PHYLOGENETIC RELATIONSHIPS AMONG DATE PALM (*PHOENIX DACTYLIFERA* L.) CULTIVARS

Nadia Haider
Department of Molecular Biology and Biotechnology, AECS, P.O. Box 6091, Damascus, Syria.

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Contents

Summary

Date palm (*Phoenix dactylifera* L., Arecaceae) is probably the most ancient cultivated tree in the world. The ability of the date palm to withstand adverse environmental changes as well as its use in prevention and control of desertification, makes this crop a unique plant in the horticultural world. After domestication, date palms diversified across the species range and at the present time there are worldwide, countless varietal strains and cultivars. The present-day date palm cultivars are the outcome of thousands of years of selection practice of seedlings holding desired features. The lack of information about these cultivars reduces the usefulness of the potential diversity present among cultivars by institutions and potentials users. Therefore, understanding the genetic diversity in date palm is the key to its targeted genetic improvement programs through breeding for desired traits. Phylogeny is an important powerful tool for plant breeders because it points to a few close relatives as focal points for crop improvement. To aid the characterization of genetic diversity among date palm cultivars in order to determine their phylogenetic relationships based on this diversity,

morphological or/and molecular characterization of cultivars are carried out. Molecular markers (protein- and DNA-based) are powerful tools that have contributed immensely to assess genetic variations in the elucidation of genetic relationships among date palm cultivars. These markers have largely complimented morphological approaches in phylogenetic studies on date palm cultivars. This chapter will introduce the reader to all methods that have been used for detection of diversity among date palm cultivars and the construction of their phylogeny based on data generated.

1. Introduction

1.1. Date Palm Classification, Origin and Domestication

The first cultivated fruits must have been indigenous species that had obvious human value. This is clearly seen in Egypt where the indigenous date palm `tree of life` was the earliest species cultivated, followed by a succession of introduced fruits such as the sycamore fig and pomegranate. Date palm (*Phoenix dactylifera* L.) is a perennial monocotyledon fruit tree that belongs to the genus *Phoenix* L. This genus is the single member of tribe Phoeniceae in the palm family Arecaceae, formerly referred to as Palmae that also includes the commercially important oil and coconut Palms. *Phoenix* contains 14 species that are endemic to various regions stretching from islands off West Africa to East Asia.

The botanical or scientific name of date palm is presumably derived from the Greek meaning for color (*Phoenix*, purple or red) and shape (*dactylifera*, finger-like appearance) of its fruit. Date palm (normally n=18; 2n= 36) is a dioecious plant and hence requires cross-pollination either naturally (wind pollination) as in the case of traditional oasis or artificially by hand. In present-day commercial plantations, one male tree to 100 females is sufficient if pollination is performed by hand.

Date palm is probably the most ancient cultivated tree in the world and was well-established during the Bronze Age in the Middle East. Hence, it is intimately tied to the history of human migration. The history of its diffusion by the human population remains unclear, since the domestication remains poorly understood and still holds uncertainty due to its prehistoric presence and because few studies have attempted to document genetic diversity across multiple regions. It is also difficult to know the exact origin of date palm due to its complicated historical background, extensive cultivation trend, distribution, and exchange of date cultivars. Numerous hypotheses for the origin of date palm and the location of its domestication have been presented with little conclusive results. Because date palm is playing an integral role in the livelihood of the people living in the hot and barren desserts since ancient times, and was exclusively mentioned and depicted in old sculptures in many ancient civilizations, many countries claim themselves its center of origin. The Middle East has long been proposed as a primary center of date palm domestication. Archaeological remains of dates have been found on a number of Neolithic sites, particularly in Syria and Egypt. Based on the archeological record, its domestication, or earliest cultivation, is frequently believed to have occurred ∼5000-3000 BC in the Fertile Crescent (a crescent-shaped region in the Middle East, spanning modern-day Iraq, Syria, Lebanon, Palestine, Jordan, Egypt, together with the southeastern region of Turkey and the western fringes of Iran). Cyprus has been also included by some authors. Genetic analysis of *Phoenix* chloroplasts (plastids, organelles that conduct photosynthesis) suggested that date palm is genetically distinct enough to likely not have been domesticated from other *Phoenix* species.

