Synthesis and assessment of date palm genetic diversity studies

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Abstract

A thorough assessment of genetic diversity and population differentiation of *Phoenix dactylifera* are critical for its dynamic conservation and sustainable utilization of its genetic diversity. Estimates of genetic diversity based on phenotypic, biochemical and molecular markers; and fruit quality traits were utilized in assessing the population differentiation of date palm populations throughout its center of diversity. Some phenotypic traits may not exhibit variation in response to environmental or management factors and can be used as stable descriptors of date palm cultivars and for cultivar identification. The majority of analyzed studies based on isozyme and microsatellite markers, reported larger within-population than among-population genetic diversity levels. Most variation estimated for fruit quality traits was found among populations; however, substantial differences in genetic diversity components were found among and within populations. The overall partitioning of genetic diversity, based on phenotypic, biochemical, molecular, and fruit quality traits suggested that date palm cultivars represent a complex gene pool within which historical movement of germplasm, recent introductions and human selection are shaping their genetic structure. The empirical evidence derived from this assessment suggested that the genetic structure of date palm populations is controlled by the environment, isolation by distance, and the biological characteristics of the tree.

Key words: Cultivar characterization, Genetic diversity, Germplasm, Molecular markers, Phenotype, Population differentiation

Introduction

Date palm genetic diversity and its link to the properties of the oasis agroecosystem have scientific, cultural, intellectual, aesthetic and spiritual values that are important to the oases dwellers and the general public (Jaradat, 2011). The date palm genetic diversity represents heritable variation within and between wild and domesticated populations grown in oases or modern plantations. On the other hand, phenotypic diversity represents the interaction effect between the genetic diversity and the environment, and it is an apparent indicator of date palm diversity. The latter represents the basis for selection and conservation, as well as for date palm improvement for sustainable utilization (Chao and Krueger, 2007; Eljuhani, 2010). Intra-specific genetic diversity provides the basis for any evolutionary changes and therefore, it is the most fundamental level of biodiversity (Pauls et al., 2013). On the other hand, biodiversity of date palm is a pre-requisite for the proper functioning of the oasis ecosystem (Nabhan, 2007), which is a complex ecosystem characterized by horticultural, ecological, economic, social and cultural dimensions (Elshibli and Korpelianen, 2009; Pintaud et al., 2013).

High levels of genetic diversity of date palm within the oasis agroecosystem can be expected, on average, to give rise to ecosystem stability; however, diversity is not the driver of this relationship; rather, ecosystem stability depends on the ability of the oasis to contain different species, or functional groups (e.g., different cultivars of date palm, different species and cultivars of fruit trees, forage crops, annual grain crops, vegetable crops, semi-domesticated crops, weedy relatives of crops, etc.) that are capable of differential responses to biotic and abiotic stresses and to different management practice. Oasis diversity at the species level has functional consequences because the
number and kinds of species, besides *Phoenix dactylifera*, present in an oasis determine the traits that influence a multitude of processes within, and services provided by, the oasis agroecosystem (Pauls et al., 2013).

It is increasingly recognized that poor management has caused some oasis agroecosystems to pass ecological thresholds, leading, in a few, well-documented instances (e.g., Sijilmasa in the oasis of Tafilalet, southern Morocco, and Timbuktu in Mali) to irreversible changes in the ecosystem and the loss of its ecosystem services, including the date palm cultivars. In-depth assessment of the genetic vulnerability of date palm to many threats (e.g., climate change, desertification and salinity stress) requires knowledge of the extent and distribution of its genetic diversity, both of which depend on the species evolution and its unique breeding system; past genetic bottlenecks; and ecological, geographical and anthropogenic factors (Wrigley, 1995; Pintaud et al., 2013).

Rational and sustainable utilization of date palm genetic resources requires extensive characterization, evaluation and documentation of the germplasm available at a local or regional level. Characterization is defined as the assessment of the presence, absence, or degree of specific traits that are little influenced in their expression by varying environmental conditions; while, evaluation is defined as the assessment of plants for potentially useful genetic traits, many of which may be environmentally variable (e.g., pest or disease resistance, fruit quality, flavor) (IPGRI, 2005). Genetic diversity of date palm is expressed at different levels, including genetic differences between species, subspecies, cultivars, populations or individual clones and may be measured at the morphological, physiological, biochemical or molecular levels. Moreover, this diversity is not randomly or uniformly distributed in space or time. The amount of genetic diversity differs between oases and populations, or between regions and localities, and several key historical, geographical, ecological and anthropogenic factors determine its magnitude and distribution (Rivera et al., 2008). Genetic diversity and genetic structure of the gene pool complex of date palm (wild, feral, and domesticated) have been shaped and greatly altered by natural and human selection, clonal propagation, and spatiotemporal exchange and movement of germplasm (Zohary and Hopf, 2000).

Discrimination among closely related date palm cultivars and clones for genetic diversity studies is often extremely difficult. Identification of date palm cultivars is usually based on plant and fruit morphology. However, morphological traits are often unreliable and may not precisely correlate with the genotype of the date palm. These traits usually are influenced by environmental conditions or they vary with the developmental stage of the date palm. However, a number of leaf and leaflet morphological qualitative and quantitative traits of a selected number of elite cultivars have been reported to be stable and did not exhibit variation in response to environmental or management factors. Such morphological traits can be used as stable descriptors of date palm cultivars and for cultivar identification at any growth stage (IPGRI, 2005).

Biochemical markers (i.e., isozymes), due to their low level of polymorphism, appear to be of limited value in discriminating between cultivars (Ould Mohammed Salem et al., 2008; Akkak et al., 2009).

Molecular markers (i.e., based on DNA structure and molecular analysis) are more precise and can accurately identify cultivars and quantify their genetic diversity and phylogenetic relationships; these markers have been extensively used to study the genetic variation of date palm cultivars. These include randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) (Al-Khalifa and Askari, 2012), and microsatellite markers, restriction fragment length polymorphisms (RFLP), inter simple sequence repeat (ISSR), and simple sequence repeat (SSR) (Ahmed et al., 2013). Each method has its own advantages and disadvantages and limitations; however, nuclear microsatellites or SSR seem to fulfill most requirements for an accurate analysis of date palm diversity and phylogeny (Khanam et al., 2012). A number of newly isolated microsatellite markers are expected to provide a valuable and highly informative resource for genetic mapping and diversity analysis in date palm (Elhoumaizi et al., 2006; Hammadi et al., 2011; Khierallah et al., 2011).

In this study, appropriate statistical analyses techniques were used to synthesize a general trend in the genetic diversity of date palm using the large body of literature which has appeared on the subject in recent years (2000–2013), especially with the extensive use of molecular markers.

The objectives of this study were to (1) compile and critically review key studies on genetic diversity of date palm in its center of origin and center of diversity, (2) collate, analyze, contrast, and evaluate the experimental procedures and results on morphological, fruit traits, biochemical and molecular markers and (3) synthesize the results of the review and statistical analyses within the context of a global assessment of and
recommendations for the future of date palm genetic resources in a changing climate.

