Research Article

Utility of Morphological Features, Chemical Composition of Fruit and Chloroplast Genes in Date Palm (*Phoenix dactylifera* L.) Characterization of

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Abstract | Date palm, a dioecious evergreen woody tree of the Arecaceae family is an important fruit crop of Pakistan. In this study seven date palm cultivars (Dhaki, Aseel, Halawi, Qantar, Hamin wali, Kupra and Shakri) grown in Pakistan were characterized on the basis of seventeen morphological traits, five parameter of proximate composition of date fruit and sequence analysis of two genes (*maturase k* and *geranyl geranyl biphosphate reductase*) from chloroplast region. Analysis of variance has revealed significant variation among the studied date palm samples pertaining to the studied physical traits and chemical composition of fruit. Sequences of the *maturase k* gene has complete identity to the reference genome of 'Khalas' cultivar. While *geranyl geranyl biphosphate reductase* has one synonymous single nucleotide polymorphism in Qantar, Hamin wali, Kupra and Shakri, suggesting a lack of divergence in these genes in the studied cultivars. Tree plots generated showed inconsistencies in grouping of the cultivars thus showing no significant relationship of the plant morphology with the chemical composition of fruit.

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Introduction

Date palm (*Phoenix dactylifera* L.), a cross pollinated ever green woody plant with very long productive life, belongs to family *Arecaceae*. It has approximately 700Mb genome (Al-Dous et al., 2011). Date palm is exceptional among the fruits with the largest number of varieties (Afzal, 2005). In Pakistan more than 300 varieties of date palm exist (Jamil et al., 2010). Globalization has necessitated the need of standardized information about date palm germplasm for characterization, evaluation and conservation (Rizk and Sharabasy, 2006). Date palm is distributed widely and it occurs in diverse climatic, soil and geographical conditions. Domesticated and wild species have quite similarity in morphology and ecology but the fruit of wild plant is small in size and not edible (El-Hadrami and Al-Khayri, 2012). The edible portion of the date fruit is its pulp which is an important aspect of fruit quality (Iqbal et al., 2012).

Dates are rich in nutrients and are economical in production and preservation (Al-Shahib and Marshall, 2003).

Variation in a population, breeding line or germplasm accessions can be analyzed on the basis of pedigree data, agronomic traits, biochemical data, morphological attributes and DNA based molecular data. Characterization is important for genetic variability analysis of the cultivars and identification of the parental combination which may provide maximum diversity for selection, introduction of the desirable genes from diverse origin into the existing germplasm and in the identification of varieties for their protection (Mohammadi and Parasana, 2003). Female date palm trees are identified by their fruit traits while morphological characters are used for cultivar identification. Male plants are mostly seed grown so they do not resemble their female parents and are difficult to identify. Farmers identify them through their experience (Simozrag et al., 2016).

Rizk and El-Sharabsy (2006) suggested a set of descriptors for characterization of date palm to be helpful in diversity studies, establishment of gene bank and to conserve the specie. Characterization of various commercially important germplasm is primarily done by measurement of physical properties, yield parameters and fruit characteristics (Markhand et al. 2010; Jamil et al., 2010; Nadeem et al., 2011; Iqbal et al., 2011a, 2012b; Haider et al., 2013a, 2015b; Bashir et al., 2014; Naqvi et al., 2015).

Commercial cultivars of date palm have been disseminated by offshoots from oasis situated in lower Mesopotamia and Eastern Arabia. Cultivars propagated by offshoots are almost similar. 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 (Jaradat, 2011). Date palm cultivars vary in their reproductive traits so fruit qualities are commonly used for their identification (Hammadi et al., 2011).

The constant change in traits as a result of interaction between the genotypes and environment necessitates the regular update of morphological properties of plants. This knowledge can be used for crop improvement and to design equipment to be used for sorting, grading, cleaning and packing of fruit after harvest (Jahromi et al., 2007; Nadeem et al., 2011 and Odewale et al., 2012). Fruit chemical composition is important for physiological and technological studies. Quality of the date fruit changes with cultivar and depends on climatic conditions and farming practices (Hasnaoui et al., 2012).

