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REVIEW ARTICLE

Hybridization in the genus *Phoenix*: A review

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Abstract

The genus *Phoenix* is composed of 14 species naturally distributed in the Old World. This genus comprises the date palm, *Phoenix dactylifera* L., cultivated for its fruits, the dates, while other species are grown for food, ornament and religious purposes. *Phoenix* species were, for these reasons, spread out of their natural distribution area. It is therefore common to find species not naturally sympatric, growing together, in cultivation or in the wild. *Phoenix* species are interfertile and crossing distinct species leads to fertile hybrid offspring (interspecific hybridization). The introduction of a species in the wild generates gene flows leading to the creation of new hybrids and has conservation implications. In cultivation, such crossings may be spontaneous or are the result of artificial pollination, as several reasons impel doing so. Crossing gives rise to beautiful hybrids and is also useful for the conservation of old palm groves threatened by pests. Moreover, artificial pollination of date palms using another *Phoenix* species can be of interest given the metaxenic pollen effects. In addition, this process may have some potential benefits in date palm improvements, by the creation of hybrid cultivars. Thus, an increasing need of hybrid detection and characterization exists, particularly as morphology alone is not sufficient for this task. Besides new methods such as traditional and geometric morphometrics that may bring new clues, the advent of genetic and molecular markers helps to detect hybrids, especially based on the combination of nuclear and chloroplastic data. The application of methods such as near-infrared reflectance spectroscopy is currently under examination to estimate their potential use for hybrid characterization.

Key words: *Phoenix*, Date palm, Hybridization, Metaxenia, Varietal improvement

1. Introduction

The genus *Phoenix* belongs to the Arecaceae or Palmae family. It is composed of 14 species, the most famous being the widely cultivated date palm, *Phoenix dactylifera* (Figure 1a, WCSP, 2013). *Phoenix* palms fulfil various roles, from food and religion to construction and ornamentation (for review see Munier, 1973 and Barrow, 1998). The date palm is cultivated mainly for its fruits but it also provides a favorable environment for the cultivation of other species such as olives, figs, vegetables and so on in oasian agrosystems (Tengberg, 2012). The sap of *Phoenix sylvestris* (Figure 1b) is boiled down to produce a sweet juice (Newton et al., 2013). Several species are used for ornamentation especially *P. canariensis* and *P. roebelenii* (Figure 1c). Leaves of the date palm, *P. canariensis* or *P. theophrasti* are essential for Palm Sunday celebrations in the Christian religion.

Phoenix species are naturally distributed in the Old World (Figure 2). According to their distribution area and habitat (Munier, 1957; Barrow, 1998; Henderson, 2009), some species may probably be found together in the wild (sympatric areas). Additionally, given the different purposes described above, the species were dispersed out of their natural ranges creating artificial sympatric areas. *Phoenix* species are interfertile and the crossing of two distinct species leads to fertile offspring, most of the time (Corner, 1966; Greuter, 1967; Hodel, 1995; Wrigley, 1995). Interspecific hybridization is the process whereby two distinct species cross, creating hybrid individuals. Gene flow, i.e. the incorporation of a gene from one species to the gene pool of another, is called introgression and is the result of interspecific hybridization.

In this review, an overview of the genus *Phoenix* is given with a focus on the natural and artificial distribution of the species. It is followed by the description of the different kinds of interspecific hybridization events that can occur in the genus. The implication in term of conservation and varietal improvement are discussed. To conclude, the different methods that can be employed to detect and characterize hybrids are described and appraised.

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Figure 1. Some *Phoenix* species: (a) *P. dactylifera* (Photo: M. Gros-Balthazard), (b) *P. sylvestris* (Photo: S. Ivorra), (c) *P. roebelenii* (Photo: M. Gros-Balthazard), (d) *P. atlantica* (Photo: Henderson et al., 2003).