Materials and Methods
Selection of key studies
The statistical analysis started with the selection of appropriate studies for inclusion in the analysis using the USDA-National Agricultural Library “Navigator” with access to nine databases including AGRICOLA, AGRIS, BIOSIS, and CAB Abstracts. I used the listed key words above and selected the most relevant and up-to-date (2000–2013) published data for the study. Publications based on morphological, fruit, biochemical, and molecular diversity were included in this analysis.

Data extraction
Diversity measures such as Nei’s gene diversity index, similarity index, dissimilarity index, average genetic distances, population differentiation and heterozygosity, were extracted from the text, tables or figures of the selected papers. Some papers reported more than one diversity measure. In addition to the diversity indices, other information extracted from the papers included the number of date palm cultivars studied, the number of quantitative or qualitative traits, the number of loci, the marker system(s) used, average estimates of the polymorphism information content (PIC), the quantitative value of the marker(s), and the country where the study was carried out.

Several papers which were originally selected for the analysis had to be rejected at closer inspection as the data were unsuitable for the analysis. Also, it was found that the same date palm cultivars were used several times in different studies using different phenotypic and molecular markers.

Data analyses
Two approaches have been followed in data analysis, these were: (1) an assessment of genetic diversity studies based on results presented by researchers and (2) analysis of secondary statistics extracted from tables and figures reported in selected references were subjected to a number of statistical analyses procedures; the results were tabulated or summarized as figures to reflect relationships between different diversity parameters in each study.

Results and Discussion
Assessment of genetic diversity studies
Genetic diversity and genetic structure of the gene pool complex of date palm (wild, feral and domesticated) have been shaped and greatly altered by natural and human selection, clonal propagation, and spatiotemporal exchange and movement of germplasm. A better understanding of the intraspecific genetic variation of the date palm and its distribution in the oasis ecosystems is essential for the proper conservation and sustainable use of its genetic resources and biodiversity (Pintaud et al., 2013). Over recent decades, date palm polymorphism and genetic diversity have been studied extensively, generating a wealth of information and knowledge about this ancient and unique fruit tree. The following is a chronologically ordered comprehensive summary of research results using several markers and carried out at several countries within the center of origin and diversity of date palm.

Phenotypic markers
Phenotypic markers of the tree, leaves and fruits of the date palm have been used in developing descriptor list (IPGRI, 2005) and in quantifying phenotypic diversity long before the advent of biochemical and molecular markers. Examples of tree, leaf, seed and fruit morphological descriptors are presented in Table 1 (IPGRI, 1995; Eissa et al., 2009). Four studies were selected to illustrate the use of these markers in studying date palm phenotypic diversity. Multivariate statistical analyses were employed in data reduction of six fruit qualitative traits, along with a final score of fruit quality scored on fruits of 203 date palm cultivars grown in 19 ecogeographical regions in six countries of the Arabian Peninsula (Jaradat and Zaid, 2004). Fruit color and shape were highly diverse across ecogeographical regions, whereas fruit softness and consumption stage were the least diverse. It was found that fruit color, shape, size and ripening and their interactions predominantly reflect differences in consumer preferences in these countries (Table 2).

Long-term intra- and inter-country selection for specific fruit quality traits was quantified as a diversity index and ranged from zero in one region of Oman for fruit softness to 1.7 for fruit color in one region in the UAE. Trait richness, at an ecogeographical or country level, reflected farmers' and consumers' preferences and allowed for targeted selection and introduction of date palm cultivars. The Central region in Saudi Arabia has the highest trait richness (6.08) for fruit color whereas the Dakhliya region of Oman has the highest trait richness for fruit ripening (3.91), fruit size (3.9) and fruit texture (5.86).
Table 1. A short list of date palm tree, leaf, seed and fruit morphological descriptors.

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Morphological variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tree</td>
<td>Trunk diameter, Crown shape</td>
</tr>
<tr>
<td>Leaf</td>
<td>Length, width, Color, Midrib color, Color of leaf base abaxial surface, Curvature and curvature point, Base width, Blade length, Blade/leaf ratio, Blotches on leaf base abaxial surface.</td>
</tr>
<tr>
<td>Petiole</td>
<td>Length, Width, Thickness, Shape, Petiole/leaf ratio.</td>
</tr>
<tr>
<td>Pinnate</td>
<td>Number/leaf, Density/unit length, Length, Width, Shape, Apex, Rachis angle.</td>
</tr>
<tr>
<td>Spine</td>
<td>Area length, Spine area/leaf ratio, Shape, Length, Base, Type, Color, Rachis angle.</td>
</tr>
<tr>
<td>Seed</td>
<td>Length, Width, Weight, Volume, Shape, Color, Apex, Base, Surface, Micropyle position and elevation, Transverse grooves, Ventral furrow shape and ends.</td>
</tr>
<tr>
<td>Fruit</td>
<td>Length, Width, Weight, Volume, Density, Apex, Shape, Base, Color at Khalal, Color at maturity, Skin texture at maturity, Skin thickness, Flesh thickness, Flesh color, Flesh texture, Flavor, Taste, Pulp, Maturity (Early, Medium, Late)</td>
</tr>
</tbody>
</table>

Source: Adapted and compiled from IPGRI, 2005.

Table 2. Fruit quality statistics based on phenotypic descriptors of 203 date palm cultivars from six countries in the Gulf Cooperation Council region.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Country</th>
<th>Bahrain</th>
<th>Kuwait</th>
<th>Oman</th>
<th>Qatar</th>
<th>KSA</th>
<th>UAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correct class</td>
<td></td>
<td>63%</td>
<td>88%</td>
<td>75%</td>
<td>55%</td>
<td>70%</td>
<td>79%</td>
</tr>
<tr>
<td>Total diversity</td>
<td></td>
<td>0.55</td>
<td>0.76</td>
<td>0.88</td>
<td>0.62</td>
<td>0.82</td>
<td>0.93</td>
</tr>
<tr>
<td>Variance components (percent of total variance)</td>
<td></td>
<td>28%</td>
<td>0.0%</td>
<td>48%</td>
<td>21%</td>
<td>42%</td>
<td>45%</td>
</tr>
<tr>
<td>Eco region</td>
<td></td>
<td>29%</td>
<td>40%</td>
<td>20%</td>
<td>27%</td>
<td>21</td>
<td>30%</td>
</tr>
<tr>
<td>Population</td>
<td></td>
<td>42%</td>
<td>60%</td>
<td>32</td>
<td>52</td>
<td>37</td>
<td>25%</td>
</tr>
<tr>
<td>Cultivars</td>
<td></td>
<td>42%</td>
<td>60%</td>
<td>32</td>
<td>52</td>
<td>37</td>
<td>25%</td>
</tr>
<tr>
<td>Economic value predictor</td>
<td></td>
<td>Fruit Color</td>
<td>Dark- Red</td>
<td>Light Red</td>
<td>Red</td>
<td>Yellow</td>
<td>Yellow</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>61%</td>
<td>55%</td>
<td>58%</td>
<td>64%</td>
<td>70%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fruit texture</td>
<td>Soft</td>
<td>Soft</td>
<td>Semidry</td>
<td>Soft</td>
<td>Semidry</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100%</td>
<td>72%</td>
<td>72%</td>
<td>60%</td>
<td>60%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Use</td>
<td>Rutab</td>
<td>Rutab,Tamar</td>
<td>Rutab</td>
<td>Rutab</td>
<td>Rutab,Tamar</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>78%</td>
<td>83%,33%</td>
<td>70.5%</td>
<td>100%</td>
<td>47%,45%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quality model</td>
<td>Color-shape</td>
<td>Color-shape</td>
<td>Color-shape</td>
<td>Color-shape</td>
<td>Color-shape</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Color-size</td>
<td>Ripening-softness</td>
<td>Color-shape</td>
<td>Shape-ripening</td>
<td>Color-ripening</td>
</tr>
<tr>
<td>Shannon I</td>
<td></td>
<td>0.866</td>
<td>0.989</td>
<td>0.962</td>
<td>0.772</td>
<td>0.885</td>
<td>1.122</td>
</tr>
<tr>
<td>Hot spots for fruit traits (%)</td>
<td></td>
<td>Ovate (66.7%)</td>
<td>Ovate (44.0%)</td>
<td>Semidry</td>
<td>Yellow fruit</td>
<td>Ovate-elongate</td>
<td>Red fruit</td>
</tr>
</tbody>
</table>