1.2. Horticultural and Economic Importance of Date Palm

The ability of date palm to withstand adverse environmental changes such as drought, flooding, and extreme temperature fluctuations as well as its use in prevention and control of desertification, make this crop a unique plant in the horticultural world. Date palm covers a surface of about 800.000 ha and it is important for the life of about 100 millions of inhabitants. The regions that are used for the cultivation of dates occur from the South Western borders of Asia to the coasts of North Africa and from the Northern lands of Syria to the Indian Ocean. The total annual world production of dates amounts to 8.5 million metric tons, countries of the Middle East and North Africa being the largest producers. Date palm is an important horticultural crop as well as heart of oasis cultivation systems in the date palm-growing countries. It is widely cultivated for its edible sweet fruits mainly in the arid or semiarid zones of the world as the date fruit ripening requires long and hot summer with very low humidity. It is also cultivated for its medical uses (sore throat, colds, bronchial catarrh, cystitis, gonorrhea, edema, liver, and abdominal troubles) and many other purposes (religious, feed, brooms, and wood), making use of almost every part of the tree. The chemical composition of date palm fruits and the values of dates in food and its by-products as feed for livestock were described in literature. Date palm has a great socio-economic importance as it represents a source of income and nutrition to oases inhabitants, and creates favourable conditions for improving secondary crop culture like barley, alfalfa and cloves as forage. It also plays an essential role in the bionetwork, economy, and sociology of the Saharan ecosystem, representing a potentially vital function in future arable and modified environments. Potential of dates as natural antioxidant source and functional food for healthy diet and utilizing the waste of date palm fruit to biosynthesize polyhydroxyalkanoate bioplastics with favorable properties have been recently illustrated.

1.3. Date Palm Reproduction

The production of offshoots (suckers) by date palm tree is one of the principal characteristics of domesticates. For centuries date palm, despite its ability to outcross, has been cultivated using clonal propagation from off-shoots. Offshoots are more preferred for conventional propagation because they produce true-to-type trees with fruit quality identical to the mother tree. It is traditionally and mainly vegetatively propagated from offshoots produced by elite individual trees because cross-pollination results in new cultivars out-of-type with unknown characteristics. At present, all cultivars can be propagated in this manner and one to several offshoots may be removed each year.

Date palm can also be propagated by tissue culture or through seeds. Propagation of date palm cultivars using tissue culture provides thousands of plantlets from a single offshoot, which should be true-to type to the mother plant. Although new genotypes (a genotype is the complete heritable genetic identity) or forms of date palm, which are considered the main source of variation in date palms, are the results of propagation through seeds, about 50% of the seedlings are males when seeds are used for propagation. Seedlings that develop from seeds cannot be recognized until trees begin to bloom after 4-5 years except when using male-specific DNA markers. In the majority of countries producing date palm, however, this molecular tool does not exist, and the majority of farmers cannot afford it.

2. Date Palm Genetic Resources

Date palm genetic resources include modern cultivars, landraces (a landrace is a local cultivar that has been improved by traditional agricultural methods), obsolete cultivars (improved varieties of recent past, these are the varieties which were popular earlier and now have been replaced by new varieties), breeding lines (a pure line is a result of inbreeding where plants have certain characteristics that are the same through generations, or a group of genetically identical homozygous individuals that, when intercrossed, produce only offspring that are identical to their parents), and related wild species.