In animals, only one gene, Cytochrome Oxidase 1 (CO1) is enough for phylogenetic studies but plants lack such a universal barcode. In plants both *rbcL* and *matK* along with some other suitable regions have been suggested to be used in phylogenetic studies by the consortium for barcode of life (Patwardhan, 2014). Since plastid genes are transferred mostly from the mother line so identification of maternal lines is possible by sequencing of plastid genes (Khew and Chia, 2011). *matK* encodes maturase needed for photosynthetic like activities of the chloroplast. It is a 1500bp gene located within the trnk intron (Barthet and Hilu, 2007). Resolution power of *matK* is better than other regions like rpoC, trnH-psbA, *rbcL*, atpF-atpH (Burgess et al., 2011). Genetic distance determined by *matK* among the cultivars ranged from 0.00-0.72 thus *matK* alone or in combination has the potential to distinguish among the cultivars (Enan and Ahmad, 2014). Intergeneric and interspecific nucleotide distances determined by *matK* were higher (0-10.9% and 0-52.5% respectively) as compared to 0-3.2% and 0-17.9% determined by *rbcL* for xerothermic plant of the Central Europe (Heise et al., 2015). Similarly the gene of *geranyl geranyl biphosphate reductase* (*GGR*) has also been shown to provide additional phylogenetic information to resolve species in certain cases (Bell, 2010).

Biochemical and morphological characteristics are less reliable in assessment of diversity and characterizations of the germplasm because these traits are few and environmental factors and growth stage of the plants affect these markers (Elhoumaizi et al., 2002; Ahmed and Al-Qaradawi, 2009). This necessitates the use of genetic characterization utilizing gene sequencing which can be reproducibly employed independently to any stage of the plant growth and are not affected by environmental factors. A combination of morphological, biochemical and molecular characterization of the date palm cultivar can better assess the level of diversity and relationship among the cultivars.

Materials and Methods

Morphological characterization

Seven date palm cultivars namely Dhaki, Aseel, Halawi, Qantar, Haminwali, Shakri and Kupra were selected and properly tagged for this study. The trees were of same age and well maintained with uniform cultural practices. Data were taken in triplicates. Seventeen morphological parameters from descriptors established by Rizk and Sharabasy (2006) for date palm were selected. The parameters studied were trunk diameter, length, width and base width of leaf, midrib length, length of midrib with spines, midrib length with spines, midrib length with pinnae, length of the top pinnae. Length, weight, diameter and volume of fruit, seed weight, pulp weight, and perianth height were also measured.

Leaves from second whirl below the canopy of selected trees were used for data scoring. Distance from leaf base to tip of the last pinnae was measured as leaf length and distance across the pinnae in the mid of leaf was taken as leaf width. Distance from the first spine at the base of the leaf to the last pinnae at the top was measured as midrib length. Midrib length with pinnae was measured from first pinnae at the base to the last pinnae at the tip. Midrib length with spines was measured from first spine at the base of the leaf to the last spine towards the pinnae. Leaf base width was measured at the base of the leaf. Number of pinnae and spines on the frond were also counted. Trunk diameter was calculated from circumference measured at a height of 4ft above the ground.

Fruit (500g) from every replication of each cultivar under study were collected from July to September at 'khalal' stage in properly labeled sampling bags and were brought to the laboratory (Figure 1). Fruit of all the three replicates of each cultivar were mixed separately and data were recorded for twenty randomly chosen dates per cultivar. Fruits were thoroughly rinsed with tap water to remove dirt and foreign material and were air-dried. Fruit length, diameter and perianth height were measured with digital vernier calipers. Digital balance was used to measure weight of fruit, pulp and seed. Water displacement method was used for determination of fruit volume. Individual fruits were dipped in graduated cylinders containing water. The difference in initial and final volume of water was measured as volume of the fruit. The mean fruit morphological data were averaged over 20 replicates.

