis, P. iberica and *P. chevalierii*. The clusters do not resolve *P. chevalierii* from *P. dactylifera* cultivars (Fig. 1), suggesting that it is most likely a distinct group of date-palm cultivars. However, *P. atlantica* and *P. iberica* appear together in a distinct cluster. The four samples of *P. theophrastii* clearly appear together in a single cluster, and separated from all *dactylifera* cultivars.

When the distribution of the different taxa is plotted on a map (Fig. 3), it is evident that all wild relatives of *P. dactylifera* are situated on the periphery of the main date-palm cultivation area. To the north *P. iberica* and *P. theophrastii*, to the east *P. sylvestris*, to the west *P. canariensis* and *P. atlantica* and to the south *P. caespitosa.*

Molecular Analysis

Molecular analysis has so far produced only preliminary results, which seem to confirm the major groups of Barrow (1998).

CONCLUSION

The first morphological analysis suggests that a thorough study of multiple characters (qualitative and quantitative) will help to define the limits between *Phoenix dactylifera* and related species. The combined use of morphology and molecular markers will most likely lead to a deeper understanding of the complex relationships between cultivars, feral and wild relatives.

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Tables

Table 1. Samples and accessions used for morphological, molecular analysis and carpology. Repositories: 1. USDA-ARS, National Germplasm Repository, Riverside (USA); 2. Palermo Orto Botanico (Italy); 3. Station Phoenix (Elche, Spain); 4. Viveros Huerto del Cura (Elche, Spain); 5. Viveros Ajauque (Abanilla, Spain); 6. Palmasur (Muchamiel, Spain); 7. National Phoenix collection, Universidad Miguel Hernández (Orihuela, Spain); 8. Universidad de Murcia (Murcia, Spain); 9. Palmiye Merkesi (Mugla, Turkey); 10. Universita d. Studi Firenze (Italy); 11. Data from Barrows (1998).

Species	Varieties	Cultivars	Seeds	Seed samples	Accessions	Repositories
1. *Phoenix acaulis*	-	-	45	2	6	7
2. *Phoenix andamanensis*	-	-	0	0	1	7
3 *Phoenix atlantica*	-	-	15	2	31	7
4. *Phoenix caespitosa*	-	-	0	0	1	10
5. *Phoenix canariensis*	-	-	60	3	300	7, 8
6. *Phoenix canariensis*	-	Porphyrocarpa	25	1	2	8
7. *Phoenix chevalierii*	-	-	10	1	1	7
8. *Phoenix dactylifera*	-	-	85	3	40	8
9. *Phoenix dactylifera*	-	Candits	25	1	2	3, 4
10. *Phoenix dactylifera*	-	Maurs	25	1	2	4
11. *Phoenix dactylifera*	-	Sayir	25	1	1	7
12. *Phoenix dactylifera*	-	Maduros	50	2	2	5, 7
13. *Phoenix dactylifera*	-	Abu Faqqus	100	1	1	7
14. *Phoenix dactylifera*	-	Deglat Nour	100	1	1	3, 7
15. *Phoenix dactylifera*	-	Zahidi	100	1	1	1, 7
16. *Phoenix dactylifera*	-	Ghars	100	1	1	7
17. *Phoenix dactylifera*	-	Barhee	50	1	1	1, 7
18. *Phoenix dactylifera*	-	Medjool	50	1	1	1, 3, 7
19. *Phoenix iberica*	-	-	10	2	2	5, 7
20. *Phoenix loureiroi*	*humilis*	-	120	2	1	7
21. *Phoenix loureiroi*	*loureiroi*	-	0	0	3	7
22. *Phoenix loureiroi*	*pedunculata*	-	50	1	2	7
23. *Phoenix paludosa*	-	-	45	2	1	1, 7
24. *Phoenix pusilla*	-	-	140	2	5	1, 7
25. *Phoenix reclinata*	-	-	145	3	1	1, 2, 6, 7
26. *Phoenix roebelinii*	-	-	230	7	5	2, 7, 8
27. *Phoenix roebelinii*	-	Mekong	10	1	1	7
28. *Phoenix rupicola*	-	-	55	3	4	2, 7
29. *Phoenix sylvestris*	-	-	245	6	4	1, 6, 7
30. *Phoenix sylvestris*	-	Robusta	25	1	1	7
31. *Phoenix sylvestris*	-	Round Seeds	20	1	3	7
32. *Phoenix theophrastii*	-	Datça	100	2	3	7, 9
33. *Phoenix theophrastii*	-	Gölköy	100	1	3	7, 9
34. *Phoenix theophrastii*	-	Vai	43	3	2	2, 6, 7
35. *Phoenix theophrastii*	-	Prevali	45	2	1	6, 7
		Totals	**2248**	**62**	**437**	