Results of canonical discriminant analysis indicated that a minimum of 55% (Qatar) and a maximum of 88% (Kuwait) of the cultivars were correctly classified based on their fruit quality traits (see below). However, these percentages were not in line with results of total genetic diversity analysis or with Shannon polymorphic index (I) estimates. Nevertheless, certain variants of fruit traits were found with high frequencies in specific regions within each country included in this study. For example, ovate fruit shape was found with high frequencies in Bahrain (66.7%) and Kuwait (44.0%); whereas all fruits (100%) in one region in Oman were semidry, and all fruits were red in one region of the UAE. In addition, the two-trait models developed for fruit traits reflect local preferences for a combination of color, shape, texture and maturity stage variants across this region (Table 2).

Three predictors of fruit economic value (fruit color, softness and consumption stage) explained 65.5% of its total variation and delineated clear subregional differences in consumer preferences. A classification matrix based on discriminant analysis of all quality traits showed that Kuwaiti cultivars were unique, with percent correct classification of 88%, and may have originated from countries outside the Arabian Peninsula, whereas Qatar has the least (55%) number of cultivars correctly classified. Values for the remaining countries were: 63% for Bahrain, 70% for Saudi Arabia, 75% for Oman and 79% for UAE. A Mahalanobis distance matrix between countries of origin indicated that geographical
distance are indicative of quality differences between cultivars. However, the intra- and inter-country shared cultivars expressed as a percentage turnover of cultivars between regions within one country and among countries implied that germplasm exchange is widely practiced in these countries.

Phenotypic diversity of date palm in Oman was assessed (Al-Yahyai and Al-Khanjari, 2008; Al-Yahyai and Al-Kharusi, 2012) using physical phenotypic diversity index and concluded that there was a large biodiversity among the populations. These researchers constructed a similarity matrix indicating that similarity between Omani cultivars ranged from 74 to 90% and concluded that there is a need for chemical and molecular analysis to explore the genetic linkages among these cultivars. Diversity index for fruit weight was small (H’ = 0.43) as compared to the larger value (0.82) for fruit length. The morphological variability in 12 Mauritanian date palms (including soft, semisoft, and dry cultivars) was investigated (Ould Mohamed Salem et al., 2008) using 18 vegetative traits, 14 of which showed a high discriminatory power between cultivars and suggested the establishment of a date palm catalogue based on these traits. The 14 traits in three principal components explained 68.2% of total variation in all cultivars. Finally, a new approach was developed (Hammadi et al., 2009) for morphological identification using intracultivar stability of six morphometric traits, out of 30 traits measured or scored on leaves, pinnae and spines, in three different date palm cultivars from Tunisia and suggested using these stable phenotypic markers for cultivar identification.

A unique case was reported on fruit quality traits from the ~500 year old, seedling-derived date palm groves of Kachvhh, Gujarat, India (Muralidharan and Baidiyavadara, 2013), where farmers selected and are able to identify single date palms on the bases of fruit color, size, shape, and taste. However, due to the marginal climatic conditions of Gujarat, the farmers harvest the dates at the khalal stage to avoid fruit spoilage caused by the rainy season. Dates from this region were characterized by having oblate, round, ovate, oblong or elongated shapes; small, medium, large and very large sizes; sweet, or very sweet taste. However, color variants were the most diverse among fruit descriptors and included yellow, yellow-orange, orange, purple, grey purple, orange-red, red, red-purple and green. The red (51.5%) and yellow (19.5%) colors were predominant.

Allozymes

Allozyme electrophoresis can reveal genetic polymorphism allowing the direct study of genetic variation. However, there is a limit to the number of enzymes available for study (because of the requirements for detection), and therefore a limit to the proportion of the date palm genome which can be accessed. Due to their low level of polymorphism, allozymes appear to be of limited value in quantifying the genetic diversity of date palm populations. However, allozymes have been extensively used prior to 2000 in genetic diversity of crops and fruit trees, however, only a single study using allozyme markers was recently undertaken to assess levels of genetic diversity in 29 date palm cultivars belonging to three main date palm growing regions (Ould Mohamed Salem et al., 2008). Using data from starch and polyacrylamide gel electrophoresis of five polymorphic loci corresponding to four enzyme systems, date palm was found to have high percentage of polymorphic loci (P = 83%), strong heterozygosity (Ho = 0.561) and total genetic diversity (HT = 0.495). Genetic (population) differentiation between geographical groups was low (GST = 0.027). Multivariate analysis showed no well-defined structuring of cultivars in relation to their geographical origins. Twenty seven of the 29 cultivars could be identified by one of the 28 multilocus genotypes observed.

Amplified fragment length polymorphism (AFLP)

A large number of studies using the AFLP as molecular markers have been reported in the literature. Among them, five are cited to illustrate their utility in quantifying date palm genetic diversity. The principle behind this technique is the PCR amplification of a selected subset of all fragments produced by restriction digest of genomic DNA. The AFLP technology is sensitive enough to detect low levels of variation, allowing us to discriminate between highly related cultivars (Díaz et al., 2003). Three date palm cultivars grown at Elche in Spain were identified on the basis of AFLP using Dice coefficient which was calculated from a similarity matrix (Díaz et al., 2003). The similarity index using different primers ranged (in each paired comparison) from 0.18 to 0.70; whereas, Medjool and Boufagous, the two well-known Moroccan cultivars, were separated
by the largest genetic distances (0.80) from a hybrid known as E-85.