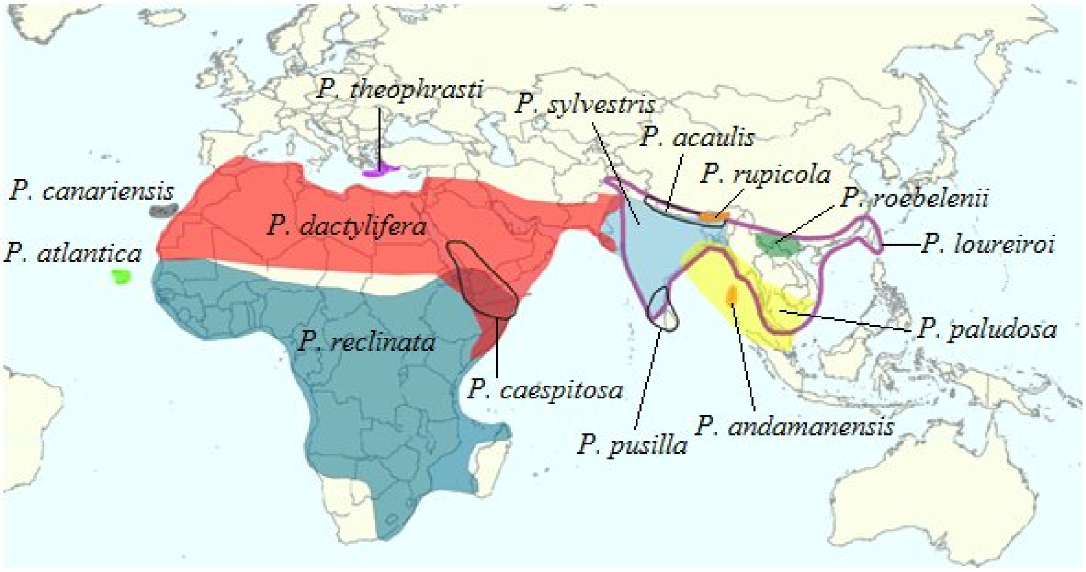

Figure 2. Distribution of the *Phoenix* species.
(Map by M. Gros-Balthazard based on Munier, 1973; Barrow, 1998; Henderson, 2009).

2. Overview of the genus *Phoenix*

The genus *Phoenix* belongs to the Arecaceae (Palmae) family, Coryphoideae subfamily and is the only genus in the *Phoeniceae* tribe. It is composed of 14 species (Govaerts and Dransfield, 2005; Henderson et al. 2006; WCSP, 2013) distributed in the Old World (Figure 2). All species are dioecious with male individuals bearing staminate flowers and female individuals pistillate flowers. The female flower is composed of 3 carpels. After pollination, only one will develop into a fruit while the other two abort. Without pollination, there is no fruit development or sometimes, fruit develops but it is parthenocarpic, that is it does not contain seed, is small and does not reach full maturity. *Phoenix* species share unique morphologic characteristics in the Arecaceae family, making the genus *Phoenix* the most distinctive of all palm genera. Leaves are pinnate; leaflets are V-shaped in cross-section (induplicate) and at the leaf base are modified as sharp spines called acanthophylls. At the molecular level, the genus *Phoenix* also appears highly divergent from other palms but it remains hardly classifiable in phylogenetic analysis (Asmussen and Chase, 2001; Hahn, 2002; Baker et al., 2009).

2.1. Interspecific relationships in the genus *Phoenix*

Within the genus, species are morphologically close to each other, sometimes hardly distinguishable (Barrow, 1998; Henderson et al., 2006; Pintaud et al., 2010). Thus, the number of recognized species has changed during recent decades. In the last monograph, Barrow (1998) identified 13 species. *Phoenix atlantica* (Figure 1d), which is morphologically very close to the date palm, was later distinguished as a distinct species (Govaerts and Dransfield, 2005; Henderson et al., 2006), bringing the number to 14 species. Two other species, supposedly endemic to southern Spain, have been described: *Phoenix iberica* and *P. chevalieri* (Rivera et al., 1997). However, these species are not recognized by the World Checklist of Selected Plant Family of Kew and considered synonyms of *Phoenix dactylifera* (WCSP, 2013).

