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Genetic Diversity of Nigerian Date Palm (*Phoenix dactylifera*) Germplasm based on Microsatellite Markers

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Abstract

Characterization of date palm cultivars is a complex task using morphological traits alone since morphological markers are dependent on plant developmental stage and influenced by the environment. However, DNA fingerprinting can complement and enhance the discriminatory power of morphological traits. The study was conducted to investigate genetic diversity amongst fourteen cultivars of date palm (Phoenix dactylifera L.) from Nigeria and Saudi Arabia using microsatellite markers. The aim was to determine the genetic and geographical patterns of Nigeria and Saudi Arabia date palms. Molecular study conducted using six microsatellite markers employed on fourteen cultivars, ten from Nigeria and four from Saudi Arabia, revealed 83.3% polymorphism which indicated high genetic diversity among the cultivars studied. The amplified products ranged in size from 127 to 304 bp. A total of 42 alleles with an average of seven alleles per locus were scored. Two of the markers, MpdCIR025 and MpdCIR050, distinctively characterized six cultivars. This study indicated that variation observed among the cultivars followed a geographical pattern. However, this study was not able to show any alleles that might be linked to gender in date cultivars. Inclusion of more molecular markers in such a study might provide more accurate differentiation and possibly gender discrimination in date palm.

Keywords: *Characterization, Genetic diversity, Date palm, Microsatellite markers, Nigeria*

1. Introduction

Date palm (*Phoenix dactylifera* L.) of the family Arecaceae is dioecious trees and one of the most important members of the family. It is also one of the oldest cultivated crops grown in the desert and semi-desert areas, extending from North – West Africa and Asia.

Date palm cultivars have large number of homonyms and synonyms, due to its wide distribution and human influence through cultivation and selection that make it adaptable to wide environments [1, 2]. This also makes blurred distinction between wild and cultivars [3].

There are about 5,000 date palm cultivars [4], but farmer's preference choice has drastically restricted its genetic materials leading to predominance varieties with excellent fruit quality over others with poor fruit quality. For example, [5] reported genetic erosion in date palms in Tunisia caused by large cultivation of *Deglet Nour* variety which had higher preference over other varieties date farmers adjudged of poor quality. From the view above, it is obvious that most of the description of date cultivars is based on farmer's selection which

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is why there is no evidence of majority of cultivars being breeder's clones [6]. Also, their vulnerability to biotic and abiotic stress suggests an urgent need for their genetic conservation.

The date palm reproduces sexually and asexually. Offshoot propagation is the most common type of vegetative propagation because it maintains true to type cultivars, but numbers of offshoots produce in the date life span is limited and a good number of them do not survive adult stage [7-8]. It is contended that sexual reproduction is not a proper method of propagation because of its dioecy [7], heterozygosity and seed dormancy which consume time and money. Efforts have been made to use tissue culture propagation techniques to overcome the impracticality of sexual propagation but with limited success [1, 9-12]. For instance, there are reports of growth abnormalities like crop failure, loss of chlorophyll and failure to flower and fruit [13-15]. This method of propagation will also minimize the diversity of local genotypes. Groups that attempted to find sex- linked markers in date palm only identified markers that segregate sex in a few varieties [16], [17] only identified one region that was linked to gender but in broad sense, none have worked across the date genome [18]. Disease and drought are also reported to have contributed to a decline in date palm genetic diversity in North Africa [19-20]. The Bayoud disease resistant genotypes used are being propagated by tissue culture which is rather expensive and has its own constraints.

Date palms can be identified by presence of induplicate pinnate leaflets, spine pinnae, lower leaves modified to spine and a large bract in the inflorescence; as a result most of the characterization of different cultivars was based on fruits and morphological characters. Several authors reported genetic variation using morphological traits in dates. Both anatomical and morphological characteristics were used as taxonomic evidence to classify dates cultivars in Egypt [21], the result supports the general classification but could not establish characters that can discriminate, while [22] distinguished early maturing and late maturing varieties of date palms in Tunisia using vegetative descriptors, he also established six stable morphological characters not affected by environmental factors. While Fruits characters is mostly used to characterize date cultivars and landraces [23], long gestation period and environmental factor contributes to difficulty in date identification using fruit morphology. Date palm takes 5 – 7 years to fruit [7]- meaning that most of features for characterization could only be observed in adult plants and cannot be used to distinguish any date variety at seedling stage. Even though some morphological variation was reported to be influenced by genetic variation [24] some have narrow distinguishing morphological characters which complicate cultivar identification so requires further evidence like genetic markers [25]. Since many phenotypic studies carried on dates to unveil the genetic variation among date cultivars cannot precisely discriminate between them [22] and many of them recommended further study beyond only morphological features to distinguish between cultivars [6, 22, 26-28].