Five date palm cultivars from Egypt were characterized (Elkhishin et al., 2003) using AFLP which resulted in an average of 72.17 amplicons per assay and the total number of unique markers per cultivar ranged from 13 to 51 as an indication of molecular diversity. A similarity matrix based on Jaccard coefficient was used to construct a dendrogram which separated the five cultivars into three groups with similarity indices ranging from 64.9 to 76.7%.

The AFLP markers were used to discriminate between 18 date palm cultivars from Iraq (Jubrael et al., 2005). A total of 122 polymorphic AFLP loci were scored, with an average of 17.4 per primer combination. Jaccard’s coefficient ranged from 0.108 to 0.756, indicating moderate to highly diverse relationships between these cultivars. A larger number of accessions (66) of the Moroccan cultivar Medjool and four accessions from California were examined by AFLP (Elhoumaizi et al., 2006). The Moroccan accessions shared a minimum of 79% genetic similarity suggesting that Medjool is a landrace and is not genetically uniform. The authors suggested that other date palm cultivars may be landraces composed of several genotypes and are not pure cultivars. Finally, Khierallah et al. (2011) carried out an assessment of genetic diversity for 11 female and seven male Iraqi date palm cultivars using AFLP markers. The study detected 83 polymorphic AFLP fragments with an average of 13.8 per primer combination and separated males and females cultivars (Figure 1).

These researchers estimated genetic distance using Jaccard’s coefficient (ranged from 0.07 to 0.75). Clustering based on these data brought some male and female cultivars together in one cluster, whereas most female cultivars were clustered separately in others. Based on primer combinations, major allele frequencies ranged from 0.7 to 0.78, average gene diversity from 0.29 to 0.30, and average polymorphism information content (PIC) from 0.23 to 0.28. Genetic distances between cultivars were smallest between those cultivars which shared similar names (0.075) and largest (0.681) between geographically distant cultivars.

**Random amplified polymorphic DNAs (RAPDs)**

The Random Amplified Polymorphic DNA technique observes the variation in non-specific regions throughout the plant genome. There are variations of the technique in which different lengths and concentrations of primers are used, and certain other protocol steps are modified. In a study using RAPD markers, Askari et al. (2003) identified 42 out of 140 primers which detected polymorphism in seven date palm cultivars from Saudi Arabia. A total of 213 bands were detected with an average of 5.6 RAPD markers per primer. Cultivars were clustered based on their banding patterns and a Nei’s distance ranging from 0.79 to 0.83 as a measure of similarity between cultivars was reported. In a follow-up study, Alkhalifah and Askari (2003) carried out a molecular phylogeny of 13 different date palm cultivars from Saudi Arabia using DNA fingerprinting. All cultivars were distinguishable by their unique banding with genetic similarity (using Nei and Li’s coefficient) ranging from 18.6 to 86.0% and concluded that the present RAPD data generated by different primers suggested narrow genetic diversity among these cultivars.

The RAPD markers have been used (Gonzalez-Perez et al., 2004) in a unique study and presented genetic evidence of hybridization between seven populations of the endemic *Phoenix canariensis* and the widespread *P. dactylifera*. The study assessed the impact of this ‘gene flow’ on both species in the Canary Islands and concluded that the hybridization between both species would pose clear threats to the survival of the endemic species (*P. canariensis*), whereby some of the trees that had been characterized as *P. canariensis* on the basis of tree morphological traits, were actually hybrids when assessed using RAPD analysis (Figure 2).
The researchers reported a wide range of percent classification based on individual characterization (mixed hybrids of 14.7–72.2% in *P. canariensis* and 5.5–64.7% in *P. dactylifera*), and concluded that RAPD markers were efficient in distinguishing these species genetically and provided, for the first time, convincing evidence of inter-specific hybridization between them. The study outlined the genetic diversity and genetic erosion consequences of such hybridization on both species. Based on several factors, if hybrid progeny and progeny from advanced hybridization (through natural gene flow) are vigorous and fertile, the endemic species (i.e., *P. canariensis*) will be at risk from genetic assimilation with the common species (i.e., *P. dactylifera*); however, if the hybrid progeny are sterile or have reduced vigor, then the endemic species will become at risk from outbreeding depression.

A large study involving 37 female and 23 male accessions of date palm from Sudan, in addition to eight female cultivars from Morocco, concluded that high genetic diversity was revealed using 6 SSR markers (Elshibli and Korpelainen, 2008). The study detected a total of 343 alleles at the 16 loci, with an average of 21.4 (range from 14 to 44) alleles per locus, and reported a high level of expected heterozygosity for female (0.841) for male (0.799) cultivars from Sudan, and 0.82 for Moroccan cultivars. The population differentiation based on molecular markers ($F_{ST}$) estimates were different between Sudanese and Moroccan cultivars, and the genetic diversity was strongly represented within populations (i.e., countries) rather than between populations of these cultivars. New SSR markers have been recently presented for the assessment of genetic diversity in date palm (Elmeer et al., 2011). A study based on these SRRs found that out of 30 primers, only seven failed to amplify the expected PCR fragments, 13 amplified monomorphic banding patterns, and the remaining 10 primers generated polymorphic banding patterns. The researchers observed 77 alleles with a mean of 7.7 alleles per locus, and estimated a mean genetic diversity of 0.80 in the whole germplasm (range 0.6 to 0.9, depending on the molecular marker). These new markers are expected to help researchers in the genetic mapping and diversity analyses of date palm at a large scale.

The genetic diversity among sub-populations of 27 Tunisian cultivars based on their fruit texture was estimated (Hammadi et al., 2011) using SSR markers. The study concluded that genetic variation was significant among fruit sub-populations. The researchers found 36 polymorphic alleles with an average of 7.2 (from 6 to 8) alleles per locus. Average expected heterozygosity was 0.63 and observed heterozygosity ranged from 0.34 to 0.88 depending on the marker used. Also fixation index was positive (range from 0.04 to 0.47 depending on marker used). Sub-population differentiation was estimated for fruit texture (soft, semisoft, heterozygosity and fixation index in date palm. Hamwieh et al. (2010) developed 1000 microsatellite markers across the date palm genome using 30 well-defined reference date palm cultivars from Iraq. They speculated if derived microsatellite markers could be used to screen for sex in date palm. Also they suggested that these markers would be used for genetic mapping and genetic diversity analyses in date palm.

![Figure 2. Principal components analysis based on the correlation matrix of RAPD markers of *Phoenix dactylifera*, *P. canariensis* and their hybrids in the Canary Islands, Spain. (Reanalyzed and redrawn from Gonzalez-Perez et al., 2004).](image)

**Microsatellites (SSRs)**

Microsatellites or simple sequence repeats (SSRs) are tandemly repeated units of 2–10 base-pairs which can be found dispersed throughout the date palm genome (Ahmed et al., 2013; Figure 3); they are locus-specific, co-dominant, highly polymorphic and highly reproducible (Akkak et al. 2009). Polymorphism in microsatellites arises when the number of sequence repeats in tandem increases or decreases. The SSRs are more reliable and powerful tool for diversity analyses, they are locus-specific, co-dominant, highly polymorphic and highly reproducible (Akkak et al., 2009). An increasing number of studies used SRRs to study population differentiation, sex identification,
semidry and dry) with 7% among and 93% within sub-populations; whereas all variation for maturity period (early, medium and late) was found within sub-populations.