The interspecific relationships within the genus *Phoenix* remain poorly understood. Based on morphological, anatomical and molecular data (5S spacer sequences), Barrow (1998) identified one clade comprising *P. dactylifera*, *P. sylvestris*, *P. canariensis* and *P. theophrasti*. Another study based on nuclear and chloroplastic microsatellites and minisatellites does not reach this conclusion and although it allows distinguishing species, affinities are still speculative (Pintaud et al., 2010). A new study based on chloroplastic sequences (psbZ-trnM and rps3-rpl16) permits, for the first time, discussion of the phylogeny and the biogeography of the genus (Pintaud et al., 2013; Gros-Balthazard et al., unpublished data). The so-called date palm clade is identified as comprising *P. dactylifera*, P. *sylvestris*, *P. atlantica*, *P. canariensis*, *P. theophrasti* and *P. rupicola*. The date palm appears most closely related to *P. sylvestris* and *P. atlantica*. Although this study constitutes a great advance, the *Phoenix* phylogenetic relationships remain to be investigated with more variable molecular data such as nuclear sequences.

2.2. Distribution of *Phoenix* species

The genus *Phoenix* likely originates from Eurasia as attested by *Phoenix*-like fossils dating back to the Eocene (Munier, 1973; Dransfield et al., 2008). Today, *Phoenix* species are distributed in the tropical and subtropical regions of the Old World, in southern Europe, Africa and South Asia (Figure 2). They grow in a wide range of habitats, from sea level up to 2,000 meters, but all require moisture around the roots. For instance, *P. reclinata* and *P. roebelenii* grow in seasonally-inundated areas, *P. paludosa* is found in mangroves while *P. dactylifera* grows in oases in hot, arid deserts. According to the different reports of the geographic ranges and habitats of *Phoenix* (Barrow, 1998; Henderson, 2009), it is likely that contact zones between different *Phoenix* occur (Figure 2). For instance, *P. loureiroi* and *P. acaulis* are both reported in Northern India and Nepal in similar habitats (open forest and pine forest) according to Henderson (2009). Additionally, three species (*P. dactylifera*, *P. caespitosa* and *P. reclinata*) are mentioned in southern Arabia and the Horn of Africa (Barrow, 1998). The date palm is reported in mixture with *P. sylvestris* in India, sometimes in the same field (Newton et al., 2013). Nonetheless, it is important to note that the natural distribution of the date palm is unknown as the long history of its cultivation has likely extended its original distribution (Barrow, 1998). The area represented in Figure 2 corresponds to its *historical* or *traditional* cultivation area, where it has been cultivated for centuries or even millennia. Thereby, sympatric areas of date palm and other *Phoenix* should be regarded with caution as they may not be natural. To conclude, as far as I know, no natural sympatric area between *Phoenix* has been noted to date. This may be a reality or an artefact due to lack of intensive research and the difficulty to differentiate species.

Due to their many uses, *Phoenix* species have been dispersed from their original distribution area. As mentioned above, the date palm was introduced in many regions for fruit production, as far as California and Australia. For instance, the date palm was introduced in the Italian Riviera during the Middle Ages for religious purposes (Castellana, 1998). *Phoenix roebelenii* and *P. canariensis* are of great ornamental value and therefore are grown in many botanical gardens around the world (Figure 1c). In this way, species that are not sympatric in the wild are brought into contact; such areas are here referred as anthropogenic or artificial zones of contact. They exist in cultivation, especially in botanical and ornamental gardens such as in San Remo gardens and in the *Phoenix* collections of the U.S. Department of Agriculture in California (Figure 3). Additionally, *Phoenix* spp. may be introduced into the wild where a native species naturally grows. This is the case in the Canary Islands where *P. canariensis* is endemic, whereas the date palm was introduced, probably after the Spanish Conquest (Morales, 2007).