Characterization on the basis of chemical composition

Sample preparation

After recording data for morphological parameters,

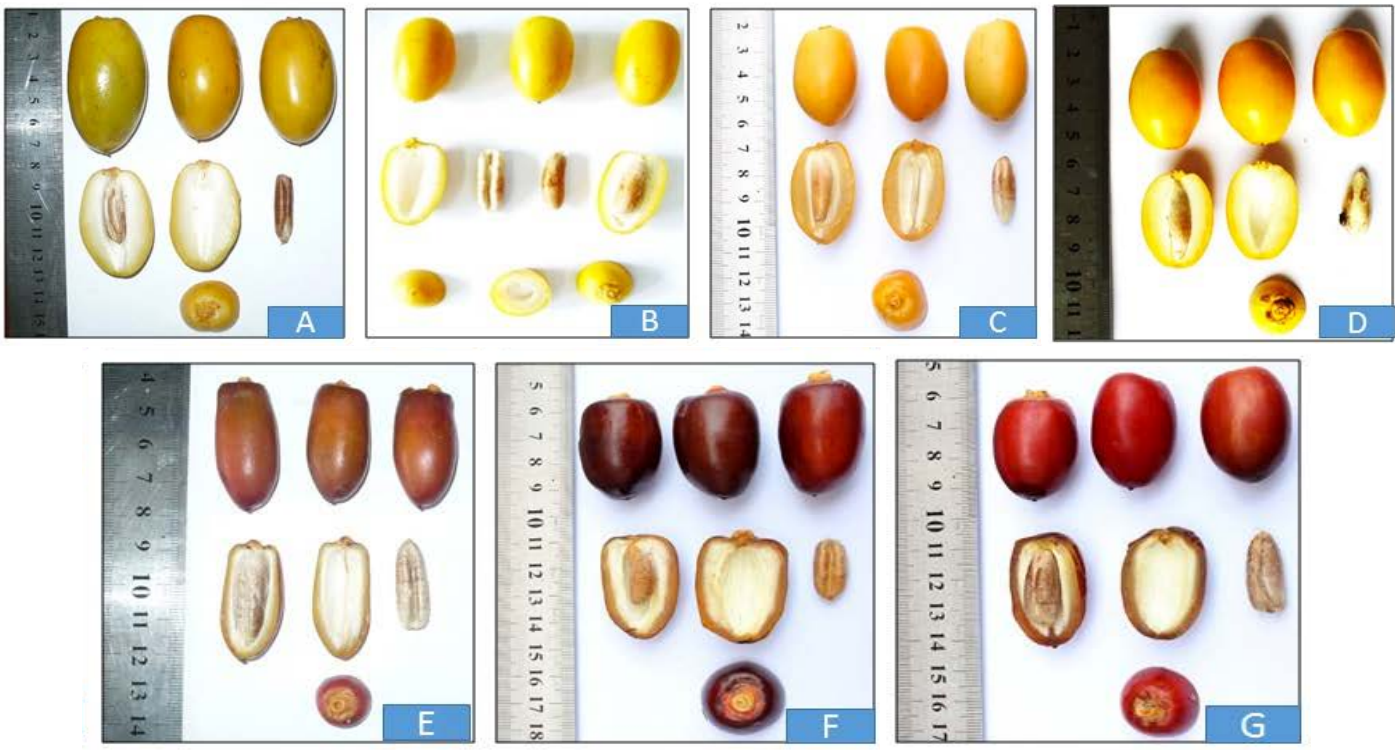

Figure 1: *Fruits of seven date palm cultivars at 'Khalal' stage.*
A: Dhaki, B: Halawi, C: Aseel, D: Qantar, E: Hamin wali, F: Kupra and G: Shakri.

the fruits were chopped finely and immediately subjected to moisture and ash determination. Remaining samples were stored at 4°C for further analysis.

