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Review article

Analysis of molecular marker-based characterization and genetic variation in date palm (*Phoenix dactylifera* L.)

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Abstract

Date palm breeding is challenging because of its long juvenility and dioecity. Genetic variation between cultivars is a pre-requisite to develop improved varieties. DNA fingerprinting is an effective method for date palm cultivar identification, examining genetic diversity and phylogenetic analysis. This review discusses the different markers used in DNA fingerprinting and phylogeny analysis of date palm varieties and the advances achieved. The date palm fingerprint analyses reported so far are neither comprehensive nor particularly clear because of variable variety nomenclature, a large number of uninvestigated new introductions, and uneven geographic sampling, which itself leads to inconsistent nomenclature. Most of the molecular markers utilized such as RAPD, RFLP, AFLP, ISSR and SSR have some limitations related to their cost, ease of use, robustness, dominance/co dominance and polymorphism level. Nuclear Microsatellite or (SSR) markers seem to fulfill most of the requirements to achieve accurate analysis of date palm fingerprints and phylogeny. The need for coordinated international, or at least regional, efforts to establish a comprehensive DNA fingerprint data set and phylogeny of all date palm cultivars is discussed in this review.

Keywords: Date palm, DNA fingerprints, Molecular markers, Phylogeny, Tissue culture.
Abbreviations: Amplified fragment length polymorphisms (AFLP), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphisms (RFLP), inter simple sequence repeat (ISSR), simple sequence repeats (SSR), tissue culture (TC).

Introduction

Date palm (*Phoenix dactylifera* L., 2*n*= 2*x* = 36) is a dioecious, perennial, monocotyledonous fruit tree that belongs to the family Arecaceae, and is of major socio-economic importance in west Asia and Africa not only for its fruit as staple food but also as an ornamental plant (Morton, 1987). The tree plays an important role in the development of sustainable agriculture in many drought and saline-affected regions of the world (Wellmann et al., 2007; Kurup et al., 2009). Date palm has been cultivated for at least 7,000 years and is believed to have originated in Mesopotamia (Wrigley, 1995). Its ancient dissemination around the Mediterranean coast lead to the development of new cultivars, including along the south-eastern coast of Spain, where commercial date culture developed by offshoot exchange between date producing countries and by means of seeds (Mahmud, 1958). Other current regions of cultivation include the desert valleys of south-central Asia, north Iran and Afghanistan, desert regions of Australia, South Africa, Namibia, and tropical and sub-tropical regions of the New World, from California to South America (Nixon 1951; Zaid & de Wet 2002a; McCubbin, 2007), and Australia (www.gurradown.com.au; www.canaryislandpalm.com.au). The number of known varieties distributed all over the world was reported to be approximately 5000 (Bashah, 1996), out of which 650 are cultivated in Iraq (Ibrahim, 2008), 340 in Saudi Arabia (Al-Mssallem, 1996) and 135 in United Arab Emirates (Ghaleb, 2008). Based on botanical descriptions, there are about 400 cultivars grown in Iran, 370 in Iraq, 244 in Morocco (Zaid & de Wet 2002c), 400 in Sudan (Osman, 1984) and 250 in Tunisia. Recently, United Arab Emirates has been officially recognized as the world's leading grower of date palms (FAOSTAT, 2009), with 42 million trees and a minimum of 200 cultivars, of which 68 are the most important commercially (Jaradat & Zaid, 2004). Date palm can reproduce both sexually and asexually. Its true-to-type propagation is traditionally obtained only through offshoots. This method maintains the genetic integrity of date palm cultivars. One limitation to this type of propagation is the minimum availability of offshoots. Numbers of produced offshoots vary greatly among cultivars with the typical range being 20-30. However, not all offshoots make enough roots to survive (Zaid & de Wet, 2002b). Seeds are breeding material with long backcrossing cycles. The first flowering of a seed-derived tree takes place at the age of about 5-7 years (Baaziz et al., 2000; Zaid & de Wet, 2002b). Therefore, the reproductive characteristics of the date palm render it difficult to breed for new cultivars or to compensate for the rapid decline of some cultivars due to natural disasters. Extensive efforts have been made to propagate date palms through tissue culture (TC) (Zaid & de Wet, 2002b; Al-