Figure 3. Artificial contact zones between *Phoenix*. (a) *P. dactylifera* and *P. acaulis* in the collection of the U.S. Department of Agriculture in California. (b) *P. theophrasti* and *P. rupicola* in San Remo.
(Photos: M. Gros-Balthazard)

3. Hybridization in the genus *Phoenix*

In the genus *Phoenix*, interspecific barriers appear to be very limited (Pintaud et al., 2010). When *Phoenix* species are brought into proximity, crossings occur (Corner, 1966; Greuter, 1967; Hodel, 1995; Wrigley, 1995). For most crossings, these hybrids appear fertile. However, this is not always the case, as reported by Sudhersan (2010): *P. dactylifera* fruits, obtained after pollination with *P. pusilla,* appear infertile as seed development is arrested at the Khalal stage (Figure 4). The ease with which *Phoenix* hybridize has produced a range of hybrids of different kinds. Crossing between very different species is possible such as the dwarf *P. roebelenii* and the imposing *P. canariensis* (Figure 5).

This ability to hybridize sometimes leads to misinterpretation of species. For instance, the cultivated date palm was thought to be of hybrid origin **(**Munier, 1973**)** as well as *P. atlantica* (Barrow, 1998) while we now know that they are distinct pure species (Henderson et al., 2006; Pintaud et al., 2010**).** On the contrary, many taxa (species or varieties) described are invalid and refer to putative hybrids of *Phoenix* (Barrow, 1998). This is the case of *P. macrocarpa* and *P. intermedia* which refer to hybrids between *P. dactylifera* and *P. canariensis* (Beccari, 1890). Introgression is so common that it is sometimes questionable what the true species is supposed to look like as reported by Bergman (2005) concerning *P. roebelenii*.

Here, we differentiate three types of interspecific hybridizations. First, the possibility of interspecific gene flows in natural sympatric areas is treated. In the second part, the spontaneous hybridization events between *Phoenix* in anthropogenic contexts are addressed. Finally, hybridization events achieved by artificial pollination are discussed.

3.1. Natural interspecific hybridization

As mentioned above, it is possible that contact zones of different *Phoenix* exist (Figure 2) but none has been clearly identified to date. Consequently, gene flows between different species have never been reported in nature. This may be due to an artefact induced by a lack of research to identify natural contact zones. Additionally, *Phoenix* species are sometimes hardly distinguishable and the identification of hybrids is therefore challenging (see section 4, Detection and characterization of hybrids). When species are sympatric, their integrity, that is the avoidance of interspecific gene flows, may also be preserved by temporal isolation. Indeed, even in case of contact, gene flows are impossible if males of a species and female of another do not flower at the same time. Interspecific hybridization events in natural context remain to be carefully assessed with first the identification of natural sympatric areas followed, if existing, by the identification of hybrids or by the processes limiting hybrid occurrence.

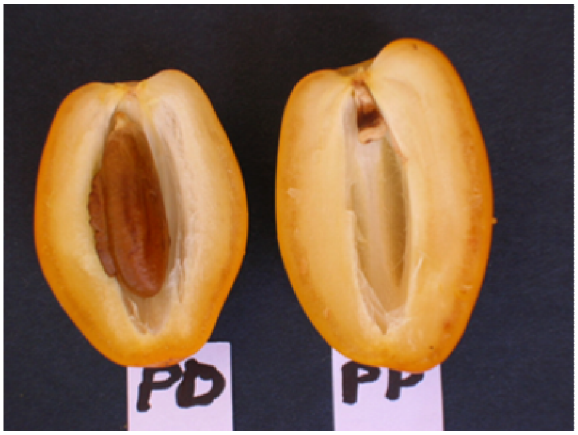

Figure 4. Fruits of Medjool cultivar. On the left, after pollination by *P. dactylifera* (normal seed). On the right, after pollination by *P. pusilla*. The seed appears very small and rudimentary.
(Photo: Sudhersan, 2010)

Figure 5. Hybrid *P. canariensis* x *P. roebelenii* in a garden of Medellin, Colombia.
(Photo: J.-C. Pintaud)

3.2. Spontaneous hybridization in anthropogenic contexts

Spontaneous gene flows are possible if one male of a species and one female of another flower at the same time and are close enough for natural pollination (wind and insect). The female thus bear fruits containing seeds of hybrid origins. This type of spontaneous event is possible in cultivation but also in the wild.