Apart from studies that found variation among date palms using anatomical and phytochemical characters of fruits by [29], biochemical studies using isozymes were used to characterize date cultivars in Tunisia, Morocco and Algeria [30-31]. However, these markers cannot give a direct assessment of genetic variation and have low level of polymorphism which made them only indirect techniques for assessment of genetic variation [6].

Molecular markers have been used to evaluate genotypic and phenotypic variation in date palm in the last decade. A gene of interest can be linked from molecular markers thereby allows identification of commercial varieties and indirect selection of desired genotypes [32]. DNA markers have yielded good results in some breeding programmes [33] and in studying crop genetic relationships [34-36]. Techniques used for DNA fingerprinting in date palm research are AFLP [37], RFLP [38], RAPD [39] and microsatellite [40-41]. These markers

had been used to identify different date cultivars singly or in combination with other markers for in-depth studies to see if they can complement one other [42-43]. RFLP was used in Egypt to identify 5 cultivars [44], while RAPD was used to identify various date cultivars in Morocco [28], Egypt [45] and Saudi Arabia. AFLP was used to identify date cultivars in California [46], Spain [32] and Egypt [47]. Microsatellite markers were used in Tunisia [5], Egypt [43], Qatar [48] and Sudan [6]. However, some of these markers could not distinguish cultivars according to geographical pattern [25, 46-47] while some detected low level of genetic diversity [42-43]. Probably, that is why recent studies used combination of molecular and morphological markers to increase the discriminating power for date palm identification [24, 49-50]. The studies using both markers are still new and may raise a new hope for proper identification of Date palm genotypes.

All the DNA markers have their merits for instance, AFLP and RAPD markers allow identification of large amount of number of loci without sequence knowledge and they are time saving and provide useful information about the genetic relationship and diversity in many fruit crops [44, 48, 51]. However, of all the markers used, microsatellite markers have been noted to be the most efficient strategy [52] because it can detect more genetic relationships and is more suited for population-based investigations [53], more informative for DNA fingerprinting [54] has high level of polymorphism and because of their abundance throughout Eukaryotic genome [40].

In Nigeria, Date Palm is restricted to compound homestead and orchards in the Northern parts where it is one of the most important tree crops [55]. It has annual production of 21, 700 Metric tonne [56]. A female date palm tree produces 10 – 75kg of fruits annually at maturity with a mean population yield of 255.4kg [57]. Date palm was introduced to Nigeria in the 17^{th} century through Trans – Sahara trade route from North Africa and Middle East and cultivated for edible fruits [58]. However, its long cultivation in Nigeria has provided several genotypes within the country upon which National germplasm collection depends on [59]. Nigerian Institute for Oil palm Research (NIFOR) has date palm as one of its six mandate crops and has embarked on several collections of land races and exotic varieties to establish a germplasm both for conservation against genetic erosion and for crop improvement studies. These collections have been used to establish seven gene banks [59]. Some of these landraces have been morphologically characterized based on fruit characters and broadly classified in to large, medium and small fruits [60]. Further evaluation of these land races identified superior genotypes capable of yielding up to 60 kg and above annually [57]. There is insufficient data on the description and variability in date palms grown in different part of Nigeria. However, recent review by [59] recommended further evaluation by molecular analysis to diagnose the genetic variability of the cultivars and facilitate selection for future breeding programmes and germplasm conservation.

Introduction of date palm to Nigeria and between countries has caused genes recombination and create genetic variation within and between countries making understanding of date cultivars at regional level important [25]. However, since use of molecular characterization of date palm in Nigeria is yet to be comparatively advanced, this is aimed at developing a date palm SSR identification toolkit for Nigerian germplasm and determines the genetic relationship between them. It will broaden source of information on gene bank of Date palm of the Nigerian Institute for Oil Palm Research (NIFOR) for proper characterization and selection for breeding programme.