% Moisture determination

Finely chopped pitted sample (20gm) was taken in a pre weighed empty moisture dish, the sample was heated in oven at 71°C for 72hrs. The sample was then kept in desiccator for cooling up to room temperature and was weighed again. Moisture content was determined by the formula of Horwitz and Latimar, (2007).

% Ash determination

Pitted chopped fruit sample (20 gm) was taken in pre weighed ash crucible. Sample was heated to 585°F in furnace for overnight. After cooling in desiccator, the sample was weighed again. Ash content was determined by the formula of Horwitz and Latimar, (2007).

Total soluble solid (TSS) determination

Digital Abbe Refractometer was used to measure total soluble solids by putting 2-3 drops of the fruit extract (obtained by squeezing 5 dates from each sample) on the prism of refractometer and recording the reading in °Brix.

Sugar content determination

Extraction of sugar

Finely chopped and pitted sample of date fruit (10gm) was taken in a beaker and 75 ml of water and ethanol each was added to it. This mixture was boiled for 1hr on flux system. After 1hr the balls of flux system were removed to let ethanol evaporate. When the volume reached to 100ml, the sample was removed from flux system, allowed to cool and then filtered. Distilled water was used to make the volume upto 100ml and this solution was neutralized using NaOH and HCl. The solution was divided into two halves of 50ml each to determine sugars by Lane and Eynon method described by Kirk and Sawyer (1991).

Molecular characterization

Sampling and DNA Isolation and quantification

Soft immature leaves from the suckers of trees tagged for morphological analysis were sampled in properly labelled sampling bags. Leaf samples were brought to the laboratory and preserved at -20°C till DNA extraction. DNA was extracted using CTAB method modified by Hyder et al. (2011). Gel electrophoresis and Nano spectrophotometer were used for quantitation of DNA.

Primer design/selection

Two chloroplast genes *geranyl geranyl biphosphate reductase* gene (*GGR*) and *maturase K* (*matK*) were chosen for molecular characterization. Primers (Table 1) were designed manually on the date palm sequences of these genes available online (www.ncbi.nlm.nih.gov/).

Sequence based molecular analysis of chloroplast DNA

For sequence based molecular evaluation the genes of *geranyl geranyl biphosphate reductase* (*GGR*) and *maturase K* (*matK*) from date palm chloroplast genome were amplified from the seven samples. For amplification of the selected chloroplast genes PCR reaction mixture and PCR thermal profile of Abdullah et al., (2016) with little modification was used. For confirmation of PCR, 1% agarose gel was used. The PCR product was then purified using commercially available kit by Promega, Madison, USA and sequenced commercially by MACROGEN Korea.

Data analysis

Morphological and proximate composition data analysis

Analysis of variance and Fisher's LSD test were done through Minitab version 16. The mean data for morphological traits and proximate composition were then converted to interval data and tree plots were calculated through NTSYSpc version 2.10J software.

Molecular data analysis

The sequence of both strands of every fragment of chloroplast DNA amplified from each sample was assembled separately using DNA Dragon-DNA Sequence Contig Assembler Software version 1.5.2 (www.sequentix.de) and when found identical in the

Table 1: *Sequences and details of primers for amplification of chloroplast gene/gene fragments*

Primers	Primer sequences	Tm (°C)	Coordinates of the primers (nt)	Reference sequence
matK	F 5'-ATGGAAGAATTACAAGGATATTTAGA-3' R 5'-AAGTCTCATCACGTCAACAAACCAATT-3'	53	1714-1740 3282-3256	NC_013991.2

GGR	F 5'-CCAAGTCATCAATGGCCTCT-3' R 3'-GACTACGACTACGCCATCGC-5'	60	230757-230776 230966-230947	NW_008246734.1

Table 2: *Accession numbers of sequences of chloroplast gene fragments submitted to the genbank*