Wild relatives of date palm include *P. sylvestris*, *P. theophrasti*, *P. atlantica*, *P. canariensis*, and *P. reclinata*. Of these, the closest to date palm are *P. sylvestris* and *P. atlantica* according to chloroplastic data. Using whole plastome (the genome of a plastid) assemblies, phylogenetic analyses revealed that cultivated date palm accessions share the same clade (a taxonomic group of organisms classified together on the basis of homologous features traced to a common ancestor) with *P. sylvestris*, *P. pusilla* and *P. acaulis*, which are native to the Indian subcontinent, and *P. caespitosa* that is native to the Arabian Peninsula and the deserts of Somalia. The evolutionary history of date palm and its wild relatives was investigated by re-sequencing the genomes of date palm cultivars and five of its closest relatives. Populations of the wild ancestor species of date palm were discovered in remote and isolated mountainous locations of Oman. These populations were found to be genetically more diverse than and distinct from a representative sample of Middle Eastern cultivated date palms and exhibit rounded seed shapes resembling those of a close sister species and archeological samples, but not modern cultivars.

Most of the wild date palm germplasm (a germplasm is a collection of genetic resources for a crop) is already lost, and only a few natural populations are believed to exist. Wild date palms often produce small and nonpalatable fruits, while the domesticates are associated with larger size and considerable amounts of sweet pulp. There are possibly no examples of wild date palm due to the long history of exploitation and selection of date palm, although there may be a few apparently wild groves still growing around oases, springs, or seepage areas, most of which are the end result of an unknown number of acts of selection.

After domestication, date palms diversified across the species range and at the present time there are worldwide, countless varietal strains and cultivars. A cultivar is a subspecies classification describing plants varieties which are produced through artificial selection. Cultivar, the word, comes from a combination of cultivated and variety (culti (vated) + var (iety)). Different forms of the same species are considered varieties. When these varieties are then artificially selected by humans for particular traits, they become a cultivar. Cultivar is more formally defined as certain plants which can be distinguished from others by any characteristic. These characteristics always remain “true” in reproducing a cultivar either sexually or asexually. This means that the characteristics are controlled by a homozygous gene for that cultivar. Adapted cultivars have resulted from human and natural assortment. Human collection is built on characteristics of fruits and ability of plant to confront biotic and abiotic pressures. While noncommercial cultivars propagated through seeds have also gone through natural selection.

Date palm is exceptional among the fruit trees with the largest number of cultivars. Date palm cultivars exhibit substantial variation in fruit-related traits such as color, size, moisture, and sugar content. Early evidence of the diversification of fruit traits comes from archeological date stones which changed in size and shape consistent with selection for larger fruits. Increased size and quality of the fruit is associated with selection carried out under domestication.

The present-day date palm cultivars are the outcome of thousands of years of selection practice of seedlings holding desired features. Each cultivar is derived from a unique single seed, cloned and multiplied vegetatively. There are about 3000 or 5000 date palm cultivars exist globally. Of these, 60 are widely grown worldwide, some have been known for a thousand years, and about 10% of them have a commercial importance for their distinct fruit shapes, colors and sizes (mainly in arid lands from the west of North Africa to India). Each country has got its own top elite cultivars of commercial value. When the same cultivars are cultivated in other countries by replacing or altering its Arabic names into their local languages, the task of knowing the exact number numbers of cultivars found in the Arabian Peninsula, Northern Africa, and Middle East becomes difficult. The situation gets more critical when the same cultivar (e.g., Deglet Noor) exhibit different fruit types in different countries. In other words, various countries use similar names for different fruit or different fruit may have the same name.

Selection practice on date palm has altered fruit quality, storage, suckering, salt tolerance, and spininess. Dates are categorized into soft, semidry, and dry cultivars as per their moisture content, texture, fruit appearance, and sugar content.

The date cultivars most commonly grown worldwide are listed in Table 1.