2. Materials and Methods

2.1. Plant Materials

The study included 14 date palm (*Phoenix dactylifera* L.) cultivars representing 10 cultivars from Nigeria and 4 cultivars from Saudi Arabia. Nigerian materials comprise of 7 females and 3 males, while materials from Saudi Arabia are all females (Table 1). The cultivars were selected based on relative better yield performance.

Table 1. Name, Label and Origin of Date Palm (*Phoenix dactylifera* L.) Genotypes Studied

Varieties	Sex	Label	Location	Coordinates
Danmali	Male	18R20	Dutse, Nigeria	11 47 9.93 N, 9 27 08. 40 E
Deglet Noor	Female	18R1	Dutse, Nigeria	11 47 9.93 N, 9 27 08. 40 E
Makekashi b	Male	6R14	Dutse, Nigeria	11 47 9.93 N, 9 27 08. 40 E
Shuwarin b	Female	3R10	Dutse, Nigeria	11 47 9.93 N, 9 27 08. 40 E
Mangyare	Female	4R14	Dutse, Nigeria	11 47 9.93 N, 9 27 08. 40 E
Zabiya A	Female	9R8	Dutse, Nigeria	11 47 9.93 N, 9 27 08. 40 E
Zabiya b	Male	3R12	Dutse, Nigeria	11 47 9.93 N, 9 27 08. 40 E
Zabiya b	Male	3R12	Dutse, Nigeria	11 47 9.93 N, 9 27 08. 40 E
Shuwarin A	Female	3R7	Dutse, Nigeria	11 47 9.93 N, 9 27 08. 40 E
Tirgal	Female	7R21	Dutse, Nigeria	11 47 9.93 N, 9 27 08. 40 E
Anbara	Female	AN	AL-Madinah, Saudi Arabia	24 27 16.07 N, 39 37 45.51 E
Sukkrat	Female	SUK	AL-Madinah, Saudi Arabia	24 27 16.07 N, 39 37 45.51 E
Barni	Female	BA	AL-Madinah, Saudi Arabia	24 27 16.07 N, 39 37 45.51 E
Shaishee	Female	SH	Al-Ahsa, Saudi Arabia	25 27 41.53 N, 49 33 59.33 E

2.2. Location

Cultivars from Nigeria were collected in June, 2012 from the gene bank of Nigerian institute For Oil Palm Research (NIFOR) Date Palm Research Substation, Dutse, Jigawa State, Nigeria while Saudi materials were collected at Al - Ahsa National palms and Dates Research Centre, Al-Ahsa and Ba- Teb farms, Madinah region both in Saudi Arabia.
The Nigeria Substation is located between 11 47 9.93 N, 9 27 08. 40 E with altitude 454m, covering an area within the Sudan Savannah with annual rainfall of about 600mm per annum, average annual temperature of 32^0 C with sandy to loam soil. The climate is characterized with long dry season between October and May and short wet season between June and September which favors production of date palm. Al-Ahsa in Saudi Arabia is coastal plain and lowlands of Oasis situated on 25 27 41.53N, 49 33 59.93E with altitude of 147m [26]. The second location 24 27 16.07N 39 37 45.51E with altitude of 606m [26] has high topography influenced by volcanic activities and mineral rich soil.

2.3. DNA Extraction

DNA extraction was performed using modified CTAB protocol (Doyle & Doyle, 1987) and quantified by spectrometer (Nanodrop ™ lite model, Thermofisher scientific inc., Madison, USA). The isolated DNA were suspended in 100µL of TE (Tris-HCL[PH 8.0]

10mM, EDTA 1mM) and kept at 4^0 Cuntil required. The quality of DNA was assessed by running on 1.5% agarose gels in 1 × TAE buffer stained with ethidium bromide. Gels were illuminated with UV lights and photographs were taken to record the presence of DNA in the sample.