Khalifah & Askari, 2005; Al Kaabi, 2009; Al-Ruqaishi et al., 2008; Nitish et al., 2010). However, clonal propagation with TC techniques still has some uncertainties concerning the true-to-type nature of propagated material, especially when somatic embryogenesis and callus formation are considered. Somaclonal variation is the main constraint for TC-derived date palm as it is sometimes relatively high, and mechanisms causing these variations are unclear and under investigation (Djerbi, 2000; Gurevich et al., 2005). Reports on growth abnormalities in *in-vitro* derived date palm plants propagated by organogenesis include a wide range of traits, like failure to flower or fruit, dwarfness, loss of chlorophyll in leaves (albino), and crop failure (Al-Wasel, 2005; Al Kaabi et al., 2005). Hence, there is a need for a much expanded and integrated analysis of date palm germplasm. Date palm production is encountering serious problems such as low yields due to the lack of sufficient research to develop improved cultivars, the spread of pests and disease, and marketing constrains (El-Juhany, 2010). Thus, genetically improved cultivars need to be identified. With the advent of DNA fingerprinting, low cost and effective solutions to these problems are foreseeable. Because of its cross pollination, date palm progeny are highly diverse (Fakir & Munier, 1981). Morphological markers based mainly on fruit characteristics (shape, weight, colour, skin aspect, consistency and texture) plus the morphology of leaves and spines, have been used to describe many varieties. However, using morphological traits alone, discrimination among closely related cultivars is often unreliable, especially because of the influence of environmental conditions (Elhoumaizi et al., 2002). Hence, DNA typing has proven to be the most convenient method for accurately identifying date palm cultivars and for analyzing their genetic diversity and phylogenic relationships. DNA fingerprinting in plants is primarily used for identification of gene diversity, protection of biodiversity or germplasm conservation, and identifying markers associated with specific traits. Genetic preservation is dependent on understanding the amount and distribution of the genetic diversity present in the existing germplasm (Jubrael et al., 2005). Moreover, because of the availability of sequences of the nuclear (Al-Dous et al., 2011) and chloroplast (Yang et al., 2010) genomes of date palm, several molecular marker systems have become more effective tools for the assessment of genetic diversity for germplasm conservation and utilization of true-to-type elite material (Khierallah et al., 2011). These sequences will also empower studies of somaclonal variation of TC-derived materials (Saker et al., 2000, Al Kaabi, 2009). This review discusses the different markers used in DNA fingerprinting of date palm varieties and the advances achieved.

Molecular marker-based characterization (DNA fingerprinting) of Date Palm

DNA fingerprinting, also known as DNA typing or genetic fingerprinting, is a method for identifying individuals by the particular structure of their DNA. DNA fingerprinting techniques have the advantage that the DNA content of a cell is independent of environmental conditions, organ specificity and growth stage (Ainsworth et al., 1996). The basic technology of DNA profiling involves extraction of DNA. The next steps are of four types; (a) PCR-based gel technologies, (b) non-PCR-based gel technologies, (c) hybridization Chip - based, and (d) sequence-based (e.g., full genome sequence analysis). To understand and analyze the genetic relationships and genetic diversity among and within date palm varieties, Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphisms (RFLP), Amplified Fragment Length Polymorphisms (AFLP), and Simple Sequence Repeats (SSR) marker have been used widely and efficiently to characterise many date palm varieties. Table (1) summarizes the characteristics of the molecular markers used to fingerprint date palm. These markers were utilized to examine date palm from Algeria (Benkhalifa, 1999), Egypt (Soliman et al., 2003; Saker et al., 2006), Morocco (Sedra et al., 1998), Oman (Al-Ruqaishi et al., 2008), Saudi Arabia (Al Khalifah & Askari, 2003), Sudan (Elshibli & Korpelainen, 2008, & 2010), and Tunisia (Trifi et al., 2000, Zehdi et al., 2004a, b).

