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Proximate Compositions Evaluation and Variability among Cultivars of Date Palm (*Phoenix dactylifera* L.) in Nigeria

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Authors' contributions

All the authors were involved in all aspects of this study.

Original Research Article

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ABSTRACT

Genetic variability among the fruits of 22 date palm cultivars was studied using 6 nutritional characters to enable us classify the available germplasm into distinct groups on the basis of their genetic diversity using their nutritional characteristics from proximate composition. The main date palm-growing areas of Nigeria were surveyed in 2011 with the objective of characterizing cultivars as to the quality and economic value of their fruits. Descriptive statistics and some multivariate analysis techniques were used to classify the 22 date palm cultivars. The genotypes based on studied traits were grouped into three clusters. Discriminant function analysis was used to confirm the accuracy of grouping that was produced by cluster analysis. Sugar and crude protein content were identified as important traits that could be used to differentiate the genotypes as revealed by both principal component and discriminant analysis. Genetic distance between Daushenga 1 and Saberari 2 exhibited the greatest dissimilarity followed by Daushenga 1 and Hausawa. Hence the use of these parents for hybridization should be given greater emphasis for the production of transgressive segregants with high nutritional potential. The level of variability observed suggested a high diversity among the cultivars. The result

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of the principal component analysis indicated that the contribution of the first three factors with Eigen value greater than one accounted for 73.03% of the total variation. The moderately high ash content shows that date palm fruit can effectively serve as a source of inorganic minerals and good adsorbent in the removal of metallic ions, odour, colours and other particulate matter from aqueous medium of water and waste water thus making date palm fruit an effective material precursor in water and waste water treatment among other uses. The high soluble carbohydrate content indeed gives an indication that it compares favourably with other fast energy giving food stuffs and thus it can be added to some food content as an additive. This work identified the existence of inherent variability in the local germplasm collections, and the traits that could be used to exploit the observed variability, eliciting important relationships among the traits in the process.

Keywords: *Date palm fruit; nutritional characters; cluster analysis; discriminant function analysis; genetic diversity and principal component analysis.*

1. INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is one of the earliest cultivated tree crops [1]. It belongs to the Palmae (Arecaceae) family. The Date Palm is a palm extensively cultivated for its edible fruit [2]. Due to the fact that date palm fruit has high nutritional value, great yields and its long life the date palm has been mentioned as the "tree of life" [3]. Date pulps hold easily digestible sugars (70%), mostly glucose, sucrose and fructose; dietary fiber and enclose less proteins and fats [4]. They also provide a good source of rapid energy due to their high carbohydrate content (70-80%). Most of the carbohydrates in dates are in the form of fructose and glucose, which are easily absorbed by the human body [5]. Fibers are claimed to prevent many metabolic or digestive diseases. It is usually defined as the part of the diet which is resistant to human digestive enzymes. Dietary fiber (DF) diets are associated with the prevention of some diseases such as constipation, colonic cancer, diverticular disease, coronary heart disease, cardiovascular disease, atherosclerosis, diabetes and obesity [5,6]. They also enclose vitamins like riboflavin, biotin, thiamine, ascorbic and folic acid that are essential for the body. Dates have significance as a stable food as well as a desert fruit, whilst their use in date products and industrial applications has increased [7]. Bedouin Arabs, who eat dates on regular basis, show an extremely low rate of cancer and heart disease [8]. Surplus dates are made into cubes, paste, spread, powder (date sugar), jam, jelly, juice, syrup or vinegar. Cull fruits are dehydrated, ground and mixed with grain to form a very nutritious stock feed [9]. To date, limited data are available regarding compositional and functional characteristics of date palm fruits grown in Nigeria. Currently, several human health problems are related to diets and nutrient imbalances is now very common among individuals in Sub-Sahara Africa with increasing rates of obesity. Just as yield varies among varieties of crops including the date palm, so does the nutritional contents and even within a variety, there could also be some variations among years in nutrient contents as the environment changes, therefore, there is a need to furnish the nutritionist and consumers of date palm fruits with yearly updates on the nutritional contents of the fruits and promote the use of dates and their corresponding fibres in food formulations. In spite of its economic importance, improvement programs in this crop are very limited in Nigeria and therefore, it becomes important for plant breeders to also study the variations that exist in the nutrient contents of date palm fruit since dates vary widely in their final appearance, organoleptic, physical and chemical characteristics. The effectiveness of selection depends primarily upon the magnitude of genetic variability in the breeding material at hand. Multivariate statistical techniques which simultaneously analyse multiple measurements on each individual under