In the Italian Riviera, there are many hybrids (Figure 6) that appear to be adapted to local conditions (Pintaud, comm. pers.). For several years now, the ornamental groves of this region are under threat by the introduced red palm weevil (*Rhynchophorus ferrugineus*). The hybrid populations could be used to regenerate the plantations with resistant genotypes and therefore appear very useful for ornamental palm grove management.

In the wild, spontaneous hybridization events have been noted in the Canary Islands between the introduced date palm and the endemic *Phoenix canariensis* (Figure 7, González-Pérez et al., 2004a; González-Pérez and Sosa, 2009). The authors stipulate that this poses a clear threat to the survival and conservation of the endemic species. Indeed, the genetic purity of the local *P. canariensis* is endangered and introgressed populations are at risk of outbreeding depression (i.e. loss of adaptation) or genetic assimilation. Furthermore, gene flow impacts conservation programs, as not only introgressed population should be the target of conservation programs. In the Cape Verde Islands, the date palm has also been introduced and is a threat to the endemic *P. atlantica*, a subject that remains to be elucidated (Henderson et al., 2006).

3.3. Hybridization by artificial pollination: purposeful hybrids

Purposeful hybrids are obtained by artificial pollination. Manual pollination consists of collecting male inflorescences and pollen and their application to female flowers. Therefore, it may concern two individuals that are spatially close to each other but not necessarily given that pollen may be transported. Moreover, temporal interspecific barriers can also be broken (while this is not the case for spontaneous hybridizations) as the pollen can be stored for months. Several reasons impel artificial production of *Phoenix* hybrids.

The result of interspecific hybridization gives different phenotypes, and crossing of distinct species is a subject of curiosity. Hybrids are unique phenotypes and some may be of great ornamental values, demonstrated by their presence in private and public gardens (Figures 5, 6).

In fruit cultivation, female date palms are manually pollinated using pollen usually from the closest male tree. This improves the pollination efficiency and therefore fruit yield, and decreases the number of males in cultivation (2-3%) therefore giving more space in a plantation to the female fruit-bearing palms (Swingle, 1928). The pollen appears to have an influence on the characteristics of fruit and seed although these tissues are of maternal origin; this is called metaxenia (Swingle, 1928; Sedgley and Griffin, 1989). This is probably due to hormones or soluble substances produced by the embryo or the endosperm or both which diffuse into these maternal tissues and affect them (Swingle, 1928).

Figure 6. *Phoenix* hybrids in Bordighera, Italy (a) *P. canariensis* x *P. dactylifera*, (b) *P. reclinata* x *P. canariensis*.
(Photos: J.-C. Pintaud).

Figure 7. Presumed hybrid *P. canariensis* x *P. dactylifera* in the wild, in the Canary Islands.
(Photo: P. Sosa)

Different metaxenia experiments have been carried out. They consist of pollinating different inflorescences of a female with pollen of different males and comparing the resulting fruits and seeds. The physical properties like size and shape may be evaluated, as well as the time of ripening, yield and chemical properties. Most of these experiments employ only male date palms (Nasir et al., 1994; Al-Khalifah et al., 2006; Al-Muhtaseb and Ghnaim, 2006). However, some used the pollen of other *Phoenix* spp. (Nixon, 1928a, 1928b; Ahmad et al., 1962; Sudhersan et al., 2010). Different species have been tested: *P. canariensis* (Nixon, 1928a; Ahmad and Ali, 1960), *P. roebelenii* (Nixon, 1928b), *P. sylvestris* (Ahmad et al., 1962), *P. loureiroi* (Ahmad and Ali, 1960) and *P. pusilla* (Sudhersan, 2010).