Genes	Dhaki	Aseel	Halawi	Qantar	Haminwali	Kupra	Shakri
matK	KT803890	KT803889	KT803891	KT803892	KT803893	KT803894	KT803895
GGR	KT983259	KT983260	KT983261	KT983262	KT983263	KT983264	KT983265

**matk: maturase K, GGR: Geranyl Geranyl biphosphate Reductase*

triplicate samples of each cultivar was represented by a single sequence and used for sequence analysis. The consensus sequences for each cultivars for chloroplast gene/fragment were aligned in ClustalW Sequence Alignment program (Thompson et al., 1994) implemented in the Molecular Evolutionary Genetics Analysis Program (MEGA) version 6 (Tamura et al., 2013). The sequences were submitted to National Centre for Biotechnology Information (NCBI) Gen Bank database (Table 2).

Result and Discussion

Morphological characterization

The present study was conducted to characterize seven date palm cultivars on morphological, chemical and molecular basis. Significant variation among the cultivars studied was shown by analysis of variance. Trunk diameter ranged from 45.43-56.58cm (Table 3) showing variation among the cultivars but principal component analysis has not mentioned it to be a marker of varietal identification (Faqir et al., 2016). El-Merghany and Al-Daen (2014) while evaluating date palm cultivars grown under 'Toshky' conditions found no significant difference in trunk diameter of the studied cultivars. Similarly in an attempt to compare vegetative Barhee cultivar of date palm and its two seedlings strains with respect to vegetative morphological traits El-kosary et al. (2009) found slight variation in the trunk girth that was non-significant. In contrast Elsafi (2012) found trunk aspect to have the highest percent and cumulative variation in date palm accessions in Sudan. Although varieties differ in their trunk diameter to various degrees but this is not a good criterion for discrimination unless the trunk has a big difference (Afzal, 2005).

Leaf length of the date palm cultivars under study ranged from 317.3-385cm (Table 3). Al-wusaibai et al. (2014) recorded maximum leaf length of 470cm in Saudi date palm variety. Leaf length is an important characteristic that can discriminate among the cultivars (Faqir et al., 2016). Leaf width ranged from 68.7cm-94.7cm and statistically it is among the traits causing variability. Saleem et al. (2008) also found leaf width to be important discriminant among the cultivars. Leaf base width was found to be 5.33cm-8.67cm and is also a measure of variation among the studied cultivars. This is in contrast to El-Merghany and Al-Daen (2014) who found no significant difference in leaf base width of date palm cultivars under Toshki conditions. Spine number of the studied date palm cultivars ranged from 16-26.33. Afzal (2005) has mentioned thorns or spines of date palm to be important for date palm cultivars characterization. Hammadi et al. (2011) found spine number as useful for determination of maturity period and fruit consistency characteristics as this character is less affected by the environmental factors. Less number of spines and more leaflets area were used as distinguishing characters in date palm (Al-Wusaibai et al., 2014).

Highest fruit weight (26.03gm), volume (26.00cm^3), length (5.45cm), diameter (2.94cm), pulp weight (25.03gm) and seed weight (1.46 gm) were observed for Dhaki (Table 3). Nadeem et al. (2011) while studying date palm cultivars from Pakistan also observed Dhaki variety to have maximum weight and volume. Dhaki has more fruit weight than the highest fruit weight (12.78gm) observed in date palm cultivars from Sudan by Al Yahyai (2008). Fifty five percent of the date palm varieties from Qatar and 88% from Kuwait were classified on the basis of their fruit traits (Jaradat, 2014). Fruit characteristics like length, reducing and non reducing sugar were significantly different in the studied cultivars belonging to soft and dry types of dates of Toshky (ElMerghany and Al-Daen, 2014). Farag et al. (2012) found that fruit characteristics of Zaghloul cultivar of date palm are influenced by the type of pollinator used for pollination of the mother tree. Information about the qualitative and quantitative properties of fruit and its chemical composition is of equal importance to date processors and traders because these qualities determine the end

use of the raw material. Consumers are concerned about the taste and nutritional value of the date fruit only (Saleem, 2005). Physical properties are important to sort the fruits and to determine how many of these fruits will be placed in a package for transportation. This information is also helpful in machines for sorting, cleaning and kernel removal (Jahromi et al., 2008).

