Research Article

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Genetic diversity of Libyan date palms cultivars using amplified fragment length polymorphism and biochemical analysis

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Abstract

Phoenix dactylifera L. is a flowering plant species commonly known as the date palm and is widely cultivated in most Middle East countries, including Libya. The present study analysed the biochemical and genetic diversity of fully mature eight Libyan date palm cultivars grown in different regions using the amplified fragments length polymorphism (AFLP) technique. Six pairs of AFLP molecular marker combinations were utilised to discriminate the eight date palm genotypes. Fruit dimensions (length x diameter) varied based on the type; Majhool Alheelo fruit had the highest value (15.29 cm^2), while the lowest value was for Alkhadraya fruits (7.9 cm^2). Reducing sugar content ranged from 10.4 per cent of flesh dry weight in Umfetity cultivars to 61.2 per cent in Sufeer-genab, which also showed the highest polymorphism percentage (P%=4.9), while Alkhadarya was the lowest (P%=0.519). The phylogenetic tree indicated that the most distantly related cultivars were Sufeer-genab, Alhamraya and Majhool Alhelo. The two most closely related cultivars were the Alsaeedy Show and Alkhadarya, grown in different regions. Our results indicate that the nutritional and genetic diversity of Libyan cultivars is not closely matched with the growing region.

Keywords: Biochemical properties, genetic diversity, Libyan date palm, phylogenetic tree

Introduction

Date palm (*Phoenix dactylifera L.*) is a perennial, dioecious crop belonging to Arecaceae. The palm predominantly grows in arid regions of Northern Africa and the Middle East (Al-Alawi *et al*., 2017). Besides its economic importance as a food crop, its nutritionally rich fruit contains about 44 to 88 per cent carbohydrates rendering palm fruit a great source of renewable energy (Siddiqi *et al*., 2020). The ability of this plant to tolerate heat stress attracted many researchers and breeders to investigate its genome. Recently, the complete nuclear and plastid genomes of this plant were fully sequenced, which facilitated the identification of the inter-varietal relationships and stress-related genes and the mechanism of sex determination (Moussouni *et al*., 2017).

Many PCR-based techniques such as amplified fragments length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), and inter simple sequence repeat (ISSR) markers were utilised to characterise the diversity of date palm trees in different countries (Azim, 2021). AFLP is a molecular genetic technique that detects DNA polymorphisms among different organisms by selectively amplifying DNA fragments digested with restriction enzymes to detect unique fingerprints for a particular genome (Zargar *et al*., 2017). This technique was mainly developed to assess genetic variation within or among closely

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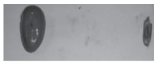
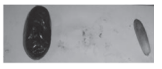
related species (Restrepo *et al*., 2018). AFLP technique can produce a large number of marker fragments in any organism without the need for a whole-genome sequence/annotated. It involves pre-selective amplification followed by selective amplification that generates specific molecular markers for a particular sample. AFLP analysis is a robust, multi-locus PCR-based DNA fingerprinting technique that can provide the most efficient, reliable, and economical population genetics analysis (El-Demerdash *et al*., 2019). AFLPs detect nuclear DNA markers inherited in a Mendelian fashion and provide a much greater level of polymorphism that cover a wider genomic area when compared to RAPD and ISSR (Zhao *et al*., 2018). Huda and coworkers (2019) reported that 90 million date palm trees are present in the world with an average life expectancy of 100 years each, and more than 70 per cent of these trees are grown in the Arab countries. For instance, it has been shown that date palms exhibited low genetic diversity in Morocco, Saudi Arabia, and Algeria but rather high diversity in Tunisia (El Kadri *et al*., 2019)**.**

Not many studies have been undertaken that have explored the genetic structure and diversity of date palm in Libya, where date palm cultivation is dominant compared to other crops due to favourable environmental conditions and the presence of many oases like Jalo, Aujla, and Ejkara. In the 1990s, around six million palm trees were cultivated in Libya to produce dates (Racchi and Camussi, 2018). The present work aimed to distinguish between eight palm trees commonly cultivated in Libya, focusing on the physical and chemical characteristic features of their fruit, such as fruit dimensions, seed and flesh weight, sugar and tannin contents and yield. The study also aimed to genetically discriminate the eight cultivars using the AFLP-PCR based technique, which can help in improving the economic value of palm cultivation.