Pollen appears to have an effect on the size of fruits (Nixon 1928a; Ahmad et al., 1962). Nixon (1928a) showed that fruits and seeds of Deglet Noor are smaller when pollinated by *P. canariensis*. The same result was observed when the pollen of *P. loureiroi* was used (Ahmad et al., 1962). To the contrary, fruits are bigger when *P. sylvestris* pollen was used (Nixon, 1935). The shape of fruits differed from oval when pollinated by date palm to dumbbell-shaped when pollinated by *P. pusilla* (Sudhersan, 2010). Pollen also affects the shape of seeds (Nixon 1928a). According to Nixon (1928a), the seed color changes when *P. canariensis* is used as a source of pollen, compared to date palm pollen; whereas Sudhersan (2010) did not notice any color change in fruits pollinated by *P. pusilla*. Ripening time is also influenced by pollen. When pollinated by *P. roebelenii*, *P. canariensis*, or *P. pusilla*, fruit ripening is delayed (Nixon, 1928a; 1928b; Sudhersan, 2010) while the fruits ripen earlier when pollinated with *P. loureiroi* (Ahmed, 1962) compared to *P. dactylifera*-pollinated palms. It is important to note that the response to various pollens depends on the cultivar (Sudhersan, 2010). For instance, the pollen of *P. pusilla* induces smaller fruit in Barhee cultivar while they are of equal size in Medjool (Sudhersan, 2010). Very interestingly, the seed stops growth in fruits developed from *P. pusilla* pollen (Figure 4) and this result was observed in the three cultivars tested (Barhee, Medjool, Sultana) (Sudhersan, 2010).

It thus appears that the choice of the species from which the pollen is extracted for pollination is of importance in date palm cultivation. It is possible to have larger fruits (with *P. sylvestris*, Nixon, 1935). Additionally, the possibility to impact ripening time is of interest as it would be possible to speed up or delay the time at which dates are fully mature according to the market demand. Last but not least, the formation of seedless fruits using *P. pusilla* pollen (Sudhersan, 2010) appear very promising as consumers prefer these types of fruits since seeds are hard and inedible.

Date palm propagation is mainly achieved by cloning, which is the planting of offshoots that are clones of the mother plant and therefore give individuals bearing the same fruits. The planting of seeds presents several disadvantageous. Indeed, the sex ratio is 50/50 and the sex identification is possible only after the first flowering at 5 or 6 years of age. Moreover, the fruits are of the same quality or better than the mother plant in only 4% of the individuals (Peyron, 2000). This explains the preferred way of propagation using cloning. However, this decreases the genetic diversity within the species and thus its capacity of adaptation to disease or climate change. The creation and selection of new cultivars presenting great organoleptic characteristics or disease resistance necessitate sexual reproduction. When the date palm is pollinated by other *Phoenix* species, it yields fruits containing hybrid seeds that if planted give hybrid individuals. Interspecific gene flow is a major source of genetic variation that counterbalances the detrimental effects of genetic drift observed in clonally propagated crops (Lynch, 2010). Such gene flows have long been noticed in many crops (Ellstrand et al., 1999), especially perennial crops such as almonds (Delplancke et al., 2012) or grapevine (Myles et al., 2011). Concerning date palm, no hybrid cultivars have been reported so far. However, this could be due to the lack of genetic research involving not only the date palm, but other *Phoenix* as well. We know that the cultivated date palm is not of hybrid origin but derived from wild populations of the same species (Pintaud et al., 2010; Gros-Balthazard et al., unpublished data); nonetheless, the role of other *Phoenix* species during the diffusion of the phoeniciculture remains to be investigated. Additionally, the potential beneficial role of interspecific gene flows from wild *Phoenix* to the date palm needs to be assessed.

4. Detection and characterization of hybrids

Phoenix hybrids, when spontaneous, are hard to characterize since even distinguishing species is sometimes difficult. Speculation about the parent species is easier when the origin of the seed from which the palm emerges is known as it may be possible to identify the parents. However, in botanical gardens when several *Phoenix* are close to each other, the origin of the seed does not help. Moreover, they could be hybrid of several generations complicating the parental identification. In most situations, the identification of parents is therefore straightforward.