2.4. Polymerase Chain Reaction (PCR) Analysis

In other to identify primers that can reveal clear polymorphism, sixteen microsatellite (SSR) primers developed for date palms by [61] were used for this study. These microsatellites have previously reported to have revealed high genetic diversity among date cultivars [25, 48]. Initially, all samples were assayed with primers using reported protocol and seven primers that give clearer bands were later selected to amplifying specific fragments of expected size (Table 2). The PCR reaction was performed in a total reaction volume of 10.0µL containing 2 µL (25ng) of DNA as template, 5.0 ul of 2X PCR mater mix (Biomix, Bioscience, UK), 0.1µL each of the 10uM reverse and forward primers and 2.8 µL of nuclease free water. Amplification was performed in a thermo cycler (Applied Biosystems) with the following reaction conditions: Initial denaturation step at 95^0C for 5 min followed by 35 cycles of denaturing at 95^0C for 1 min; Annealing at 48.5^0C – 55.9^0C (depending on annealing temperature of primer) for 60s and 72^0 C for 2 min; and final elongation at 72^0C for 8 min. Forward primer for all seven primer set was labelled with florescent dye for accurate sizing through fragment analysis.. All PCR amplifications were run on a GeneAmp® PCR System 2700 machine (Applied Biosystems, Foster City).

The PCR products were separated on 2% agarose gels in 1 × TAE buffer stained with ethidium bromide. Gels were illuminated with UV lights and photographs were taken to record the presence of the PCR products (Fig 1). Approximate size of the PCR products was determined using HyperLadder IV (running from 100 bp to 1013 bp) as a marker. PCR products were sent for fragment analysis on GeneAmp® PCR System 2700 machine (Applied Biosystems, Foster City) using commercial services.

Table 2. Characteristics of 7 SSR Loci in 14 Cultivars of Date Palm (*Phoenix dactylifera* L.)

	Forward primers	Reverse Primers	Repeat motif	Annealing temp. ^{0}C/60sec.	Allelic bp
mpdCIR016	AGCGGGAAATGAAAAGGTAT	ATGAAAACGTGCCAAATGTC	$(GA)_{14}$	51.70	130 - 132
mpdCIR025	GCACGAGAAGGCTTATAGT	CCCCTCATTAGGATTCTAC	$(GA)_{22}$	49.30	210 - 230
mpdCIR032	CAAATCTTTGCCGTGAG	GGTGTGGAGTAATCATGTAGTAG	$(GA)_{19}$	51.50	300 - 305
mpdCIR050	CTGCCATTTCTTCTGAC	CACCATGCACAAAAATG	$(GA)_{21}$	48.50	170 - 210
mpdCIR057	AAGCAGCAGCCCTTCCGTAG	GTTCTCACTCGCCCAAAAATAC	$(GA)_{20}$	55.40	260 - 270
mpdCIR070	CAAGACCCAAGGCTAAC	GGAGGTGGCTTTGTAGTAT	$(GA)_{17}$	48.70	189-205
mpdCIR085	GAGAGAGGGTGGTGTTATT	TTCATCCAGAACCACAGTA	$(GA)_{29}$	50.40	160 – 180

2.5. Data Analysis

The bands were scored using Peak Scanner software (V 1.0 Applied Biosystems) and scored manually ‘1’ for present, ‘0’ for absent and ‘9’ for missing data. Out of the seven primers scored, one did not give a good amplification and have up to 60% missing data. As a result, only six primers were scored. These were used to generate binary data on excel spread sheet were used to estimate genetic similarity using Dice coefficients [62].

A dendrogram based on genetic distance was constructed from the similarity matrix using Unweighted Pair Group of Arithmetic Means (UPGMA) using NTSys pc2.2 (Exeter Software, Setauket, N.Y.) (Rohif, 2000).

The number of polymorphic bands was also scored, and this is tabulated and the percentages of polymorphism were obtained from this in order to estimate the genetic diversity.