Characterization on the basis of chemical composition

Nutritional value and medicinal importance of date fruit depend upon chemical composition (Taain et al., 2013). Chemical composition of fruit changes with variety, environment, fruit developmental stage and the postharvest conditions. Moisture content was found to be maximum (76.23%) in Aseel (Table 3) with least total sugar percentage (18.44%). Ash percent is a measure of the nutritive quality of the food. Ash content of the seven date palm cultivars ranged from 1.49 to 3.55%. Herchi et al. (2014) while analyzing the date fruit and seed found that moisture content (9.23-11.17%) was the major component of date fruit after carbohydrates (78.69-83.46g/100g dry matter) while ash content varied from 1.18-1.64g/100g dry matter). Shaba et al. (2015) while investigating the nutritional content of date palm fruit, found the ash content in the range of 1.88±0.03% and moisture content in the range of 1.16±0.16%. Hamza et al. (2014) while evaluating the proximate composition variability in the date palm cultivars from Nigeria found ash content (22.1%-25.7%) more than the moisture content (20.3%-25.0%) after the major component of carbohydrates. Moisture content is directly related to perishability of the fruit (Yahaya et al., 2015). The high moisture content leads to a short storage life and vice versa and fruit will also be prone to microbial attack. Ash content was found to have non-significant difference among the studied varieties with maximum value of 1.80±0.01. Mohamed *et al.* (2014) found significant difference in date palm varieties from Sudan with respect to their chemical composition with moisture and ash content varying from 8.78-10.68% and 1.96-2.50% respectively.

Molecular characterization based on DNA sequencing of chloroplast genes

The genes of *matK* and *GGR* from date palm chloroplast genome from each cultivar were sequenced using primers specially designed for this purpose (Table 1) and submitted to GenBank (Table 2). The *matK* have a complete identity to reference date palm genome of Khalas and among the studied seven cultivars from Pakistan, while *geranyl geranyl biphosphate reductase* has a single synonymous SNP (A>G) present at 627nt in codon 209 in Qantar, Hamin wali, Khupra and Shakri, while rest was identical to reference Khalas genome. The sequencing of chloroplast genes has not enabled us to find genetic differences among cultivars grown in Pakistan. Akhtar et al. (2014) analyzed fifteen date palm cultivars from Sindh province of Pakistan on the basis of *Rps14* gene of chloroplast. They found very little genetic distance (0.001), low average evolutionary divergence (0.008) and low nucleotide diversity (0.007) thus concluding that the studied date palm varieties have high degree of similarity. This is in contrast to previous findings where *maturase K* (*matK*) was utilized for studying diversity among various plant species (Burgess et al., 2011; Patwardhan et al., 2014 and Heise et al., 2015) and cultivars (Enan and Ahmed, 2014).