The recognition of hybrids and the characterization of the parent species are of interest for different purposes. In cultivation, some hybrids are of great ornamental value and their characterization could be directed toward interesting interspecific mating. The characterization of hybrids is also important to implement appropriate conservation programs. For example, as previously noted, the endemic *Phoenix canariensis* of the Canary Islands is a threatened species and there is a need to differentiate pure and introgressed individuals in order to target for conservation pure plant while eliminating introgressed ones (González-Pérez et al., 2004a).

4.1. Morphological and morphometric studies

The morphology of hybrids is a mosaic of intermediate parental traits (Rieseberg and Ellstrand, 1993). The size of the trunk, the color and size of the leaves and the display of the leaflets may be useful to identify hybrids. Another criterion is the ability to sucker. For instance, *Phoenix canariensis*-like palms that are suckering may be identified as hybrids given that the pure species is not suckering (Bergman, 2005). In this way, it is likely that botanists or genus *Phoenix* experts can distinguish hybrids of first generation between two very different species, e.g. *P. roebelenii* and *P. dactylifera*. Nonetheless, the criteria listed above are subjective and hybrids of several generations must be even harder to distinguish by morphology alone. Moreover, the variability is important within species, especially in *P. dactylifera* thus differentiating hybrids from ecotypes of a pure specimen based on morphology alone is challenging (González-Pérez et al., 2004a). The characterization using genetic markers of cryptic hybrids *P. canariensis* x *P. dactylifera* in the Canary Islands, while they were morphologically described as pure *P. canariensis*, illustrate the difficulty of differentiating hybrids using morphology alone.

Characterizing hybrids based on morphology should therefore be based on quantitative traits rather than qualitative ones. This is the field of morphometrics. Morphometrics is the study of the size and of the shape of an object. Recently, it was proven that *Phoenix* species might be differentiated based on their seed size (traditional morphometrics) and shape (geometric morphometrics) (Terral et al., 2012; Gros-Balthazard et al., unpublished data). This method appears of great interest since seeds are easily sampled and stored. Studies are currently underway at the Center for Bio-Archaeology and Ecology in Montpellier, France to test the suitability of seed morphometrics to identify the parents of hybrids. First results indicate that hybrids have seeds of intermediate size between their parents (Figure 8). The effect of hybridization on seed shape remains to be investigated. This technique has limitations because only females could be identified using seed morphometrics. Moreover, it is not suitable to characterize hybrids of several generations or hybrids of more than two species.

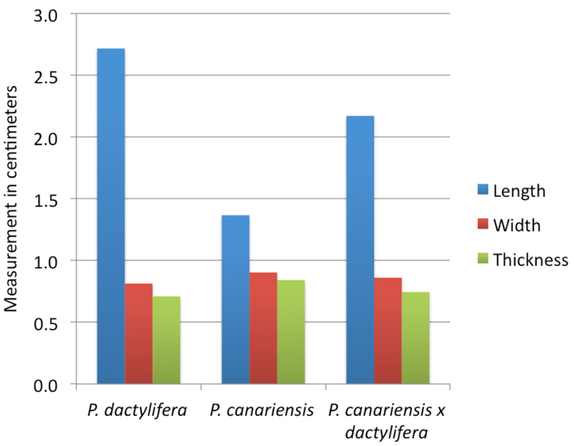

Figure 8. Seed measurement of *P. dactylifera* cv. Deglet Noor, *P. canariensis* and hybrid between *P. dactylifera* and P. *canariensis*. Length, width and thickness where measured over 20 seeds for each and averaged.

4.2. Molecular tools

A wide range of genetic markers are now available to detect hybrids based on DNA variations. They need to be variable between the potential parent species and to allow the characterization of pure species (exclusive molecular markers). For example, González-Pérez et al. (2004b) tried to identify *Phoenix dactylifera* x *P. canariensis* hybrids in the Canary Islands using allozymes but it appears unsuitable as both pure species have the same profile. Using Random Amplified Polymorphic DNA (RAPD) and isozymes, *P. canariensis* and *P. dactylifera* appear to have different profiles and hybrids were detectable (González-Pérez et al., 2004a; González-Pérez and Sosa, 2009).