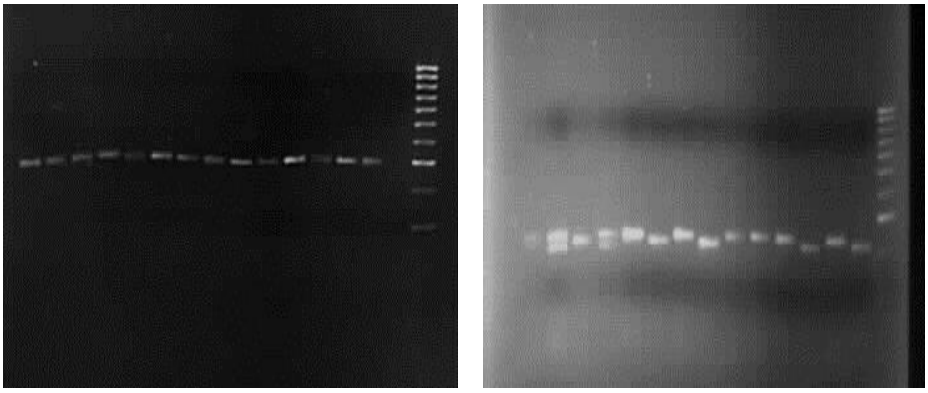

Figure 1. Images of PCR Products on Gels

3. Results and Discussions

3.1. DNA Extraction and PCR

The DNA extracted from 14 cultivars were quantified using spectrophotometer (Nanodrop ™ lite model) at wavelengths 260nm and 280 nm produced good results with DNA concentration ranging from 106. 7 – 219 ng/µl, A260 values ranges from 2.039 – 4.347 while A260/280 ranged from 1.9 – 2.05. All the 16 primers developed by [61] were tested initially out of which seven produced distinguishable bands in all the genotypes. These were then used for the final PCR and all produced distinct bands in accordance with expected base pair sizes (Fig 1).

3.2. SSR Amplification

The PCR products were run with florescent dye-labelled SSR primers were run on genetic analyser for fragment analysis and later identified using peak scanner software (Applied Biosystems v 1.0). The primers produced a uniform distribution at the peak region analysed with range 127 - 304 bp. All the primers used showed amplification of DNA fragments.

3.3. Level of Polymorphism

The six primers used to estimate genetic relationships among the selected 14 date cultivars produced several polymorphic bands and the result is given in Table 3. A total of 42 alleles were scored with an average of seven alleles per locus. The number of alleles varied among the markers and across the genotype. The numbers of polymorphic alleles are 35 with an average of 5.83 polymorphic alleles per locus. The average percentage of polymorphism is 83.33 which indicate a high genetic diversity. The number of alleles per locus ranged from 4 for locus mpdCIR016 to 11 for mpdCIR050. All genotypes have 2 alleles per locus across all the primers except primer mpdCIR070 in which most genotypes had a single allele. MpdCIR050 locus produces the highest number of alleles that are all polymorphic. Therefore, this primer will be useful in the routine characterization of date palm cultivars.

Table 3. Summary of SSR Allele Data Revealed by Six SSR Loci in Fourteen Cultivars of Date Palm

Locus	Allelic range (bp)	Numbers of alleles	Polymorphic alleles	% polymorphism
mpdCIR016	127 – 135	4	2	50
mpdCIR025	200 – 234	6	6	100
mpdCIR032	288 – 304	7	5	71.43
mpdCIR050	152 – 204	11	11	100
mpdCIR070	186 – 209	6	3	50
mpdCIR085	156 – 178	8	8	100
Total		**42**	**35**	
Mean		**6**	**5.83**	**83.33**

3.4. Genetic Diversity

The band pattern was converted in to binary data in Excel work sheet and was analysed using Dice coefficient to produce a similarity matrix showing the genetic distance between the fourteen cultivars studied (Table 4). The similarity matrix was clustered in to dendrogram (Figure 2) to separate the cultivars in to different groupings. Average similarity coefficient ranges from 0.13 – 0.83, the highest similarity was between Anbara and Shaishee cultivars (83%) from Saudi Arabia, while the least similarity (most dissimilar) was between Mangyare and Barni Al-madinah (10%). Deglet Noor cultivar showed the most dissimilarity from all other cultivars.

The dendogram showed the genetic relationship among the 14 studied cultivars. It consists of two main clusters A and B, with two sub clusters within cluster B. both groups clearly distinguish between Nigerian and Saudi Cultivars. However, Deglet Noor from Nigeria is separated from the rest of the group while Tirgal is another Nigerian cultivar that clustered with the Saudi group but with a long genetic distance from the Saudi cultivars.