Random Amplified Polymorphic DNAs (RAPDs)

RAPDs are DNA fragments amplified by the polymerase chain reaction using short (usually 10 bp) synthetic primers of random sequence. RAPD markers have been used for identification and DNA fingerprinting of the date palm varieties, although the exhibited polymorphism was low (Sedra et al., 1998; El-Tarras et al., 2007) in comparison with other cultivated species (Koller et al., 1993; Yang & Quiros, 1993; Farooq et al., 1994; Akkak, 1996). Moreover, RAPD analysis is not particularly robust because the results are influenced by experimental conditions, often making reproduction of results between labs difficult or impossible (Munthali et al., 1992; Lowe et al., 1996). This technique has been used for cultivar genotyping (Ben-Abdallah et al., 2000; Trifi et al., 1998 & 2000) and for analyses of phylogenetic relationships and genetic diversity (Al-Khalifah & Askari, 2003; Al-Moshileh et al., 2004; El-Tarras et al., 2007). Using this technique, most of the examined Saudi grown cultivars were observed to have a narrow genetic base; that is, more than 50% genetic similarity (Askari et al., 2003; Al-Khalifah & Askari, 2003; Al-Moshileh et al., 2004; El-Tarras et al., 2007), except Barhi which exhibited only 34% genetic similarity in the study by Al-Khalifah and Askari (2003). Using the experimental conditions and the particular collection of varieties in their study, Sukkari Asfar (genetic similarity 66-85%) clustered with other Saudi cultivars (Ajwa, Rothanah and Nabtet Ali) that exhibited a narrow genetic base (66 - 96.3% similarity). It is worth mentioning that Barhi and Sukkari are Iraqi cultivars, while Sukkari Asfar cv. is a Saudi-grown biotype of Sukakari (Ghaleb, 2008). In another study, by Al-Moshileh et al. (2004), Sukkari and Barhi were in separate clusters. This suggests that the sensitivity of RAPDs to experimental conditions means that this technique does not always resolve genetic phylogenies accurately. Estimating the genetic distance assists in studying genetic diversity, a trait that is important for parent selection associated with genetic mapping and for marker-assisted selection in breeding programs (Lapitan et al., 2007; Trethowan and Kazi, 2008). The grouping identified by cluster analysis was rather weak for Tunisian cultivars (Sedra et al., 1998; Trifi et al., 2000). These authors suggested that most of the cultivars from Tunisia or Iraq were associated with accessions grown in Morocco. The organization deduced from the RAPD-marker analysis did not appear to correlate with available date morphological characteristics. The fact that the cultivars from Tunisia and Iraq were not markedly separate from the genetic diversity present in Morocco indicates broad dissemination, with separate genetic integrities maintained at disparate locations over several centuries. This is the expected outcome if offshoot propagation has been the major mode for planting and replanting date palm groves. Letouze et al. (1998) and Saker

Table 1. Comparison of different molecular markers utilized in fingerprinting date palm.

Characters Marker type	RFLP Co-dominant	RAPD Dominant	AFLP Dominant	ISSR Dominant	SSR Co-dominant
Level of polymorphism	Low	Low	Medium	High	High
Quality of Template DNA needed	High	Low	Low	Medium	Low
Development cost	High	Low	Medium	Medium	Medium
Reliability	High	Low	Medium	Medium	High
Level of skill required	Medium	Low	Medium	Medium	Low