Figures

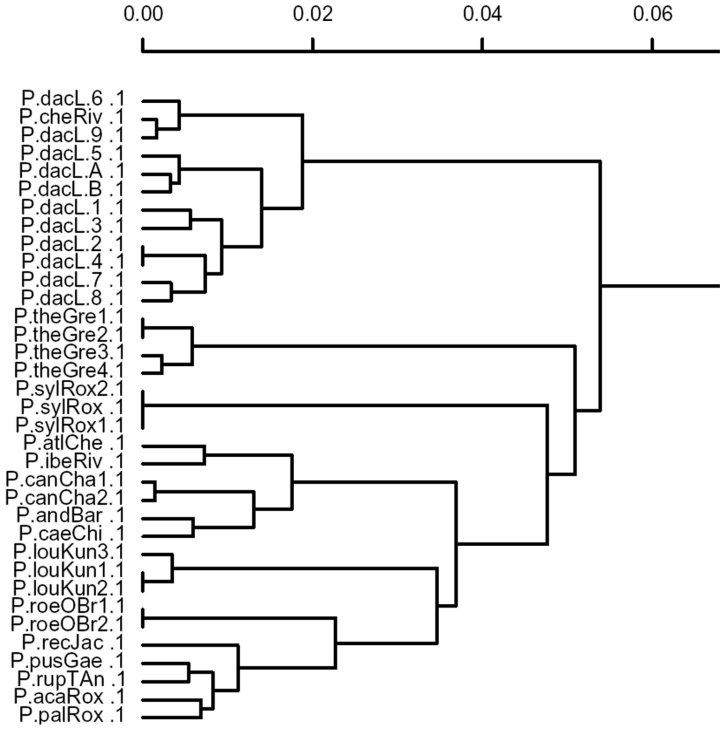

Fig. 1. *Phoenix* species and cultivars. Tree resulting of the MCA / HCA of 35 OTUs using the 36 qualitative categorical characters analysed.

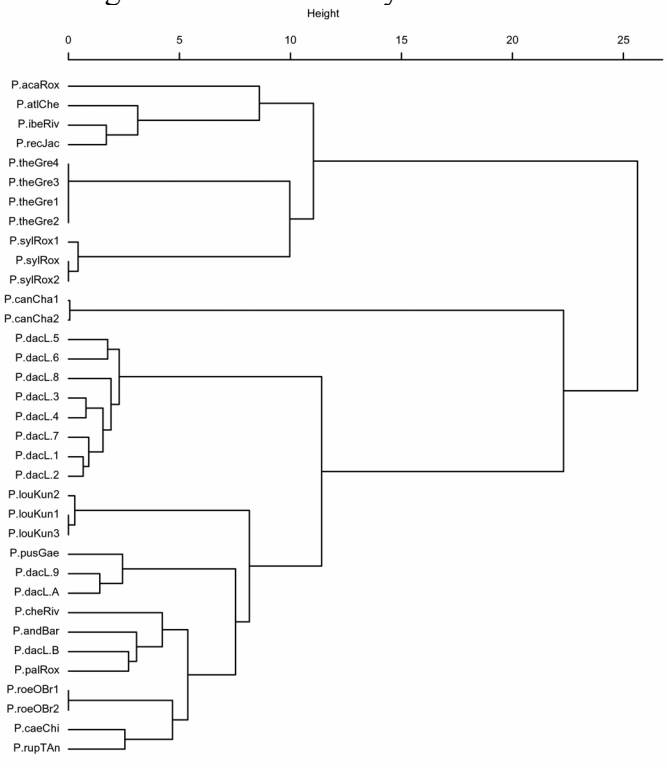

Fig. 2. *Phoenix* species and cultivars. Tree resulting of the PCA / HCA of 35 OTUs using the 37 quantitative characters analysed.

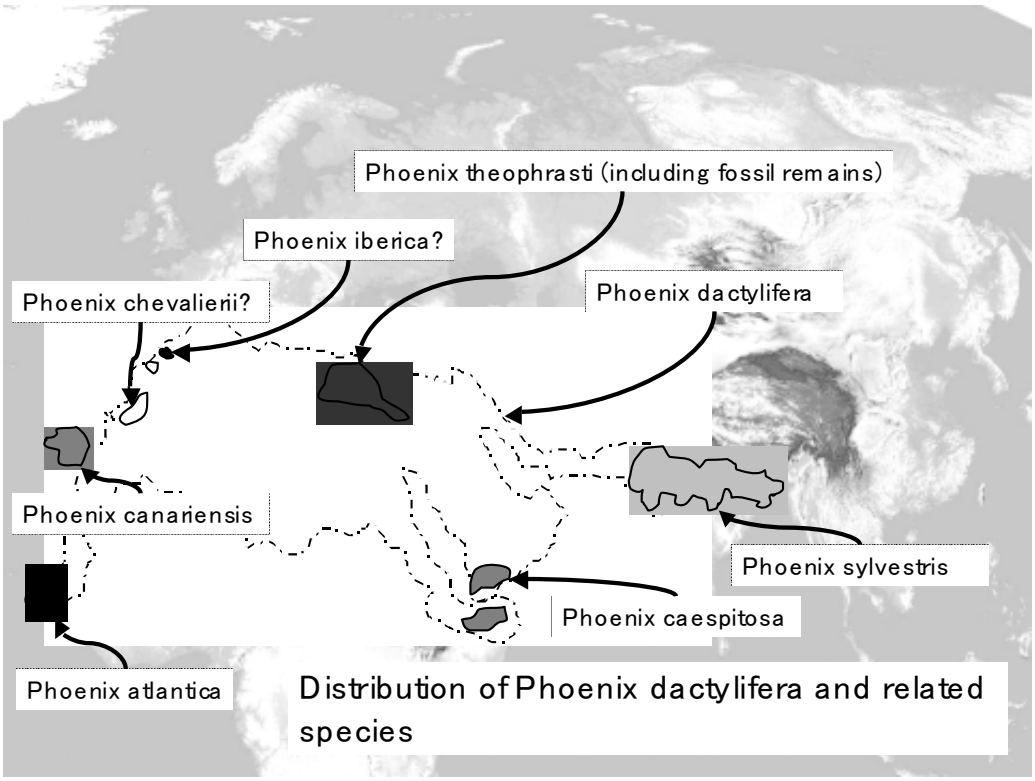

Fig. 3. Distribution of *Phoenix dactylifera* and related species (from Rivera et al., 1997; Barrow, 1998).