Cluster B mainly of Nigerian varieties has two sub clusters B1 and B2. B1 includes cultivar Shuwarin b, Mangyare , Zabiya A and zabiya B while B2 includes Shuwarin a, Danmali, Makekashi A and Makekashi B. This indicates that these cultivars are having close genetic relationship and cannot be useful for future breeding programmes. Saudi cultivars are nested between Deglet Noor and Tirgal which indicates a possibility of hybridization between the two cultivars. In this study, genetic variability of Date palm cultivars in Nigeria was detected for the first time using microsatellite markers. It also demonstrates a polymorphism of microsatellite markers among Date cultivars so that they can be separated into groups to allow comparison between cultivars. The percentage of polymorphism in this study (83.33%) demonstrated high genetic diversity among the fourteen cultivars which indicates interrelationship between cultivars inspite of morphological traits. This observation supports earlier recommendations on the importance of molecular tools in studies of variability in Date palms [25, 28, 52].

The study also detected a total of 42 alleles with an average of seven alleles per locus of which 35 are polymorphic. [5] Obtained similar results of 7.2 alleles per locus when they used the same type of markers for date palm cultivars from Tunisia. The number of alleles per locus detected is also higher than that obtained by [48] who detected four alleles per locus using microsatellite for fifteen Qatari Date palm germplasm. The high polymorphism reported may be due to the dioecious nature of Date palms [5].

The data obtained from the primers used to generate a similarity matrix among the fourteen Date palm cultivars using Dice coefficient revealed the highest and least similarity of 83% and 13% respectively. The cultivars that revealed highest similarity values are both from the same region of Saudi Arabia [26]. Deglet Nour variety from Nigeria which showed the most

dissimilarity with other cultivars is an internationally recognized cultivar reported to have originated from Tunisian [6]. It is presence in many countries suggests that it may have been deliberately introduced as an exotic variety in Nigeria and other country where it is found.

Also, the similarity matrix showed 100% dissimilarity between some cultivars, this may be due to some missing values arising from the type of markers used and their limited numbers. Among the six markers studied, two may be useful for characterization of Date palms. *MpdCIR025* marker characterized all four cultivars from Saudi Arabia with alleles at 214 and 216 base pair (bp) which are absent from cultivars from Nigeria. MpdCIR050 markers also characterized two Nigerian cultivars, Zabiya A showing alleles at 152 and 160 base pair (bp) which are absent from other cultivars studied; Makekashi A scored for alleles at 180 and 182 base pairs (bp) which are absent from all other cultivars studied. This also confirms that microsatellite markers are effective for DNA fingerprinting of Date palm cultivars and evaluation of their genetic variability. However, this study was not able to show any promising alleles that might be linked to gender in Date cultivars.

The dendrogram generated from the similarity matrix was able to separate Nigerian cultivars from Saudi Arabia cultivars in the two main clusters thus confirming variation observed among the cultivars follow a geographical pattern. This inference agrees with [25] who reported differentiation between Moroccan and Sudan cultivars from using microsatellite markers. This however conflicts with other reports [52] whose DNA studies did not show any defined geographic pattern was because they used only cultivars from different locations within the same countries as opposed to this study which compare cultivars from the two different countries.