A combination of molecular (SSRs) and morphological markers has been used to analyze sub-populations of Tunisian date palm cultivars (Hamza et al., 2011). The study reported significant differences among sub-populations for all morphological traits; these traits were positively correlated with fruit maturity time ($r = 0.161; p = 0.02$; using Mantel test). Also, based on molecular data, there was significant genetic variation among fruit texture sub-populations with a significant correlation ($r = 0.11; p = 0.029$, based on Mantel test) between genetic variation and fruit texture.

Genetic diversity was assessed in 24 female and six male Iraqi date palm cultivars using SSR polymorphism (Khierallah et al., 2011). They detected 188 alleles at 22 loci ranging from 3–21 with an average of 8.54 alleles per locus. Average heterozygosity was 0.503; genetic distance among cultivars ranged from 0.171 to 0.938 indicating a highly diverse germplasm. The sex of date palm trees was determined (Elmeer and Mattat, 2012) using 14 SSRs on 129 leaves from 43 immature date palm cultivars from Qatar. The study detected 254 microsatellite loci, 22 of them could be used to identify 9 of 12 male cultivars. A specific primer was exclusively associated with a female heterozygous allele, thus suggesting that male and female cultivars can be accurately predicted using SSR markers. The value of SSRs in genetic diversity analysis was demonstrated by a comprehensive study at the oasis level (Figuig in Morocco) (Bodian et al., 2012). These researchers used 121 trees from 11 female cultivars and seven male cultivars. Based on results of the Analysis of Molecular Variance (AMOVA), they estimated that 41 and 59% of diversity was partitioned among and within populations, respectively. Genetic similarity between cultivars ranged from 0.165 to 0.681. Polymorphic loci ranged from 80 to 100%, and a mean of 96.11%. The cultivar Medjool had the smallest genetic similarity estimates with the remaining cultivars (range from 0.165 to 0.5). Total variation (mean = 0.79, C.V. = 8.4%) ranged from 0.704 to 930; expected (He 0.499-684, mean 0.55, C.V. 8.3%) and observed (Ho 0.83-1.00, mean 0.92, C.V. 6.9%) heterozygosities differed substantially from each other and all fixation indices (mean = -0.68, C.V. = 18.5%) were negative due to excess heterozygosity. Estimates of the population differentiation (mean = 0.31, C.V. = 14.1%) ranged from 0.242 to 0.383 (mean of 0.303). Fixation index, but not population differentiation, was significantly correlated with total diversity ($r = 0.65; p = 0.05$); also expected, but not observed heterozygosity, was correlated with total diversity ($r = 0.73; p = 0.05$).

Figure 3. Examples of polymorphic bands that explain genetic diversity among five date palm cultivars (1-5) grown in three different locations (A, B, and C) in Qatar, using four primers (a, b, c and d).

(Reproduced with permission from Ahmed et al., 2013).
A recent study was carried out (Racchi et al., 2013) for the molecular typing of 377 female date palm trees belonging to 18 cultivars from Libya using 16 highly polymorphic SSR loci. The study reported scoring a total of 110 alleles with a mean of 6.88 alleles per locus. Results showed a high level of polymorphism among cultivars and found that 28 out of 110 alleles were fixed, and concluded that the fixation index was negative due to excess heterozygosity.

Another recent study (Zhao et al., 2013) identified 4,609 expressed sequence tag-SSRs and designed 4,967 primer pairs for EST-SSR markers for the computational data then tested 20 randomly selected primer pairs for amplification and polymorphism detection using genomic DNA from 12 date palm cultivars. As a result, 30% of these primer pairs detected DNA polymorphism and differentiated between the 12 cultivars. The study concluded that the date palm EST sequences provided a good source for developing gene-based markers in date palm. These markers may provide a valuable genetic and genomic tool for further genetic and genomic research and for the development of new cultivars through molecular breeding.

**RAPD and SSRs**

A combination of RAPD and SSR markers has been used in a few studies for different objectives; however, the complementarity between these markers was not discussed. Three examples illustrate the combined use of these markers in genetic diversity of date palm. Sex-specific DNA markers were identified in four date palm cultivars using RAPD and ISSR techniques (Younis et al., 2008). Polymorphism across cultivars was estimated at 0.70 and 0.87 as revealed by RAPD and SSRs, respectively.

A combined morphological and molecular (RAPD and ISSR) characterization of eight soft date palm cultivars from Egypt (Eissa et al., 2009) based on several diversity indices estimated on 40 tree and 36 fruit and seed traits. The Spearman correlation matrices between cultivars based on DNA markers ranged from 0.281 to 0.724 and between 0.693 and 0.807 based on the combined morphological and DNA markers. There were no similarities between both sets of data indicating that differences based on morphology are independent of those based on DNA markers. Finally, RAPD and SSR markers were used to identify 21 primers, and generated 79 markers that genotyped 45 date palm cultivars from Morocco to select tolerant cultivars to bayoud disease at an early seedling stage (Sedra, 2013). These markers showed a large level of polymorphism (~80%) and seven of them were selected as candidates to be associated with resistance to the disease. It is expected that this approach will be of value in breeding of date palm for disease resistance and contribute to developing a date palm genetic map.

**Expressed sequence tags (EST)**

A large-scale collection and annotation of gene models (based on assembled expressed sequence tags and mapped to a gene assembly) was developed for date palm (Zhang et al., 2012). Based on comparative analyses, these researchers found out that a range of date palm gene models are shared with rice (70.6%), sorghum (69.4%), Arabidopsis (68.4%), and grapevine (69.3%) then they assigned the date palm gene models into housekeeping and tissue-specific genes based on their tissue specificity. The study covered a wide range of tissues and their developmental stages. These EST markers will help designing future studies and discoveries on flower development, sex determination and prediction, and fruiting in date palm.

**Chloroplast (cp) and mitochondrial (mt) DNA**

Chloroplast (cp) and mitochondrial (mt) DNA techniques have been used recently in the study of date palm genetic diversity at the chloroplast and mitochondria levels. For example, Alquairainy et al. (2011) assessed the molecular signature from eight date palm cultivars from KSA using cpDNA sequences rpoB and psbA-trnH and concluded that the analysis clustered these cultivars into three groups with bootstrap values of 58 and 64%, and concluded that the sequences they obtained could be used as molecular signatures for potential date palm under trading and selection of genuine cultivars at the seedling stage. A recent study (Fang et al., 2012) presented a complete sequence and transcriptomic analyses of mitochondrial genome in three date palm cultivars. The researchers concluded that the mitochondrial genome encodes 38 proteins, 30 tRNAs, and three rRNAs (6.5% of gene content). The majority (93.5%) of the genome sequence was comprised of chloroplast-derived coding (10.3%) and non-coding sequences. The study showed obvious tissue-specific gene regulation patterns, especially for female and male flowers.
In vitro technique

Masmoudi-Allouche et al. (2008) managed to induce in vitro hermaphroditism in date palm female flowers, then, in a follow-up study; these researchers investigated the genetic variability related to in vitro floral hermaphroditism induction in the cultivar Deglet Noor from Tunisia. They identified two potential candidate gene markers that may enhance the understanding of flower development and sex identification in date palm.