et al. (2000) reported RAPD fingerprinting of Egyptian date palm varieties before and after long term cryopreservation to assess the somaclonal variation. The frequency of somaclonal variations was found to be age–dependent, in agreement with the general observation that duration of callus culture plays an important role in determining the level of somaclonal variation (Gaj & Maluszynski, 1987). Al-Qurainy et al. (2002) supported the concept that the RAPD technique can be successful in determining the genetic fidelity of micro-propagated date palms. In a later study by Al-Khalifah & Askari (2005), RAPD markers were used to detect somaclonal variation in TC-derived plant from four date palm varieties. RAPD markers were used by Eshraghi et al. (2005) to analyze the genetic stability of somatic embryogenesis-derived regenerates and mother plant in the Iranian-grown date palm cultivar Khinaizi. Al-Khalifah (2006) used 19 date palm cultivars from Saudi Arabia for micro-propagation studies and subjected the regenerates to RAPD for analysis of genetic variation. Very few RAPD variations were seen in these studies. However, the small variations in DNA structure are associated with the production of many elite cultivars which are highly variable in fruit size, shape, color, texture, sugar and protein content. Because most causal changes in somaclonal variation are likely epigenetic, it is not expected that direct DNA analysis (including RAPDs) would detect these changes. Hence, many of the above-mentioned results using RAPDs to detect somaclonal variation signatures are likely to be artifacts of the inconsistency of RAPD-derived results. Other RAPD analyses were used for the identification and assessment of genetic diversity for the conservation of date palm germplasm in Saudi Arabia. Many of these projects indicated that the RAPD technique is reliable for the identification and construction of genetic linkage maps, but other reports have suggested that RAPD markers have significant difficulties for cultivar characterization because of low polymorphism, irreproducibility and the construction of weak grouping associations (Yang et al., 1996; Benkhalifa, 1999; Sedra et al., 1998; Trifi et al., 2000). Aly and El-Hewiety (2009) examined 13 UAE-grown date palm varieties using RAPDs and reported their phylogeny. Later work in the same laboratory added seven varieties where the resulting dendrogram revealed different phylogenetic relationships. They reported that the more varieties and RAPD primers used, the more accurate the fingerprint and phylogeny. The most important factor for irreproducibility of a RAPD profile has been found to be low quality template DNA (Welsh & McClelland, 1994). In addition, differences between template DNA concentrations for individual DNA samples can result in the loss or gain of some bands (Bardakci, 1996). Hence, more robust techniques need to be applied to date palm genetic diversity analysis.

Amplified Fragment Length Polymorphisms (AFLPs)

The AFLP technique is based on the amplification of subsets of genomic restriction fragments using PCR. DNA is cut with restriction enzymes, and double-stranded adapters are ligated to the ends of the DNA-fragments to generate template DNA for amplification. AFLP fingerprinting has been used on date palm for constructing genetic maps (El-Kharbotly et al., 1998) and for genetic diversity and variety clustering (Cao & Chao, 2002; Jubrael et al., 2005; Rhouma et al., 2007). Moderate to high genetic diversities were found by the AFLP technique. Different dendrograms showed different types of clustering among cultivars depending on the cultivars investigated. According to El-Khishin et al. (2003), the Egyptian cultivars Siwi and Hayany were the most genetically similar among the studied cultivars, with Amhat and Samany next, while Zaghloul was the most distinct cultivar. Elhoumaizi et al. (2006) used AFLP analysis to confirm that Medjool in Morocco is not genetically uniform, indicating that it is a landrace derived from a mixture of genotypes that evolved largely by natural selection, under the environmental condition in which they were grown. This raises the possibility that other date palms may be landraces in different growing areas. This routinely observed lack of cultivar uniformity in date palm has significant implications for germplasm collection and preservation. Jubrael et al. (2005) also reported a high level of inter-varietal polymorphism among 18 Iraqi date palm varieties which is not surprising, that enabled them to estimate genetic relatedness with only 27% similarities between the examined varieties, indicating that all the varieties are genetically distinct and there are not multiple names for the same variety in Iraq. The varieties were clustered independent of their geographic origin in spite of their phenotypic similarities. Based on offshoot identification, and to determine genetic diversity and phylogenetic relationships, Khierallah et al., (2011) used AFLP fingerprinting to evaluate 18 date palm varieties (11 females and 7 males) collected from the centre of Iraq. The varieties were observed to cluster independently of their geographic origin and of their phenotypic characteristics. Diaz et al. (2003) reported the development and efficiency of AFLP technique in date palm. Out of 64 primer combinations, five were successfully used for comparing and identifying genetic distance of three different varieties (offshoots) and three in vitro- derived plants from Medjool variety. Accordingly, they were able to establish whether a TC-derived plant is genetically the same (at this level of sensitivity) as the donor plant or not. Observed varieties were genetically distant but in vitro plants were identical with the donor plant. Using only five pairs of primers cannot accurately confirm whether two plants are identical as numerous differences could be missed, where only one difference means plants aren't identical. Like any other marker system, AFLP analysis can be used to examine linkages between molecular markers and agronomically important traits, and also to identify genetic variation at different stages of the breeding process. El-Assar et al. (2005) studied 47 date palm accessions from eight locations of Egypt. AFLP profiling revealed genetic differences among accessions of the same variety, which suggested that these