Cultivar	**Country where the cultivar is popular**
Aabel	Common in Libya
Ajwah	From the town of Medina in Saudi Arabia
Al-Khunaizi	From the town of Qatif in Saudi Arabia
Amir Hajj or Amer Hajj	From Iraq
Aseel	From Pakistan
Abid Rahim	From Sudan, in Nigeria it is called *Dabino*
Barakawi	From Sudan
Barhee or barhi, Bireir	From Sudan
Dabbas	From United Arab Emirates

Datça	In Turkey
Deglet Noor	A leading date in Libya, Algeria, the United States, and Tunisia
Derrie or Dayri	From southern Iraq
Empress	Indio, California, United States
Fardh or Fard	Common in Oman
Ftimi or Alligue	Inland oases of Tunisia
Holwah (Halawi)	Common in Egypt
Haleema	In Hoon, Libya
Hayany (Hayani)	From Egypt
Iteema	Common in Algeria
Kenta	Common in Tunisia
Khadrawi or Khadrawy	A cultivar favored by many Arabs
Khalasah	Saudi Arabia
Khastawi (Khusatawi, Kustawy)	The leading soft date in Iraq
Khenaizi	From United Arab Emirates
Lulu	From United Arab Emirates
Maktoom, Manakbir	A large fruit that ripens early
Mazafati	From Iran
Medjool or (Majdool)	From Morocco, also grown in the United States, Saudi Arabia, South Africa, Jordan, and United Arab Emirates
Migraf (Mejraf)	Very popular in Southern Yemen
Mgmaget Ayuob	From Hun, Libya
Mishriq	From Sudan and Saudi Arabia
Mazafati or Mozafati	Iran, in particular in Kerman province
Nabtat-seyf	In Saudi Arabia
Piarom	From Iran
Rotab	From Iraq
Safawi	Mainly grown in Saudi Arabia in the Al-Madina region
Sag‘ai	From Saudi Arabia
Saidy (Saidi)	Popular in Libya
Sayer (Sayir)	Common in Khuzestan
Sukkary	Cultivated primarily in Al Qassim, Saudi Arabia
Sellaj	In Saudi Arabia
Indi	In Sri Lanka
Tagyat	Common in Libya
Tamej	In Libya
Thoory (Thuri)	Popular in Algeria
Umeljwary	In Libya
Umelkhashab	Saudi Arabia
Zahidi, Zaghloul	Essentially exclusive to Egypt

Table 1. Most commonly grown date palm cultivars worldwide.

3. Importance of Determination of Genetic Variation among Date Palm Cultivars

Genomic data suggests that interspecific hybridization contributed to the diversification of cultivated date palms based on evidence of introgressive hybridization in North Africa between Middle Eastern cultivated date palms and the wild Cretan palm *P. theophrasti*. The mixture of cultivars during sexual propagation by seeds is another very important source of variation in date palm. Variation among the different date palm cultivars can be found within a sequence of DNA which is the fundamental source of biodiversity.
The lack of information about any species genetic resources reduces the usefulness of the potential diversity present among cultivars by institutions and potentials users. Therefore, understanding the genetic diversity in date palm is the key to its targeted genetic improvement programs through breeding for desired traits. This also helps implement a strategy to strengthen cultivars preservation and restore genetic resources of date palm to control genetic erosion as well as for commercial valorisation of unknown cultivars and collecting and cataloguing the germplasm in established germplasm banks. It also allows establishment of genetic relationships among cultivars and tracing the evolutionary history of the species and understanding the process of perennial crop domestication. Hence, characterization of date palm cultivars should be the first step as it is an essential prerequisite for evaluation of this species diversity.

3.1. Conservation

Collections of date palm germplasm are fewer than for most other crops due to the relatively limited geographic area in which cultivation is possible and the relatively narrow base of genetic diversity present. Because date palm plays an important role as an income and employment source for people living in the growing areas and genetic conservation is reliant on understanding the extent, and distribution of the genetic diversity exists in the prevailing germplasm, date palm rich cultural and natural biodiversity requires a more comprehensive knowledge to keep date palm biodiversity intact through conservation of the existing material which is a prerequisite for sustainability.