Proteomics

A comparative 2-DE proteomic analysis of date palm somatic and zygotic embryos of the cultivar Deglet Noor was carried out in Algeria by Sghaier-Hammami et al. (2009). They found that most of the identified somatic embryos specific proteins belong to glycolysis pathway, whereas, those of the zygotic embryos belonged to storage and stress-related proteins. These findings will improve our knowledge on metabolism in the date palm and to understand the physiological differences between somatic and zygotic tissues. Eventually, the results may prove of value for direct regeneration of date palms from directly-sown zygotes and its value in boosting date palm genetic diversity.

Secondary statistics and genetic diversity parameters

Three kinds of parameters have been estimated from secondary statistics reported in several studies referenced in this paper. These were observed (H₀) and expected (Hₑ) heterozygosity (i.e., percent heterozygous genotypes in a population, which is a direct measure of the arrangement of alleles into genotypes), the fixation index (which measures the deviation of genotypic frequencies from panamictic expectations based on Hardy-Weinberg disequilibrium, Fis), and the polymorphic information content (PIC), which is a measure of gene diversity and ranges from 0.0 (monomorphic) to 1.0 (fully polymorphic). There were seven reports on H₀ and Hₑ, three reports on Fis, and five reports on PIC; in addition, a few papers reported on a combination of two or all three of these parameters.

Heterozygosity

Akkak et al. (2009) developed and evaluated SSRs and presented genetic diversity results (Table 3) indicating that all genetic diversity parameters were positively and significantly correlated, He was more variable than Ho, and the relationship between them was significant and non-linear. Whereas, PIC can be estimated using Ho as a predictor with R² = 98.0% but with much smaller level of certainty using He (R² = 47.6%).

\[ Hₑ = 0.4 - 1.24 \times H₀ + 2.12 \times (H₀)^2 \text{...[eq. 1]} \]

\[ R² = 49.0\% \]

The genetic diversity of the cultivated date palm is isolated from that of other Phoenix spp., but is highly structured at the phylogenetic and geographic levels (Pintaud et al., 2010). The date palm constitutes the main species and structural element of the date palm gardens that characterize the hot deserts of the Old World. The oases agroecosystems, perfectly adapted to the specific constraints of the environment, is largely shaped by the genetic diversity of the date palm.

Cherif et al. (2013) reported on male- and female-specific DNA markers and presented secondary statistics including number of alleles (A), H₀, Hₑ and Fis (Table 4). Significant differences were found between FA (0.468) and MS (1.00) for H₀ and between MS (-0.304) and each of MA (-0.012), and FA (-0.007) for Fis. Also, FS (-0.008) differed significantly from MS (-0.304) for Fis. On the other hand, both H₀ and Hₑ, but not Fis (r = 0.12; p = 0.53), were significantly correlated with A (r = 0.62 and 0.76, respectively; p<0.001).

Elmeer et al. (2011) assessed the genetic diversity of Qatari date palm cultivars using 10 SSR markers (Table 5) and presented statistics on major allele frequency, genetic diversity, Ho and PIC. Large variation (expressed as CV) was found for H₀ (62.2%), followed by major allele frequency (39.1%) as compared with the remaining parameters. Genetic diversity estimate and PIC were significantly and negatively correlated with frequency of major genes (r = -0.98; p<0.001).

<table>
<thead>
<tr>
<th>GD measure</th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
<th>Variance</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₀</td>
<td>0.66</td>
<td>0.16</td>
<td>0.85</td>
<td>0.039</td>
<td>29.7</td>
</tr>
<tr>
<td>Hₑ</td>
<td>0.58</td>
<td>0.04</td>
<td>0.96</td>
<td>0.075</td>
<td>47.3</td>
</tr>
<tr>
<td>PIC</td>
<td>0.63</td>
<td>0.15</td>
<td>0.83</td>
<td>0.04</td>
<td>31.9</td>
</tr>
</tbody>
</table>

Table 3. Genetic diversity estimates derived from secondary statistics reported on SSR markers in date palm and their transferability to other Phoenix species.

Source: Data compiled and reanalyzed from Akkak et al., 2009.
Table 4. Results of the analysis of variance between four groups of female and male autosomal and sex chromosomes in date palm genotypes.

<table>
<thead>
<tr>
<th>Variable</th>
<th>MS Effect</th>
<th>MS Error</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>11.5</td>
<td>21.0</td>
<td>0.55</td>
<td>0.66</td>
</tr>
<tr>
<td>Ho</td>
<td>0.22</td>
<td>0.052</td>
<td>4.0</td>
<td>0.04</td>
</tr>
<tr>
<td>He</td>
<td>0.06</td>
<td>0.067</td>
<td>0.88</td>
<td>0.48</td>
</tr>
<tr>
<td>Fis</td>
<td>0.07</td>
<td>0.065</td>
<td>10.9</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Source: Data compiled and reanalysed from Cherif et al. 2013.

Table 5. Summary statistics derived from genetic diversity information on Qatari date palm cultivars based on 10 SSR markers.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
<th>Variance</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAF</td>
<td>0.319</td>
<td>0.13</td>
<td>0.59</td>
<td>0.016</td>
<td>39.1</td>
</tr>
<tr>
<td>GD</td>
<td>0.797</td>
<td>0.60</td>
<td>0.90</td>
<td>0.007</td>
<td>10.5</td>
</tr>
<tr>
<td>Ho</td>
<td>0.416</td>
<td>0.00</td>
<td>0.91</td>
<td>0.067</td>
<td>62.2</td>
</tr>
<tr>
<td>PIC</td>
<td>0.773</td>
<td>0.55</td>
<td>0.89</td>
<td>0.009</td>
<td>12.3</td>
</tr>
</tbody>
</table>

Source: Data compiled and reanalysed from Elmeer et al., 2011.

Major allele frequency is expected to influence Ho as reported by Khierallah et al. (2011) who estimated genetic diversity in Iraqi date palm using two molecular SSR markers (Figure 4). A negative non-linear relationship was found between major allele frequency and Ho; with a significant correlation coefficient value ($r = -0.72; p = 0.0002$).