Table 1. Location of sample collection the eight date palms

Traditional name of dates	City	Location	Date fruit and seed figure
Umfetity	Zliten	32° 30' 0" N 14° 34' 16" E	
Bekrary	Zliten	32° 30' 0" N 14° 34' 16" E	
Alhamraya	Al-Kufra	23° 18' 40" N 21° 51' 24" E	
Sufeer-genab	Al-Kufra	23° 18' 40" N 21° 51' 24" E	
Alsaeedy Show	Al-Kufra	23° 18' 40" N 21° 51' 24" E	
Faraj Barameel	Waddan	29° 9' 41" N 16° 8' 21" E	
Majhool Alheelo	Waddan	29° 9' 41" N 16° 8' 21" E	
Alkhadraya	Waddan	29° 9' 41" N 16° 8' 21" E	

Materials and methods

Collection of plant materials

Fully mature fruits and fresh leaves were collected from well-grown Libyan date palm trees at different localities, two types from each city (Table 1). The principal dimensions of date fruits in terms of length, breadth and thickness were measured using a Vernier caliper (Mitu and Toyo, Japan). The longest dimensions in the longitudinal direction were considered as length.

Physical and chemical characteristics of the fruits

The fruit length, diameter and weight of both the seeds and flesh were measured, in addition to the yield per year from each locality as described by Altaheri *et al*. (2019). The polysaccharide content of each date fruit was measured as described by Booij *et al*. (1992). Total tannin content was estimated in the dry flesh tissue of fully mature fruit using a modified method, *viz.,* vanillin-HCl in methanol. One gram of each sample was extracted with 20 mL of 1 per cent HCl in methanol for 20 min in a water bath, followed by centrifugation at 2,000 rpm for 4 minutes. The supernatant was reacted with 5 mL vanillin solution for 20 minutes. Blank was run with 4 per cent HCl in methanol, and the absorbance was read at 500 nm using a UV/VIS spectrophotometer (Al-Farsi *et al*., 2005).

DNA extraction

Genomic DNA was extracted (Gawel and Jarret, 1991) with some modifications. Leaf tissue was homogenised in 300 µL of freshly prepared and

autoclaved extraction buffer (1.4 M NaCl, 100 mM Tris-HCl, 20 mM EDTA, 0.2% ß-mercaptoethanol, pH 8.0). DNA pellet was precipitated in 500 µL cooled isopropanol, washed with 70 per cent ethanol and redissolved in TE buffer (10 mM Tris, 1 mM EDTA, pH 8). DNA quality was checked on a 1.2 per cent agarose gel. Samples were stored at 4°C until used for AFLP analysis.

AFLP Florescent dye primer protocol

AFLP florescent dye primer protocol was performed according to Becker *et al.* (1995). About 500 ng of DNA of each sample was mixed with restriction digestion/ligation solution. The reaction mixture was incubated for 2 hours at 37°C in a MWG-Biotech Primus 96 thermocycler. The PCR product was then diluted in a ratio of 1:50 with sterile ddH_2O, and 2 µL was used as a DNA template for the pre-amplification PCR using MWG-Biotech Primus 96 thermocycler. The reaction mixture resulting from this reaction was diluted at 1:20 then 1 µL was used as a DNA template for the selective amplification using MWG-Biotech Primus 96 thermocycler. One µL of each selective amplification was vortexed with standard Genescan-500 (LIZ) (0.5 µL) and deionised formamide (2.5 µL). DNA was then denatured by heating at 95°C for 5 minutes, followed by a quick chill on ice. Samples were loaded in the ABI Prism 310. The electrophoresis was performed at 60°C, DNA was injected into the capillary, and the peak scanner V2 was used. Peaks were converted into 0/1 and used to calculate the similarity indexes and genetic relationships among the genotypes under investigation. Similarity indices were calculated using the SPSS computer program based on the banding patterns of the six AFLP primer pairs. A consensus tree of the eight date palm trees was then constructed.