Genetic diversity of date palm in the center of origin is threatened by factors such as desertification and ecosystem change. The continued selection pressures by man and shifts to fewer and more modern cultivars, beside the recent trend of establishing the date palm orchards with only few top commercial cultivars by replacing the poor yielding cultivars have also become the cause of rapid loss of numerous cultivars and erosion of natural diversity. The neglection of date palm resources for many years and the lack of effort made to expand cultivation are other factors that threat date palms. Urbanization of rural areas and biotic (Al-Wijam, red palm weevil (RPW), and bayoud) and abiotic (increased soil salinity and drought) stresses that cause a decrease in area of plantation and depletion of the gene pool of some existing cultivars may also lead to a decline in the production of dates as witnessed in Oman over the past decades. These stresses globally vary in level and nature of the problems as per cultivar, location, climate conditions, and cultural practices. Forestation, environmental pollution, and natural calamities such as cyclones, floods, or fire were also reported as possible causes of date palm extinction.

Date palm is also threatened in some countries (e.g., Algeria) by diverse factors such as the practice of monovarietal culture. The natural oasis consists mainly of seedling palms and usually produces inferior date fruits but exhibits rich diversity which needs to be maintained and conserved. These oases are frequently threatened by several pests (e.g., red palm weevil insect) and pathogens (e.g., bayoud and lethal yellowing) problems besides numerous abiotic factors which are disrupting and declining the oasis ecosystems. For instance, the Northern African countries, particularly Morocco and Algeria, are threatened by frequent and lethal infection with bayoud disease, caused by *Fusarium oxysporum* f. sp. *albedinis*. This disease has destroyed millions of palm trees in Morocco and Algeria during the last few decades and led to a decline in date palm populations and genetic diversity, and hence destabilizing the integrity of the oasis ecosystem.

An effective assessment of the genetic diversity of date palm cultivars is crucial for their conservation and exploitation of true-to-type elite material. Biodiversity of date palm can be conserved *in situ* (in place, on-farm conservation = the genetic diversity of target species is managed and maintained within the traditional agricultural or horticultural systems) and *ex situ* (seed bank, in vitro conservation/repository). *Ex situ* collections of date palm allow for a careful preservation of a specific genotype and documentation of characterization and evaluation data, reducing the chances of disease problems and permitting easier experimentation to be carried out. Cryopreservation involves maintaining of living cells and tissue organs at ultra low temperature or in liquid nitrogen (between -79 and -196 °C) for longer periods by halting all the metabolic activities and cell division.

Other than protecting date palm precious heritage from the threat of extinction, another prime goal for conserving large date palm genetic resources is for date palm breeding and improvement programs.

3.2. Breeding and Genetic Improvement

A deeper insight of the genetic diversity of date palm is becoming an urgent priority to guide the use of this diversity in breeding programs for desired traits such as higher fruit yield, disease resistance, abiotic stress tolerance, improved fruit quality, and resistance to changing climates, and hence improvement of its horticultural, nutritional and agricultural value. Therefore, a broad genetic base with a rich and diverse germplasm collection, which is the backbone of every successful crop improvement program, is required for progress in date palm breeding.

Traditional and modern date palm cultivars which have valuable traits are used by farmers and breeders to improve the productivity and quality of this crop. Since successful crosses were mostly within close phylogenetic groupings, knowledge and the assessment of the phylogenetic diversity and relationships among cultivars of date palm is critical to complete utilization of these cultivars in the rational development of breeding programs. Understanding cultivars phylogenetic relationships also provides significant information about date palm evolution.

4. Construction of Phylogeny for Determining the Relationships among Date Palm Cultivars

Phylogeny (the evolutionary history of a group of entities) is an important powerful tool for plant breeders because it points to a few close relatives as focal points for crop improvement. A phylogenetic tree is a diagram used to show how organisms are related to one another, or a series of relationships between cultivars; inferred based on their shared and unique characteristics. As long as data to be compared across different cultivars is available, it is possible to make a phylogenetic tree. In general, the more the information to be compared, the more accurate the tree will be. This means that a more accurate tree can be established by comparing entire genomes, instead of just a single gene.