accessions, of the same varietal name but collected from different locations, may derive from sexual hybridization rather than through clonal offshoot propagation. Also, genetic differences were observed in tissue culture-derived accessions of Bartmouda and Sakkoutty. Likewise, AFLP polymorphism was evident when different accessions of Bint Aisha were examined. The authors concluded that this diversity may have arisen due to the possible of existence landraces, or due to sexual reproduction from a wide hybridization background. Al Kaabi (2009) utilized AFLP fingerprinting to compare the level of somaclonal variation resulting from two tissue culture techniques; organogenesis and embryogenesis. He employed ten UAE-grown date palm varieties. AFLP analysis revealed that the level of somaclonal variation derived from organogenesis was less than that derived from asexual embryogenesis. The AFLP technique does not require previous knowledge of the DNA sequence and it can utilize florescence labelled primers that save the time required for subsequent digestion, ligations, amplifications, and analysis on a polyacrylamide gel (Savelkoul et al., 1999) and give a much higher resolution than with other AFLP detection system. Moreover, when enough markers are used, it can uncover relatively low levels of polymorphism. The major disadvantage of this marker is dominance, as it rarely detects heterozygosity and is scored as a presence/absence polymorphism (Table 1).

Microsatellites or Simple Sequence Repeats (SSRs)

SSRs are di-, tri- or tetra- nucleotide repeats shown to be abundant, dispersed throughout the genome and move highly polymorphic than other genetic markers in eukaryotic genomes. SSR resources are useful for cultivar identification, pedigree analysis, characterization of germplasm diversity and genetic mapping studies (Billotte et al., 1999). The SSR markers used for date palm (Billotte et al., 2004) revealed a high polymorphism rate, promoting the utility for germplasm diversity studies as well as cultivar identification. Elshibli and Korpelainen (2008) revealed high genetic diversity in germplasms from Sudan and Morocco, but weak genetic relationships between Sudan cultivars (Elshibli and Korpelainen, 2010). The grouping of the Sudanese and Moroccan varieties (by principal component analysis (PCA)) often did not follow a clear geographic pattern and exhibited significant varietal heterozygosity. Akkak et al. (2009) evaluated SSR loci on a set of 31 cultivars, and in clones from Algerian and Californian germplasms, and showed their transferability in 14 other species across the genus *Phoenix*. The current microsatellite markers are expected to provide a valuable resource for future diversity analyses and genetic mapping of date palm. Cross-species amplification between other species of *Phoenix* revealed clear SSR patterns and polymorphism within the expected allelic range of SSR markers investigated. Precise relationships and genetic diversity were determined among 15 different cultivars of Qatari date palm (Ahmed & Al-Qaradawi, 2009) based on SSR markers. One cultivar (Abu Maan) was found to be quite dissimilar to the other cultivars. Two cultivars (Barhi and Sultana) were found to be very closely related, so the authors suggested they could be considered as one cultivar with different names. Additional analysis suggested that Abu Maan is not a native Qatari cultivar. Alternatively, it may be that the sampled Abu Maan was mislabeled at some time during its propagation, or it may represent a Qatari germplasm component that has remained separate from other breeding materials for some unknown reason. These possible interpretations may be resolved by analysis of further Abu Maan accessions, more Qatari accessions and a broader sampling of the international date palm germplasm. Recently, 1000 SSR markers were developed (Hamwieh et al., 2011), and these should provide an excellent resource for date palm genotyping. Thirty of these new SSRs were used to assess the genetic diversity in 11 date palm cultivars from different locations in Qatar (Elmeer et al., 2011). Among the 30 primer pairs, 10 identified polymorphism, with genetic diversity ranging from 0.8 - 0.9. Genetic diversity of 30 date palm cultivars in Iraq, representing 24 female cultivars and six male cultivars, was investigated using 22 SSR primers (Khierallah et al., 2011). The tested SSR markers showed a high level of polymorphism and the average level of heterozygosity observed were 0.503 for all cultivars. Microsatellite markers (SSRs) were also used to analyse the genetic diversity among micropropagated cultivars of 21 date palm genotypes (Bahraini, Iraqi, Moroccan and Omani) and to develop a DNA fingerprints to ensure quality control for trees derived by somatic embryogenesis in Oman (Al-Ruqaishi et al., 2008). Bahraini and Iraqi genotypes exhibited close relationships, while Moroccan genotypes were distinct. Furthermore, Khalas Omani (which originated in Oman) and Khalas Bahraini (from Bahrain), which share the same varietal name, were found to be genetically quite different using molecular markers. Also, a similar situation revealed by the same research group was that Khinaizi Oman and Khinaizi Bahraini cultivars were not closely related to each other, even though they share the same name. This study also reported that the materials derived from different explants of the same genotypes yield the same fingerprint both before and after regeneration via somatic embryogenesis. This conflicts with earlier RAPD-based results, and these contradicting results as to detection of somaclonal variation in TC-derived plants may be dependent on the variations of the TC protocols followed in each laboratory or the sensitivity and artifact potential of the molecular markers used in a given investigation.