Observed heterozygosity can be predicted by the following equation:

$$Ho = 1.033 - 1.48 \times MAF + 0.49 \times (MAF)^2........[eq. 2]$$

$$R^2 = 52.0\%.$$  

A similar relationship was found in the same study between major allele frequency and genetic diversity (GD) estimate; where GD can be estimated as:

$$GD = 0.93 - 0.093 \times MAF -0.81 \times (MAF)^2........[eq. 3];$$

$$R^2 = 94.0\%.$$  

Elshibli and Korpelainen (2008) reported high genetic diversity in date palm germplasm from Sudan and presented data on number of alleles ($A_o$), besides $H_o$ and $H_e$ (Table 6). The data indicated a large level of diversity as quantified by $Ho$ (0.91) and $He$ (0.84); however, the negative correlations between $He$ and each of $A_o$ and $H_o$, although not significant, were unexpected.
Table 6. Mean and standard deviation, and correlation coefficients between several secondary statistics derived from genetic diversity analysis of date palm populations in Sudan using SSR markers.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
<th>r-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ao</td>
<td>9.56</td>
<td>1.397</td>
<td>0.68*</td>
</tr>
<tr>
<td>Ho</td>
<td>0.91</td>
<td>0.019</td>
<td>-0.26</td>
</tr>
<tr>
<td>He</td>
<td>0.84</td>
<td>0.026</td>
<td>-0.22</td>
</tr>
</tbody>
</table>

Source: Data compiled and reanalysed from Elshibli and Korpelainen, 2008.

However, a closer inspection of the data revealed a non-linear relationship between Ho and He (Figure 5). The non-linear relationship between Ho and He suggested a slight decline of He at larger levels of Ho. Expected heterozygosity can be predicted by the following equation:

\[ He = -2.9 + 8.8 \times Ho - 5.1 \times (Ho)^2 \] ....[eq. 4];

\[ R^2 = 0.05. \]

Racchi et al. (2013) genetically characterized Libyan date palm cultivars using SSR markers (Table 7). In this study, both He was found to be slightly larger than and significantly correlated (\( r = 0.79; p = 0.01 \)) with Ho; however, both were positively correlated with number of genotypes found in the study. On the other hand, there was an indication of a positive, but non-significant correlation between Ho and number of fixed alleles (\( r = 0.35, \text{ ns} \)); whereas He was totally independent of the number of fixed alleles.

**Fixation index**

The same study by Racchi et al. (2013) reported on the fixation index (Fis) and percent polymorphic loci (PPL), in addition to Ho and He. A nonlinear relationship was found between Fis and PPL, where Fis can be estimated as follows (Figure 6):

\[ Fis = 1.98 - 0.087 \times \text{PPL} + 0.0006 \times (\text{PPL})^2 \] ....[eq. 5];

\[ R^2 = 52.0\%. \]

Figure 5. Nonlinear relationship between expected (He) and observed (Ho) heterozygosity estimated on date palm populations from Sudan and based on SSR markers.
(Data compiled and reanalysed from Elshibli and Korpelainen, 2008).

Table 7. Descriptive statistics and correlation coefficients between four genetic diversity parameters estimated on Libyan date palm genetic resources using SSR markers.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
<th>r-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. genotypes</td>
<td>20.4</td>
<td>8.8</td>
<td>0.52*</td>
</tr>
<tr>
<td>Ho</td>
<td>0.714</td>
<td>0.21</td>
<td>0.71*</td>
</tr>
<tr>
<td>He</td>
<td>0.724</td>
<td>0.11</td>
<td>0.79*</td>
</tr>
<tr>
<td>Fixed alleles</td>
<td>177.3</td>
<td>55.52</td>
<td>-0.15</td>
</tr>
</tbody>
</table>

Source: Data compiled and reanalysed from Racchi et al., 2013.
Figure 6. Relationship between percent polymorphic loci and fixation index in Libyan date palm genetic resources assessed by SSR markers. (Data compiled and reanalysed from Racchi et al., 2013).

The relationship depicted in the regression line suggested that the variation in Fis increased with the increase in PPL, with a range of Fis between -0.8 to ~0.0 at the maximum (100%) PPL.

The relationship between these two parameters (i.e., Fis and PPL) and each of number of effective alleles (Ne), Ho, and He (Table 8) indicated that only Ho was not significantly correlated with Fis and PPL. The difference between the magnitude of the r-values between Ne and each of Fis and PPL is worth further exploration in date palm.

Table 8. Correlation coefficients between the fixation index and percent polymorphic loci and each of number of effective alleles (Ne), observed (Ho) and expected (He) heterozygosity in Libyan date palm genetic resources assessed by SSR markers.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ne</th>
<th>Ho</th>
<th>He</th>
<th>Fis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixation Index (Fis)</td>
<td>0.81</td>
<td>-0.15ns</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>Polymorphic loci %</td>
<td>0.57</td>
<td>0.35ns</td>
<td>0.77</td>
<td>0.72</td>
</tr>
</tbody>
</table>

The observed heterozygosity estimates reported by Cherif et al. (2013) on male- and female-specific DNA markers included secondary statistics on the fixation index of all four combinations of male and female autosomal and sex chromosomes. The relationships based on these four combinations were used to estimate Fis as a function of Ho, and plot the distribution of the four loci combinations (Figure 7).

Based on this relationship, Fis can be estimated as a function of Ho as follows:

\[ F_{is} = -0.33 + 1.47 \times Ho - 1.4 \times (Ho)^2 \]  
\[ R^2 = 18.0\% . \]

Most Fis estimates were negative and the two most heterozygous male-sex loci had the largest (most negative) fixation indices. The only study which reported on total diversity (HT), population differentiation (FST) and fixation index (Fis) was carried out on date palm cultivars of a Moroccan oasis (Figuig) and used SSR markers to determine the genetic diversity of date palms (Figure 8) in that oasis (Bodian et al., 2012).

The mean and standard deviation estimates of estimated genetic diversity parameters were as follows:

HT (0.79±0.07), He (0.55±0.05), Ho (0.92±0.06), Fis (-0.68±0.13), and FST (0.30±0.04).

Polymorphism information content

Two studies expressed the polymorphism information content (PIC) as a function of heterozygosity in Qatari date palm populations (Elmeer et al., 2011), and as a function of major allele frequency in Iraqi date palm populations (Kheirallah et al., 2011); both studies used SSRs as molecular markers. The PIC estimates ranged from 0.46 to 0.87 (Figure 9), and from 0.16 to 0.90 (Figure 10) in the first and second studies, respectively.
Figure 7. Scatter diagram of male and female autosomal and sex chromosome loci and the relationship between the fixation index (Fis) and observed heterozygosity (Ho) in Libyan date palm genetic resources assessed by SSR markers. 
(Data compiled and reanalysed from Cherif et al. (2013).)

Figure 8. Double nonlinear regression plot expressing fixation index and population differentiation coefficient as functions of total diversity in genetic diversity study of date palm population in the Moroccan oasis of Figuig. 
(Data compiled and reanalysed from Bodian et al., 2012.)