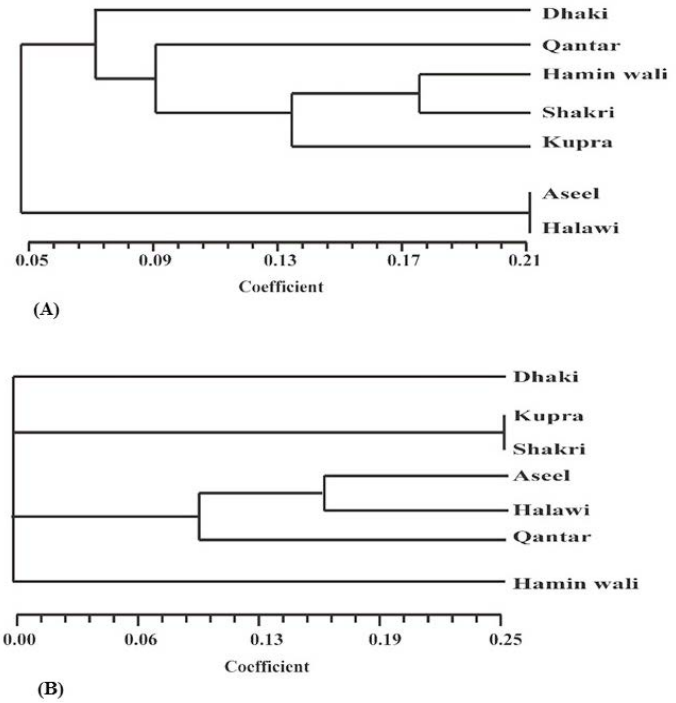

Figure 2: *A comparison of trees plots of morphological parameters and proximate analysis of Pakistani cultivars. A: The data of various morphological traits was subjected to ANOVA and means were compared by LSD test. The data were then converted into interval data for plotting tree using NTSYSpc 2.10j software, B: The tree of proximate analysis data was also plotted in similar way as described for m orphological tree.*

Comparison of tree plots generated for plant morphology and fruit proximate composition (Figure 2) revealed inconsistencies in grouping of Pakistani cultivars, suggesting that these factors were independent

of each other and no significant relationship of plant morphological traits existed with proximate composition of fruit. Morphological based phylogenetic approach is as important as molecular analysis based method as the structure of basic biomolecules is similar in all organisms and morphological characters are the reflection of their genome. Thus the combination of the two methods gives strength to the phylogenetic relationship of the organism (Patwardhan et al., 2014). Organisms which are similar phenotypically,

Table 3: *Data of important morphological descriptors and proximate composition of fruit of date palm cultivars studied from Pakistan*