The main aim of phylogeny reconstruction is to describe evolutionary relationships in terms of relative recency of common ancestry. These relationships are represented as a branching diagram, or tree, with branches joined by nodes and leading to terminals at the tips of the tree (Figure 1). There are three main types of relationship distinguished. These are monophyly, paraphyly and polyphyly. Monophyletic groups include all the descendants from a single ancestor, as well as that ancestor. Monophyletic and paraphyletic groups have a single evolutionary origin. A step by step guide to phylogeny reconstruction was presented in by Soltis and Soltis in 2003, and by Harrison and Langdale in 2006.

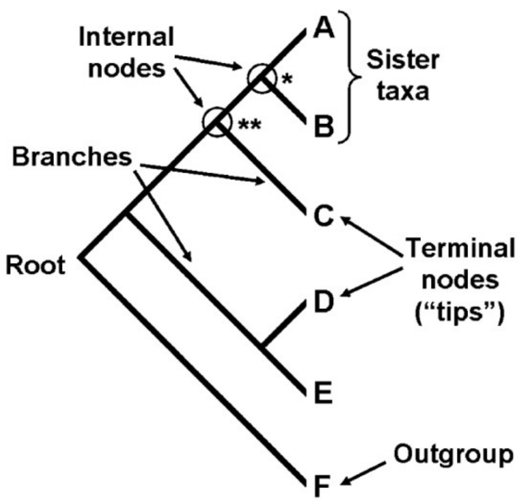

Figure 1. A branching diagram, or tree, with branches joined by nodes and leading to terminals at the tips of the tree (after Gregory 2008).

In a phylogenetic tree, each line is called a branch. The end of each branch is called a tip (this is where a cultivar is put). Each point where the two branches split is called a node. A node is the most recent common ancestor of all cultivars on those branches. And by going down to the bottom of the tree, the last node is called the root. This is the common ancestor of all the cultivars in the tree. The root (common ancestor for the whole group) refers to widely shared features, whereas the tips (individual cultivars) refer to more unique features (e.g., cultivar-specific features). Branching points (nodes) are common ancestors. Cultivars that are most closely related to each other are sister cultivars (e.g., Maktoom and Khastawi, Birbin and Ashrasi, Figure 2). Phylogenetic trees can be drawn in many different shapes. For instance, it can be rectangular or circular. The import part is how it is branched. The branching represents the differences in the relationship of cultivars.

Remember, the phylogenetic tree shows the order in which things took place and any phylogenetic tree is an evolutionary hypothesis that represents part of the greater whole. The branches do not account for length of time over which evolution occurred, unless otherwise indicated, and they reflect the evolutionary difference and the order of divergence among lineages, unless the data are specifically correlated to time with an evolutionary model. In phylogenetic trees, branches do not usually account for length of time. The individual branches do not necessarily evolve at the same rate or in the same way. Also, groups that are not closely related, but evolve under similar conditions, may appear more similar to each other than to a close relative. This means that closely related cultivars may not always look more alike, while cultivars that are not closely related yet evolved under similar conditions may appear more similar to each other.