Results and discussion

Physical characters of fruit

Results of different fruit characteristics of the eight cultivars used in this study are presented in Figure 1. The highest fruit dimension (length x diameter) was recorded with Majhool Alheelo fruits (15.29 cm^2), while the lowest was in genotype Alkhadraya (7.9 cm^2). The highest annual yield was reported in the Alsaeedy Show and Bekrary (110 kg). However, the lowest yield was recorded for Majhool Alheelo, with only 45 kg per tree. The total fruit weight ranged from 11.94 g in Alkhadraya to 34.66 g in Majhool Alheelo. The flesh weight ranged between 10.75 g in Alkhadraya to 32.91 g in Majhool Alheelo. Seed weight ranged between 1.15 g in Alkhadraya to 1.86 g in Bekrary. These results indicated that Majhool Alheelo was the best in fruit quality but with lower total production per palm.

Chemical contents of the fruit

The results of chemical analysis of date fruit flesh depicting reducing sugar, non-reducing sugar and tannin contents of each type of selected Libyan date fruits are shown in Table 2. Sugars represent the most prevalent component in date fruit, which can be used as a sugar source (Benmeziane-Derradji, 2019). However, reducing sugars, including glucose, fructose, and non-reducing sucrose, are the main types of date fruits (Maqsood *et al*., 2020). The results showed that reducing sugar contents ranged from 10.40 per cent of flesh dry weight in genotype Umfetity to 61.20 per cent of flesh dry weight in genotype Sufeer-genab. Non-reducing sugar ranged from 26.30 per cent of flesh dry weight in genotype Sufeer-genab to 56.60 per cent of flesh dry weight in genotype Bekrary. The concentration of total sugar content ranged from 42.2 per cent of flesh dry weight in genotype Umfetity to 90.4 per cent of flesh dry weight in genotype Faraj Barameel. The results of tannin content showed that it ranged from 0.03 mg per 100 g flesh dry weight in genotype Alhamraya to 0.20 mg per 100 g flesh dry weight in genotype Umfetity.

At the stage of full maturity, sucrose is converted into glucose and fructose by the enzyme invertase, which is abundant in all date fruits. An exception to this is the variety Deglet Noor of North Africa and California, in which this inversion is partial at commercial maturity (Aljaloud *et al*., 2020)**.** Interestingly, it was claimed by Besbes *et al*. (2004) that the percentage of total sugar on a dry weight basis is very consistent among date fruits collected from different species all over the world. According to this claim, the average percentage of total sugar is within 70-88 per cent. Our data, on the other hand, indicated that the percentage of total