Inter Simple Sequence Repeats (ISSRs)

The ISSR technique involves amplification of DNA segments present at an amplifiable distance between two identical microsatellite repeats oriented in opposite directions. The technique uses microsatellites, usually 16–25 bp long, as primers in a single primer PCR reaction. This targets multiple genomic loci, and usually yields dominant markers. Fourteen microsatellite primers were used to examine the genetic diversity in Tunisian date-palm germplasm (Zehdi et al., 2004b) showing a high level of polymorphism in 49 accessions from three oases, with little geographic structure within Tunisia. Karim et al. (2010) used ISSRs to estimate genetic diversity among 10 accessions of high quality fruit Tunisian date palm varieties, including two introduced (one from Iraq and the other from Algeria). Native varieties were characterized with 50% genetic divergence, whereas introduced varieties were not significantly distant from the Tunisian varieties, suggesting a narrow genetic diversity between the native and introduced varieties. Varietal relatedness has also been analyzed by isozyme markers (Bendiab et al., 1993 & 1997), especially with regard to date palm resistance to the bayoud disease. The main disadvantages of isozymes markers are the small number of investigatable alleles, the influence of environmental factors, and the fact that each isozyme requires different assay conditions.

Restriction Fragment Length Polymorphisms (RFLPs)

The variation(s) in the length of DNA fragments produced by a specific restriction endonuclease from genomic DNAs is termed restriction fragment length polymorphism (RFLP) (Soller and Beckmann, 1982). Relatively few studies have been conducted with RFLPs on date palm because of the relatively large amounts of DNA required to obtain an adequate sample, and the very high cost due to labour and reagent expenses. A PCR-based RFLP technique has been conducted to identify genotype and genetic polymorphisms in plastid DNA from 38 Tunisian date palm accessions, including 5 male ecotypes (Sakka et al., 2003 & 2004). The results showed that the plastid haplotypes of the tested accessions was independent of the tree's sex and provides a common genetic background within the implied varieties (phonetic groups composed of cultivars clustered together). An RFLP analysis has been performed on offshoot leaves of five date palm varieties that were used to initiate TC. Because polymorphism was detected, preliminary attempts were made to assess the extent of variability at the DNA level of tissue culture-derived plants (Corniquel & Mercier, 1994 &1997). By this technique, a clear-cut discrimination was observed among the five date palm cultivars. Single Nucleotide Polymorphisms (SNPs) provide a robust sequence-based marker system which is becoming the tool of choice for many applications and organisms because of the thousands of markers that can be assessed simultaneously (Brumfield, 2003; Hu et al., 2012). However, according to our knowledge, no SNP-based investigations have been reported for date palm up to the time of submitting this manuscript, although the publication of a draft date palm genome sequence (Al-Dous et al., 2011) will greatly facilitate SNP discovery and utilization.