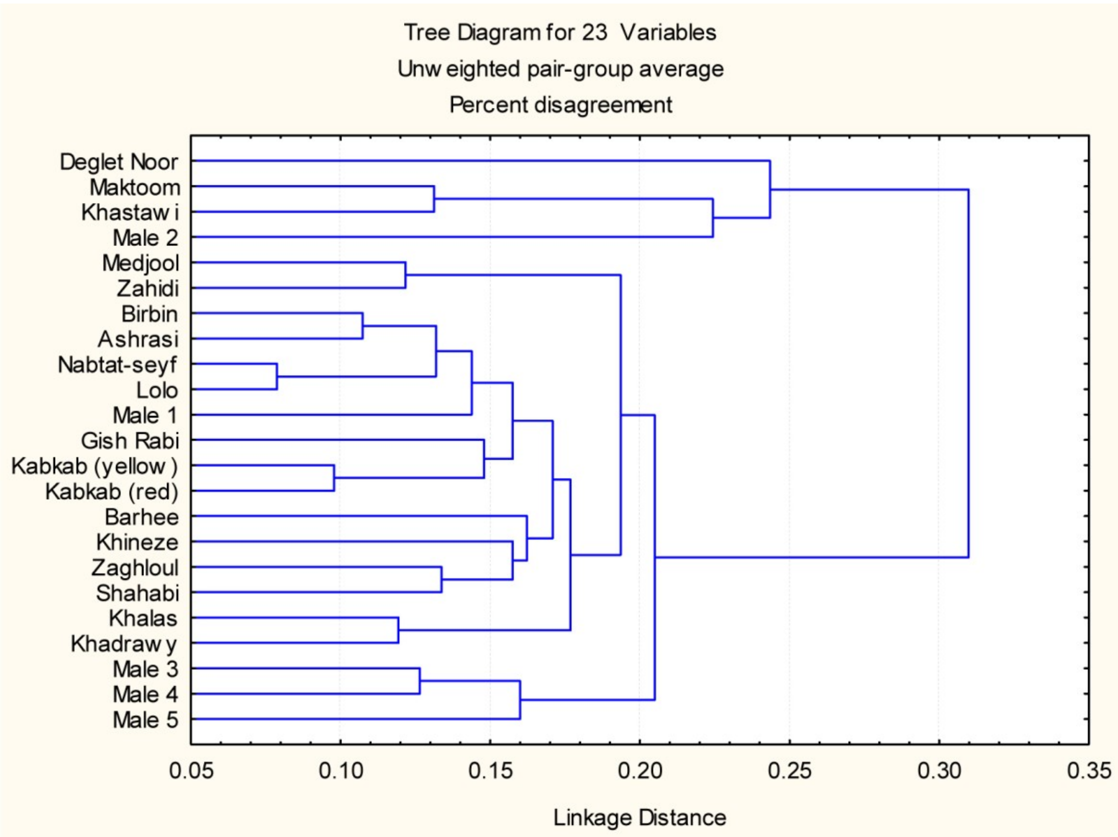

Figure 2. A phylogenetic tree of 18 date palm cultivars and 5 male date palm genotypes (after Haider *et al*. 2012).

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Biographical Sketches

Nadia Haider received her M.Sc. and Ph.D. from Reading University in Reading, United Kingdom. She was appointed as a researcher in 2004 at Department of Molecular Biology and Biotechnology, Atomic Energy Commission of Syria (AECS). In 2005, she spent six months as a postdoctoral researcher on “DNA-Barcoding of Plant Species” at Reading University, and was a co-author on two very highly cited articles on DNA barcoding of plants. She was promoted to Senior Researcher position in 2009. After 5 years, she was promoted to Research Director. She was invited by many refereed scientific journals to review manuscripts. She was registered as a peer reviewer for Physiology and Molecular Biology of Plants, and International Journal of Agriculture Sciences. She has been doing research for studying genetic diversity and identification of wheat, date palm, pear, hawthorn, orchids, legumes, iris, and Brassicas. She succeeded in designing a large set of plastid DNA-specific universal primers for flowering plants. She also worked on detection of food (coffee, tea, meat) adulteration using DNA markers. In 2015, she was a co-author on the paper that was published in Nature Communications on whole genome re-sequencing of 62 date palms cultivars. So far, she wrote 31 research papers, two research paper chapters, six review chapters, and five review articles and registered five patents in Syria, she was assigned in 2019 as the Head of Translation Authorship and Publishing Office in AECS.