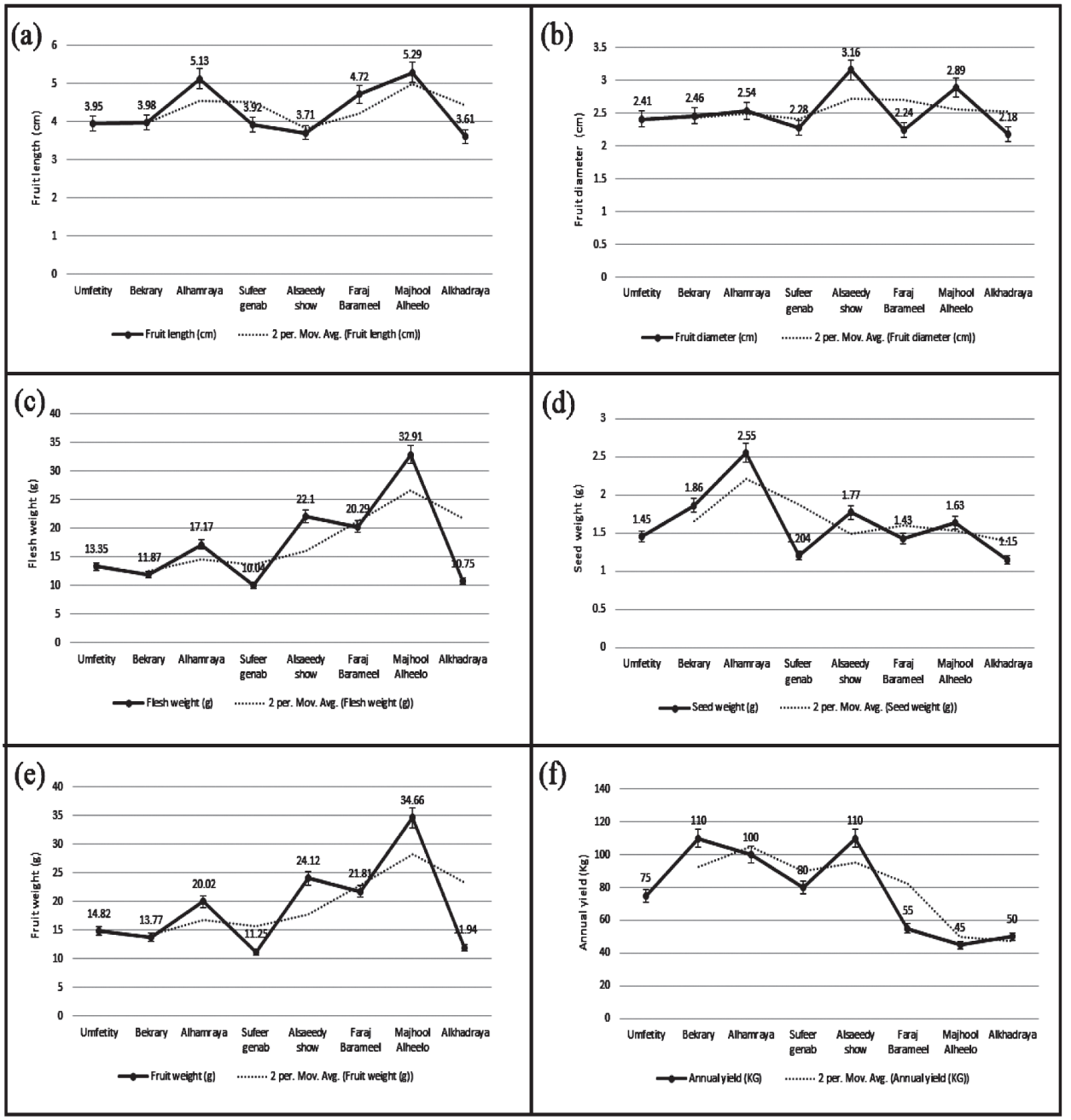


Fig. 1. The results of physical properties of date fruits; (a) fruit length, (b) fruit diameter, (c) flesh weight, (d) seed weight, (e) fruit weight, and (f) annual yield per tree

sugar in terms of dry weight is generally concomitant with this claim for Bekrary (88.5%), Alhamraya (88.9%), Sufeer-genab (88.9%), Alsaeedy show (88.4%), Majhool Alheelo (77.5%) and Alkhadraya (89.97%). However, the fruit of Umfetity showed an exceptionally low percentage of total sugars (42.18%) while Farag Barameel (90.37%) exhibited an exceptionally high amount of sugars. The higher quantity of reducing sugars in Sufeer-Genab (61.20%) and Faraj Barameel (58.69%) might be attributed to the relatively enhanced invertase enzyme activity in these

Table 2. Chemical analysis of sugar and tannin contents in eight genotypes of date palm

	Samples	Total sugars (% of flesh dw)	Reducing sugars (% of flesh dw)	Non-reducing sugars (% of flesh dw)	Tannin contents (mg 100 g^{-1})
1.	Umfetity	42.1 ± 2.1	10.4 ± 0.1	30.10 ± 2.1	0.20 ± 0.06
2.	Bekrary	88.5 ± 3.4	29.0 ± 0.6	56.60 ± 1.9	0.08 ± 0.01
3.	Alhamraya	88.8 ± 1.9	43.0 ± 1.3	43.10 ± 2.5	0.03 ± 0.02
4.	Sufeer-genab	88.8 ± 2.7	61.2 ± 2.1	26.30 ± 1.6	0.07 ± 0.03
5.	Alsaeedy Show	88.4 ± 2.6	38.5 ± 1.7	49.90 ± 3.1	0.08 ± 0.01
6.	Faraj Barameel	90.3 ± 3.3	58.6 ± 2.9	31.68 ± 2.2	0.10 ± 0.01
7.	Majhool Alheelo	77.4 ± 1.9	39.0 ± 0.8	38.37 ± 1.3	0.19 ± 0.02
8.	Alkhadarya	89.9 ± 1.6	35.3 ± 2.0	52.00 ± 4.1	0.09 ± 0.07

dw: dry weight

cultivars. A reverse situation might be assumed for the low amount of reducing sugars (10.40%) in the fruits of Umfetity.