Combined methods

Hussein et al. (2005) found low levels of intervarietal polymorphism suggesting a narrow genetic background among 14 date palm (*Phoenix dactylifera* L.) accessions collected from different locations in Egypt using a combination of RAPD and ISSR markers, with the estimated genetic similarities ranges from 91.4% to 99.6%. This type of information helps to select possible parents to generate mapping population. Combined data from RAPD, ISSR and AFLP markers revealed more accurate results than with the sole use of RAPD and ISSR markers (Adawy et al., 2002), in terms of relatedness among the varieties of Egyptian date palm cultivars by comparing markers and estimating the genetic stability among them (Adawy et al., 2005). The combined technique was used not only to identify the unknown genotypes by identifying genotype-specific markers (Moghaieb et al., 2010), but also for identifying sex-specific DNA markers (Younis et al., 2008). Genetic fidelity of micropropagated date palm (*Phoenix dactylifera* L.) plants was assayed using RAPD and ISSR markers by Nitish et al. (2010). These authors reported homogeneous (i.e., unchanged) banding patterns and concluded that the somatic embryogenesis protocol utilized in the study was the safest mode for production of true-to-type date palm plants.

Discussion

The agro-morphological traits used to characterize date palm varieties present limited discriminatory power. This has led to cultivars with somewhat similar morphological characters being given the same varietal name. The two examples mentioned above for the cultivars Khalas and Khinaizi grown in Oman, illustrate such discrepancies (Al-Ruqaishi et al., 2008). These were labeled as the same cultivar from different regions, but were found to be very different genotypes. Though this may not be surprising, it provides important information for future breeding efforts and clonal production. In some cases, the same cultivar is grown in different countries with different names or even different spelling of the name due to the different dialects from one country to the other, or even from one region to the other within the same country. To date, not enough comprehensive efforts have been directed towards the use of molecular markers to assist identification and conservation of the date palm genetic resources. This is mainly due to the relatively scant number of cultivars examined in the respective studies, and focusing on the commercial varieties in the country/region where the study is performed. In the absence of sufficient fingerpring information, possible changes in cultivar composition or purity may be endangered by new introductions. This may also be true for TC-derived palms, which may induce genetic and/or epigenetic variability. The combined use of the AFLP, RAPD and RFLP techniques is costly in time and manpower, and thus precludes analysis of a large number of individuals (Powell et al., 1996; Semagn et al., 2006). Other than SSRs, none of the diagnostic markers techniques applied, so far, to date palm has fulfilled all of the requirements in terms of cost, ease of use, cultivar discrimination, and dependability. Some molecular marker studies on date palm were not deep enough to permit variety identification (Billotte et al., 2004; Zehdi et al., 2004b; El-Tarras et al., 2007; Hamwieh et al., 2011). Even when varieties were distinguished, little geographic structuring was observed in most cases (Elshibli & Korpelainen, 2009). Because of intra-varietal variation, accurate identification of cultivars was often not achieved, meaning that the advantages of molecular markers were not fully utilized. A concentration on using more markers and especially the most dependable markers (e.g., SSRs and SNPs) may alleviate these problems. Analysis of possible genetic change in TC-derived plants yielded very different results in different studies. In some cases, DNA marker techniques revealed true-to-typeness of TC-derived date palms (Adawy et al., 2005; Cao and Chao, 2002; El-Assar et al., 2005; Elshibli & Korpelainen, 2008 & 2010, Nitish et al., 2010), while the studies showing variation are in question due to technical issues regarding the reproducibility of such markers as RAPDs. It seems likely that the methods of pollination and cultivar selection are major contributors to the weak clustering association (based on genetic similarity) found for the majority of accessions originating from different regions. This emphasizes the need for further investigation of the genetic diversity of date palm germplasm. A comprehensive study can be manifested by a detailed analysis of cultivars originating from different geographic locations. Currently, two sequence based marker systems, Simple Sequence Repeats (SSRs) and Single Nucleotide Polymorphisms (SNPs), satisfy more conditions than any other technique and are becoming the tool of choice for many applications and organisms (Brumfield, 2003). The SNP and SSR markers which can be rapidly and cheaply identified through bioinformatics have many uses in genetics, such as the detection of alleles associated with disease resistance, genome mapping, association studies, genetic diversity, paternity assessment, forensics and inferences of population history (Collins, 2004; Gong et al., 2011; Ashkani et al., 2012; Allegre et al., 2012). SSRs are powerful genetic markers, due to their genetic co-dominance, abundance,