Descriptors	Dhakki	Aseel	Halawi	Qantar	Hamin wali	Kupra	Shakri
Trunk Diameter (cm)	56.58±0.46 A	45.43±0.56 C	46.07±0.46 C	54.88±0.74 A	50.00±1.47 B	48.62±1.18 BC	48.41±1.75BC
Leaf Length (cm)	385±0.00 A	344±2.31 BC	338.7±6.96 BC	353.3±7.13 AB	333±0.76 BC	317.3±22.8 C	321±18.6 BC
Leaf Width (cm)	94.7±1.67 A	68.7±1.33 E	75.7±0.66 DE	90.7±0.66 AB	94.33±1.86 A	85.7±1.86 BC	80.0±5.13 CD
Leaf Base Width (cm)	8.00±0.00 AB	6.33±0.33 CD	7.33±0.33 BC	5.33±0.33 D	7.00±0.00 BC	7.00± 0.57 BC	8.67±0.33 A
Spine Number	24.33±0.33 A	26.33±0.33 A	17.33±0.33 C	20.66±0.33 B	25.33±1.33 A	16.00±1.73 C	25.67±1.20 A
Midrib Length with Spines (cm)	57.43±0.06 CD	100.0±0.00 A	75.33±2.33 B	59.67±1.67 CD	52.00±3.06 D	62.00±5.69 C	74.00±2.52B
Midrib Length (cm)	355.33±3.33 A	322.33±0.66 B	320.00±7.00 BC	323.33±7.33 B	315.33±3.18 BC	290.70±22.5 CD	285.00±7.77 D
Pinnae Number	189.67±1.67 BC	211.67±1.67 A	172.67±0.33 D	198.67±0.66 B	182.30±7.31 CD	150.67±5.21 E	189.33±3.18 BC
Midrib Length with Pinnae (cm)	295.00±5.00 A	120.67±0.33 F	146.33±5.67 E	071.67±8.33 G	231.67±9.28 C	251.00±1.00 B	202.00±3.06 D
Length of Top Pinnae (cm)	27.67±2.67 BC	21.33±0.66 D	23.66±0.66 CD	28.33±1.17 B	26.00±1.53 BC	26.66±0.88 BC	36.00±0.57 A
Fruit Weight (gm)	26.03±0.41 A	15.46±0.33 B	13.70±0.10 C	10.40±0.10 D	07.93±0.36 F	11.16±0.08 D	08.91±0.04 E
Fruit Volume (cm3)	26.00±0.00 A	14.66±0.66 B	14.00±0.00 B	10.00±0.57 D	08.33±0.66 E	12.00±0.00 C	09.16±0.16 DE
Fruit Diameter(cm)	2.94±0.02 A	2.58±0.00 B	2.26±0.01 C	2.26±0.02 C	2.12±0.03 D	2.53±0.02 B	2.23±0.00 C
Fruit Length (cm)	5.45±0.02 A	3.53±0.06 D	4.36±0.01 B	3.28±0.02 E	3.05±0.04 F	3.76±0.02 C	3.16±0.00 F
Pulp Weight (gm)	25.03±0.03 A	12.90±0.85 B	11.93±0.86 B	09.43±0.01 C	06.80±0.11 D	10.16±0.17 C	07.76±0.08 D
Seed Weight (gm)	1.46±0.08 A	1.00±0.05 D	1.30±0.00 B	1.00±0.00 D	1.13±0.06 CD	1.10±0.00 CD	1.20±0.00 BC
Perianth Height (mm)	2.00±0.22 BC	3.54±0.93 A	0.94±0.21 CD	2.80±0.19 AB	2.51±0.11 AB	2.27±0.21 B	0.67±0.18 D
% moisture	62.06±0.08 C	76.23±0.13 A	73.53±0.28 B	74.21±0.13 B	57.57±0.63 E	59.47±0.10 D	45.20±0.11 F
Total Soluble Sugar (Brix)	32.66±0.66 C	22.33±0.88 E	20.00±0.57 F	29.66±0.33 D	34.66±0.88 B	39.66±0.33 A	40.00±0.57 A
% Reducing Sugars	20.51±0.09 C	15.69±0.04 F	17.33±0.03 E	18.12±0.07 D	15.02±0.00 G	32.44±0.05 A	27.87±0.51 B
% Total Sugars	24.77±0.05 D	18.44±0.02 F	19.04±0.04 F	21.25±0.06 E	42.20±0.52 A	36.08±0.03 B	34.50±0.17 C
% ash	2.56±0.28 CD	3.40±0.24 AB	2.98±0.11 BC	3.55±0.04 A	1.49±0.00 E	2.41±0.03 D	2.26±0.02 D

The values are mean from three independent replicates except for fruit morphological traits where it is averaged on twenty replicates. Means that share same letter are not significantly different at 5% level of significance.

may be quite different in their molecular and biochemical characteristics. Thus determination of the phylogenetic relationship is very difficult as organisms show immense diversity through their molecular, biochemical and morphological characters (Patwardhan et al., 2014). Hammadi et al. (2011) found no correlation between molecular and morphological data. The reason may be that molecular variation is based on variation in DNA sequence only but morphological traits are affected by the environment.

Conclusions

The seven date palm cultivars studied showed significant morphological differences. A substantial amount of variation also existed in proximate composition of their fruits but molecular analysis showed almost identical DNA sequences of the studied gene fragments. Morphological and proximate analysis resulted in different grouping of the cultivars thus necessitating the need for in depth genetic study to find the molecular basis of variation among different date palm cultivars.

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Author's Contribution

Nadia Faqir: Performed the experiments, analyzed the data and wrote the article.
Aish Muhammad, Muhammad Zeeshan Hyder and Ghulam Muhammad Ali: Conceived and designed the study, contributed reagents/ material/analysis tools.
Armghan Shehzad: Analyzed the data, contributed reagents/ material/analysis tools
Hafeez Ur Rahman and Farhatullah: Conceived and designed the study

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