Tannin content affects the taste and consumer preference in the market; tannins aggregates with proteins to form a strong, insoluble complex. Tannins are present in substantial amounts within immature fruits and are responsible for the high astringency making the fruits non-edible while still green. In the present work, the total tannin contents of the mature fruits of the eight cultivars under investigation were evidently below the toxic levels stated by De Nicola *et al*. (2004). Moreover, the content of tannins recorded in the Libyan cultivars was relatively below the recorded levels for either the Egyptian or the Saudi Arabian fully mature date fruits (Al-Tamim, 2014). However, the highest tannin content in the Libyan dates was detected in the fruits of Umfetity, Majhool Alheelo, and Faraj Barameel (0.20, 0.19, and 0.10 mg 100 g^{-1}, respectively). This suggests that the astringent taste in these cultivars that may affect their market value in Libya compared with other cultivars. Generally, all cultivars studied here contain very low tannins in their mature fruits. Therefore, they are predicted to cause little to no problem when consumed at high amounts by humans. The lowest tannin content was detected in the fruit of Alhamraya (0.03 mg 100 g^{-1}), and the highest was in Umfetity (0.20 mg 100 g^{-1}), as shown in Table 4. Considering these findings, it is highly recommended to improve many features in the cultivar Umfetity to increase its yield, fruit dimensions, and sugar level and decrease its tannin content to improve the taste and quality of its fruit production.

Molecular characteristics

AFLP analysis revealed a total of 963 amplified fragments which were produced with the mean of 160.5 amplicons per assay using six primer pair combinations and the eight Libyan date palm genotypes. Unique markers among the eight date

Table 3. Total number of unique fingerprints generated by primer pair combinations with each palm genotype

	Umfetity	Bekrary	Alhamraya	Sufeer-genab	Alsaeedy show	Faraj Barameel	Majhool Alheelo	Alkhadarya	Total product/ primer pair
pp1	1	2	2	13	0	0	4	0	99
pp2	1	4	0	8	0	3	3	0	167
pp3	1	5	6	11	0	5	1	1	222
pp4	1	3	4	9	0	11	1	1	221
pp5	2	0	0	5	2	5	3	0	119
pp6	1	2	2	2	4	4	4	3	135
Total unique	7	16	14	48	6	28	16	5	
P%	0.7268	1.6614	1.4537	4.9844	0.6230	2.9075	1.6614	0.5192	