dispersal throughout the genome, multi-allelic variation, high reproducibility and high level of polymorphism (Katti, 2001, Toth, 2000). This high level of polymorphism is primarily due to mutations affecting the number of repeat units. SSRs provide a number of advantages over other molecular markers, namely that multiple SSR alleles may be detected at a single locus using a simple PCR-based screening, very small quantities of DNA are required, and analysis is amenable to automated allele detection and sizing (Schlotterer, 2000). Studies of the potential biological function and evolutionary relevance of SSRs is leading to a greater understanding of genomes and genomics (Subramaniam, 2003). SSRs were initially considered to be evolutionally neutral (Awadalla, 1997), however recent evidence suggests that some may play an important role in genome evolution (Moxon, 1999) and provide hotspots of recombination. There is now evidence to suggest that some SSRs in non-coding regions are also of functional significance (Mortimer, 2005). SNPs are highly abundant polymorphic markers which can provide a high density of markers near a locus of interest. SNPs are not homogeneously distributed, and are usually enriched in non-coding regions (Barreiro et al., 2008). SNP density can be predicted by the presence of microsatellites, because regions of thousands of nucleotides flanking microsatellites have an increased or decreased density of SNPs depending on the microsatellite sequence (Varela and Amos, 2010). SNPs can be bi-, tri- or tetra-allelic. However, tri- and tetra-allelic SNPs are rare, and in practice SNPs are generally biallelic (Doveri, 2008). This is a disadvantage of this marker over multiallelic markers such as SSRs, which limits the relative abundance of SNPs. For the application of molecular marker, both high density and uniform maps that represent whole genomes are needed. SNPs are more popular for their high density within the genome and their ease of characterization. Currently, there are several ongoing efforts to further enrich the sequence of the date palm nuclear genome (Al-Dous et al., 2011). A research project to study diversity and to partially sequence the nuclear genome of selected UAE cultivars is currently conducted in the author's labs (UAE University grant No. 31F002, Genomic tool development and full genome sequencing of date palm variety, Naghal. M. Aly, PI.). This will enable more facile fingerprinting, phylogeny and breeding studies. In addition, these studies should provide data to assist the assembly of DNA chips (Augenlicht and Kobrin, 1982) that will greatly enhance future genotyping efforts. In the perhaps not-so-long term future, genotyping will probably be most effectively performed by low pass full genome sequencing on next generation sequencing platforms.

Conclusions

The date palm fingerprints reported do not always provide highly accurate results, and are not comprehensive due to the small numbers of cultivars investigated and the focus on local commercial cultivars in the reported studies. Furthermore, the development of new molecular techniques clearly provides more accurate results, Also, the variable nomenclature of cultivars, new introductions, and different geographical distribution may also confuse varietal designations. Accordingly, there is a need for collective international, or at least regional, efforts to establish a comprehensive database of DNA fingerprints and phylogenetic relationships of all date palm cultivars. Furthermore, with the availability of the draft date palm nuclear and chloroplast genome sequences, more SNP, SSR, and other markers will be available to investigate date palm systematic, cultivar relatedness, and genetic map structure. These tools will greatly assist breeding programs, which can be tremendously accelerated by marker assisted selection. In addition, there is a major need to include wild date palm relatives in future studies to provide a more accurate phylogeny, and as a possible source of novel alleles for traits like resistance to disease. Finally, the current focus on exclusively growing, selecting and studying major commercial cultivars may lead to loss of desirable genes already existing in the current non-commercial cultivars. Thus, a major effort needs to be directed towards including the full breadth of Phoenix diversity in comprehensive studies.

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