Recently, there has been increased interest in the development of genetic markers for DNA barcoding in plants (for species and cultivars identification), especially microsatellite markers. These markers are highly variable and therefore suitable for hybrid identification and they have been widely used in the identification of hybrids in crop species such as rice (Gealy et al., 2002). A huge number of microsatellite primers for the date palm were described in recent years (Billotte et al., 2004; Akkak et al., 2009; Arabnehzad et al., 2012). They have not been tested so far for hybrid identifications but if they are transferable to other species (this is the case for microsatellites described by Billotte et al., 2004) they could be applied to achieve this goal. It is important to note that the combination of nuclear and chloroplastic data appears of interest for the following reason. The nuclear genome is inherited from both parents while the chloroplastic genome is maternally inherited. Thus, studying the nuclear profile allows identification of both parent species and reveals the degree of introgression (50% for a hybrid of first generation) while analyzing the chloroplastic genome allows identification of the mother species. There are some ongoing analyses regarding this subject at the Research Institute for Development in Montpellier, France (Pintaud, unpublished data). To conclude, the characterization of *Phoenix* hybrids using molecular markers, especially microsatellites, appears promising as primers are available and this is a lab routine work. However, it is essential to note that if one of the parent species is not included in the analysis, the hybrid cannot be detected. The implementation of hybrids detection through molecular markers thus necessitates inclusion of all potential parent species, or all Phoenix species when there is no prior information about parents and morphology is unhelpful.

4.3. Near-infrared reflectance spectroscopy (NIRS)

Molecular marker determination is expensive and requires hard work. Another possibility to identify hybrids relies on the Near-Infrared Reflectance Spectroscopy (NIRS) method which is less expensive. NIRS is based on the absorption of electromagnetic radiation by matter (Osborne et al., 1993). Leaves of a sample are dried and milled into powder and then subject to NIRS in order to obtain a spectrum. This spectrum is characteristic of individual species (Atkinson et al., 1997) and even varieties in coffee for instance (Downey and Boussion, 1996). The method appears useful for differentiating hybrids of *Betula pubescens* and *B. pendula* (Atkinson et al., 1997) and is being tested at the Research Institute for Development in Montpellier, France for characterization of *Phoenix* hybrids. Initial results appear very promising (Pintaud, unpublished data).

5. Conclusion and perspectives

Interspecific hybridization events in the genus *Phoenix* are very common and in most cases lead to fertile hybrids. The difficulties to distinguish species and their hybrids lead to misinterpretation of species and complication in resolving interspecific relationships.

In the wild and natural context, gene flows have not been reported so far but more research is required. In cultivation, when species are artificially brought into contact, spontaneous hybridizations may be useful, or at least not damageable. These events appear very common, so much so that according to Bergman (2005), to get a pure species, it is necessary to collect seeds in the wild and far from sympatric areas. Hybrids may be of great ornamental and conservational great value. In the wild, spontaneous gene flow from introduced to endemic species threatens endemic species and has conservation implications.

Crossing the date palm with other *Phoenix* is of great interest for its cultivation. Indeed, because of metaxenia, the selection of pollen from other species to pollinate females could improve yields, fruit size and even produce seedless fruits. More research and experiments in metaxenia are necessary to assess the effect of different male genotypes and understand the basis of these effects. This could lead to the selection of male cultivars specific to the pollination of given female cultivars. Moreover, other *Phoenix* appear as genetic reservoirs and hybrid cultivars could be of great interest in terms of cultivation and disease resistance and therefore more research should focus on it.

The interest in developing methods for detection of hybrids was discussed here. Several methods are available, especially molecular tools such as microsatellites, and others are being developed. This will help understanding hybridization in the genus *Phoenix*.

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