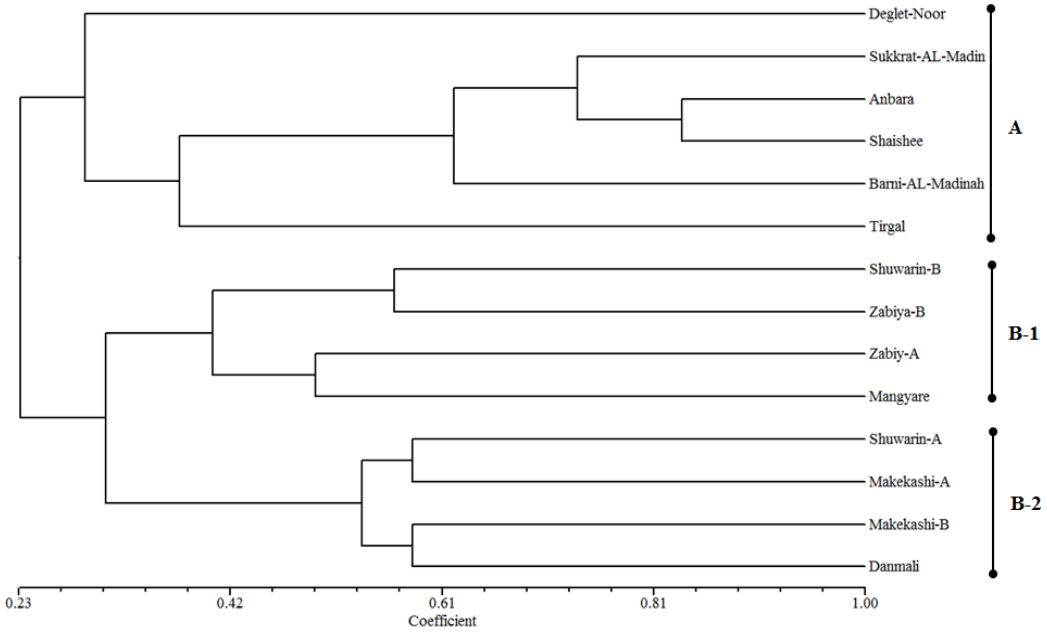

Figure 2. Dendrogram derived from a UPMGA Analysis using Dice Coefficient based on 35 Polymorphic SSR bands Showing Relationship among 14 Date Palm Cultivars

Table 4. Similarity Matrix of the 14 Date Palm Genotypes Calculated on the Basis of Simple Sequence Repeat Marker Data based on Dice (1945)

	Deglet Noor	Shuwarin B	Al Madinah	Zabiya B	Zabiya A	Anbara	Shuwarin A	Tirgal	Makekashi A	Makekashi B	Mangyare	Shaishee	B. Al Madinah	Danmali
Deglet Noor	1.00													
Shuwarin B	0.00	1.00												
Sukkrat Al Madinah	0.27	0.20	1.00											
Zabiya B	0.11	0.57	0.13	1.00										
Zabiy A	0.00	0.38	0.00	0.36	1.00									
Anbara	0.33	0.33	0.75	0.18	0.17	1.00								
Shuwarin A	0.33	0.35	0.25	0.00	0.24	0.31	1.00							
Tirgal	0.38	0.13	0.40	0.27	0.15	0.44	0.31	1.00						
Makekashi A	0.22	0.56	0.31	0.12	0.25	0.27	0.59	0.27	1.00					
Makekashi B	0.25	0.42	0.18	0.27	0.25	0.17	0.59	0.13	0.53	1.00				
Mangyare	0.21	0.44	0.14	0.44	0.50	0.25	0.59	0.40	0.48	0.21	1.00			
Shaishee	0.13	0.42	0.73	0.27	0.13	0.83	0.24	0.38	0.21	0.30	0.21	1.00		
Barni Al Madinah	0.35	0.25	0.46	0.00	0.13	0.67	0.24	0.29	0.32	0.38	0.10	0.75	1.00	
Danmali	0.35	0.35	0.31	0.25	0.24	0.38	0.56	0.29	0.50	0.59	0.38	0.59	0.38	1.00

4. Conclusion

This study detected variability among Nigerian and Saudi cultivars using microsatellite markers. This is explained by high allelic polymorphism as revealed by DNA marker. The DNA studies of fourteen Date palm cultivars provide evidence of distinct variation between cultivars from Nigeria and Saudi Arabia which follows a geographical pattern. It also indicates that genetic characters will have high discrimination power among cultivars and can increase the confidence in cultivar classification of date palms. Overall, there is a high variability among the cultivars studied which can be explained by molecular markers and that the variation which follow geographical pattern.

Since some of the DNA microsatellite markers used could detect those alleles that distinguish six cultivars, microsatellites will be effective in cultivar characterization. Additional studies on these markers are required of their discriminatory ability to identify cultivars with specific features of interest. Future studies should include more cultivars; this will assist the farmers which grow these popular cultivars to identify different cultivars using vegetative traits. This study did not predict or classify any cultivars based genders. One of the major challenges in date palm research is the identification of sex at seedling stage or using vegetative attributes. Since most of these markers were able to detect polymorphism in cultivars. The six markers used for this study are among the sixteen markers developed by [61] and are too few to amplify large numbers of Date cultivars. However, some new markers were recently developed [63-65]. Incorporation of these markers into genetic studies will provide wider scope in future molecular studies of Date palm cultivars for genotyping and geographical partitioning.

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