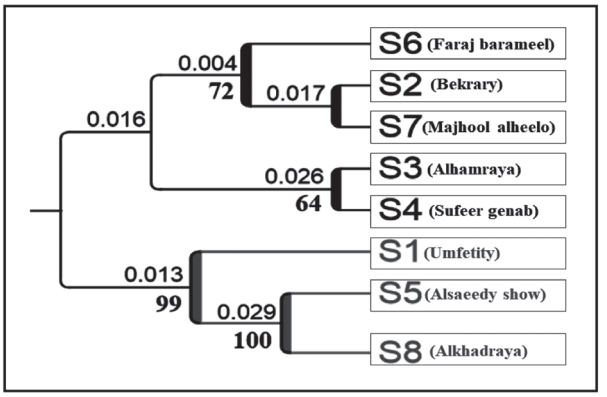


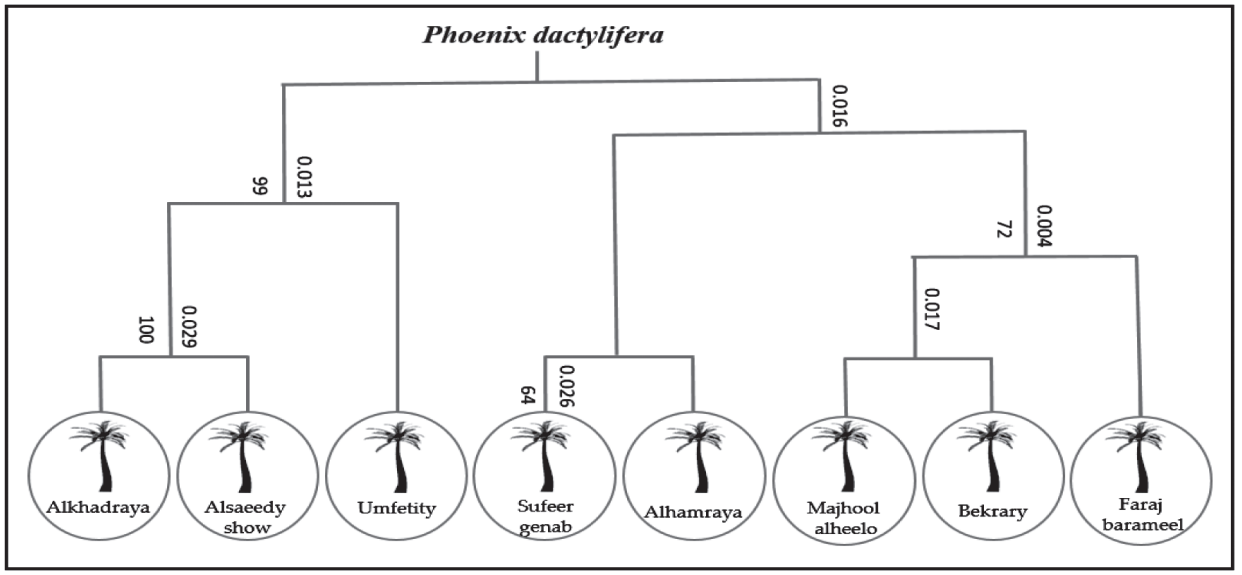
Fig. 2. Cluster analysis with UPGMA method of related eight Libyan date palm genotypes using six pairs of AFLP primer combinations of data based on Jaccard similarity matrix

palm genotypes are shown in Table 3. The total number of unique markers per genotype ranged from 5 to 48. The cultivar Sufeer-genab was characterised by the highest number of unique markers (48). In contrast, 5, 6, 7, 14, and 28 markers were specific for the cultivars Alkhadarya, Alsaeedy Show, Umfetity, Alhamraya and Faraj Barameel, respectively. The cultivars Bekrary and Majhool Alheelo showed an equal number of unique markers (16). The highest polymorphism was detected in Sufeer-genab (P%=4.9), followed by Faraj Barameel (P%=2.9), while the lowest polymorphism was detected in Alkhadarya (P%=0.519) (Table 3).

AFLP data were used to estimate the genetic similarity matrix value based on Jaccard's coefficient. The similarity values were further used to construct a dendrogram revealing the genetic relationships based on the un-weighted pair group method using arithmetic averages (UPGMA). The 963 AFLP amplified fragments grouped the palms into two main clusters, as shown in Figure 2. The first main cluster included palm genotypes Faraj Barameel, Bekrary, Majhool Alheelo in a sub-cluster and Alhamraya, Sufeer-genab in the other sub-cluster of the first main cluster. On the other hand, the second main cluster included palm genotype Umfetity in one sub-cluster while Alsaeedy show and Alkhadraya in the second sub-cluster. These results showed that Faraj-Barameel and Alkhadarya were the most distantly related genotypes and Bekrary and Majhool Alheelo were the most closely related genotypes.

Conclusion

Our results indicate that the nutritional and genetic diversity of Libyan cultivars is not closely matched with the growing region. Sufeer-genab, Alhamraya, and Majhool Alheelo were the most distantly related cultivars. This study will assist in selecting cultivars among the studied ones that span the major sub-populations for functional studies. Our findings suggest that future studies should sample, at minimum, from the three major regions



A consensus tree of the eight date palm trees

to cover better the natural Libyan date palm fruit differences, including other fruit properties. The work presented here will form an important foundation for genetic conservation and further functional analysis of the Libyan date palm genome.

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