Figure 9. Relationship between observed heterozygosity (Ho) and population information content (PIC) in Qatari date palm populations assessed by SSR markers. 
(Data compiled and reanalysed from Elmeer et al., 2011.)
Figure 10. Relationship between major allele frequency (MAF) and population information content (PIC) in Iraqi date palm populations assessed by SSR markers. (Data compiled and reanalysed from Kheirallah et al., 2011)

Total diversity (HT) was significantly correlated with He (r = 0.73) and Fis (r = 0.65), but not with FST (r = 0.43); He was significantly correlated with Fis (r = 0.62), but not with FST (r = -0.30); and finally, Ho and He were not significantly correlated (r = 0.41). The PIC in Qatari date palms can be estimated using the following nonlinear relationship with observed heterozygosity:

$$\text{PIC} = 0.6 - 0.32 \times \text{Ho} + 0.61 \times (\text{Ho})^2$$

$$R^2 = 0.35.$$

The largest variation in PIC estimates were within the 0.4 to 0.6 Ho (Figure 8). A stronger relationship and better predictive ability was found for major allele frequency as a predictor of PIC in Iraqi date palm populations (Figure 9); the latter can be predicted using the following equation:

$$\text{PIC} = 0.97 - 0.47 \times \text{MAF} - 0.46 \times (\text{MAF})^2$$

$$R^2 = 94.0\%.$$

**Synthesis and assessment**

Future research on date palm will most certainly be carried out in the Middle Eastern and North African countries where dates are an important economic commodity and the date palm is a culturally significant fruit tree (Elhoumaizi et al., 2006; Eljuhani, 2010; Al-Khalifah et al., 2012). However, new production regions in North America, South America, Namibia and Australia may become of increasing importance for research and development as the center of origin and center of diversity of date palm becomes under increasing pressure of climate change (Jaradat, 2011; Pauls et al., 2013). Accurate estimates of genetic diversity and its partitioning, at the oasis, plantation, country and global levels, especially for fruit quality traits, tolerance to biotic and abiotic stresses within and among gene pools in the center of origin and center of diversity of the date palm are important considerations for the future of a successful date palm industry (Jaradat, 2011).

Genes or gene complexes of potential use in meeting future challenges may well be present in non-elite date palm cultivars found in traditional oases but their presence is largely unknown. Therefore, traditional farmers should be encouraged to replant date palm orchards and home gardens with locally-produced, highly heterozygous and heterogeneous offshoots or seedlings. Replacement of old or dead date palm trees with only elite and foreign cultivars, as is the case in Egypt (Elkhishin et al., 2003), Tunisia (Hammadi et al., 2009; 2011), and Sudan (Elshibli and Korpelainen, 2008; 2009) for example, will diminish genetic diversity and hasten genetic erosion of locally-adapted cultivars.

The overall partitioning of genetic diversity, based on results of the preceding analyses of phenotypic, biochemical and molecular markers, and fruit quality traits, suggests that date palm cultivars represent a complex gene pool within which historical movement of germplasm, recent introductions and human selection are shaping its genetic structure. The strong artificial selection and clonal propagation of the date palm greatly altered its original genetic structure. Although the
date palm may not be immediately threatened by genetic erosion; however, several reports directly (Eljuhani, 2011), or indirectly (Elshibli and Korpelainen, 2011) indicated that the level of genetic diversity as to the number of cultivars in oases is declining due to interacting anthropogenic, biotic and abiotic stresses, including desertification, salinity, bayoud disease and the red palm weevil (Jaradat, 2011).

Although most variation estimated for multiple fruit quality traits at a regional level was found to reside among populations, substantial differences were found in genetic diversity components among and within populations. However, several studies, based on isozyme and microsatellite markers (e.g., Hamwieh et al., 2010; Hamza et al., 2011; Khierallah et al., 2011) reported larger within-population than between-population genetic diversity levels of date palms in several North African countries and Sudan. Therefore, it is postulated that the long-term intra-and inter-country selection for specific traits resulted in a highly diverse germplasm in the center of origin and center of diversity of date palm. Detailed analyses of date palm populations originating from different geographic locations will help in understanding their genetic structures and will reveal the extent of gene flow between populations and its impact on population structure and fruit quality.

The reviewed literature and multilevel analyses of data presented in the referenced manuscripts indicated ample opportunities and dedication for date palm research and development through biotechnological research to identify and quantify genetic diversity components in the species, identify and clone genes and gene complexes for biotic and abiotic stresses, and utilize the generated information for future research and development. The assessment of inter-specific hybridization and introgression between species or subspecies, although still lacking, is becoming more important for the implementation of appropriate genetic conservation strategies and for the assessment of overall biodiversity and genetic diversity.

Efficient management of wild relatives of date palm is needed to identify and conserve the remaining unique populations and to evaluate the extent to which they are endangered by the introduction of the cultivated species or climate change. The new technology of genetic manipulation may allow the transfer of selected gene(s) to a specific genotype in only a single generation that would not be possible by conventional breeding.

Although clonal propagation using offshoots and tissue culture maintains heterozygosity and genetic purity, particularly of female date palm cultivars, it promotes genetic uniformity, may accelerate genetic erosion or enhance vulnerability of the date palm to environmental stresses and future climate change. Therefore, the maintenance of genetic variation within and among oases and modern plantations remains a central question in the study of evolutionary biology and the production of genetically-diverse populations of date palm. Strongly selected traits through mass propagation using tissue culture or other mass-production methods are expected to have low levels of genetic variance and lower heritability estimates; whereas, traits that are closely associated with fitness most probably will have higher levels of genetic variation but lower heritability estimates than weakly-selected traits.

Conclusions and Recommendations

Future advances in developing elite date palm cultivars will depend jointly on identification and development of stable phenotypic and molecular markers that may assist in identifying traits and cultivars of high economic and horticultural importance. Most referenced studies, with a few exceptions assessed genetic diversity in a few date palm cultivars with a limited geographical coverage, and rarely assessed this diversity over time. Research and development questions with significant impact on the future of date palm biodiversity and genetic diversity may revolve around the ability of the date palm to interact with other components of the biodiversity complex within oasis agroecosystems, and what are the practical implications of these interactions; how is the genetic diversity partitioned within and among populations and within and among traditional oasis and modern plantations; what are the scientific and practical implications for the conservation of this genetic diversity; what are the benefits and the dangers of mass vegetative reproduction of date palm through tissue culture, and what are the consequences of this technology on total diversity and vulnerability of the species; and where are the “hot-spots” for key tree traits for biotic and abiotic stress tolerance, and for fruit quality traits, and how to utilize these efficiently?

There is a need for capacity building and strengthening of existing research centers and the establishment of regional and national date palm field genebanks in the Middle East and North
Africa. Collectively, these would enhance research on current and future problems important to local production, both in traditional oases and modern plantations. Building relational databases on wild and domesticated species, tree and fruit phenotypic and biotechnological attributes of populations and cultivars, and the development of a “Digital Atlas” will help document and provide on-line information for research, conservation and sustainable utilization of date palm genetic resources. Finally, the development of alternative markets for date palm by-products will create incentives to grow more and diverse date palm cultivars, encourage the development of a wide range of products based on phenotypic and fruit traits diversities, and enhance the role of date palm as a functional genetic resource.

Acknowledgments

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References