investigation are widely used in analysis of genetic diversity irrespective of whether it is morphological, biochemical or molecular marker-based and subsequently, classification of germplasm collections. Among the multivariate techniques, cluster analysis, PCA, principal co-ordinate analysis (PCoA) and Multi-Dimensional Scaling (MDS) are at present, most commonly employed and appear particularly useful [10]. Multivariate analysis has been used frequently for genetic diversity analysis in many crops such as barley (*Hordeum vulgare* L.) [11], Sorghum (*Sorghum bicolor* L. Moench) [12], wheat (*Triticum* spp.) [13], peanut (*Arachis hypogaea* L.) [14], vineyard peach (*Prunus persica* L. Batsch) [15], rice, [16], coconut (*Cocos nucifera* L.) [17], and in oil palm (*Elaeis guineensis jacq*) [18]. Generally, information on genetic diversity based on nutritive value of date palm cultivars in Nigeria is scanty. The proximate composition serves as a general guideline for nutritionists and dieticians, as well as for investigators concerned with the general quality characteristics of foods. This study was carried out to unravel the nutritive compositions as well as variation in proximate constituents of some of the unstudied date palm cultivars in Nigeria so as to provide current information for nutritionist and also to run a classificatory analysis on the date palm genotypes using the nutritive compositions by means of some multivariate procedures which would enable us to classify the available germplasm into distinct groups on the basis of their genetic diversity using their nutritional characteristics from proximate composition. The information, thus obtained, would be helpful to develop an effective date palm breeding programme and as such a quantification of the degree of divergence would be helpful in choosing suitable genotypes and traits of interests for on-going breeding programmes.

2. MATERIALS AND METHODS

The main date palm-growing areas of Nigeria were surveyed in 2011 with the objective of characterizing cultivars as to the quality and economic value of their fruits. 22 cultivars were collected and measured, all under cultivation and in full production in the farmers' field. The cultivars characterized were Dilaka 1, Dilaka 2, Shuwari 1, Shuwari 2, Kudai 1, Kudai 2, Hausawa, Darazo 1, Darazo 2, Tsangaya 1, Tsangaya 2, Daushenga 1, Daushenga 2, Saberari 1, Saberari 2, Zuga 1, Zuga 2, Dantaya 1, Dantaya 2, Gurduba 1, Gurduba 2 and Maratayi. One hundred fruits were randomly selected from different palms of each of the cultivars and mixed together to act as replicates and then 20 unblemished fruits were taken randomly from each cultivar and washed, seeds were removed and the remaining parts were air dried and grinded into fine powder and were sieved with 2mm mesh pore size. Moisture content was determined by the method of [19,20]. Crude protein (N x 6.25) was determined by Kjeldahl method. The recommended method of [21] was used for the determination of ash, crude lipid and crude fibre. The carbohydrate content was obtained by the difference as the nitrogen free extract.

2.1 Data Analysis

Descriptive statistics such as mean, standard deviation and coefficient of variation for each one of the 6 studied traits were calculated. Clustering of cultivars into similar groups was performed using Ward's hierarchical algorithm based on squared Euclidean distances by subjecting the 22 x 6 data matrix to cluster analysis. Discriminant function analysis was used to confirm the accuracy of grouping that was produced by cluster analysis. In order to identify the patterns of phenotypic variation, principal component analysis (PCA) was conducted. Those PCs with Eigen values greater than one were selected as proposed by [22]. The component was further rotated using the varimax method with Kaiser

Normalization. SPSS version 17 for Windows statistical software packages was used for the analysis.

3. RESULTS

Basic statistics for various nutritive compositions is given in Table 1.

Table 1. Basic statistics of proximate chemical composition of 22 cultivars of date palm

Parameter	Mean	Minimum	Maximum	SD	Variance	CV
Moisture (%)	22.84	20.3	25.0	1.299	1.687	5.69
Ash (%)	23.76	22.1	25.7	0.962	0.925	4.05
Protein (%)	5.46	4.83	6.3	0.530	0.281	9.70
Lipid (%)	5.51	3.70	6.3	0.694	0.481	12.58
Sugar (%)	40.92	37.30	44.2	2.023	4.092	4.944
Fibre (%)	0.824	0.30	1.1	0.228	0.052	27.7

Pattern of variation among the varieties was different for different traits. The largest variation was observed for fibre, lipid and protein content. The coefficients of variations for these three traits were 27.7, 12.58 and 9.70 respectively. Relatively, a low level of variability was detected in most of the remaining traits. Coefficient of variation (CV %) ranged from 4.05 to 27.7% for various traits. The highest coefficient of variation was observed for fibre content while the lowest level was showed by ash content. Based on nutritional composition, date palm cultivars showed variation in fibre, lipid and protein content but as they were collected from different geographical areas have different environmental conditions so these variations are special. Kudai 2 had the least fat content (3.7%) as against the highest value for the same traits (6.3%) in Dilaka 1 cultivar (Table not shown). Sugar content was least in Hausawa (37.3%). The cultivar with the highest sugar content was Shuwari 1. The moisture content in the studied date palm cultivars revealed that Dilaka 2 and Shuwari 1 had highest (24.0%) moisture content and Hausawa had the highest (25.7%) ash content. Although, Hausawa had the highest (25.7%) ash content but sugar content (37.3%) was the lowest.

3.1 Cluster Analysis

The clustering of proximate compositions of the 22 Date palm cultivars is presented in Fig. 1. Classification was based on similarities among the 22 Date palm cultivars for the six proximate variables. Phenogram based on squared Euclidian distance coefficients using 6 traits placed the 22 cultivars into three main clusters. First cluster consisted of a total of nine genotypes (40.9%). These genotypes were characterized by the highest moisture content and lowest crude fibre, crude protein and lipid content (Table 2). The second cluster comprised of a total of 4 genotypes (18.2%). These genotypes were characterized by lowest sugar content and highest ash content. The third cluster comprised of nine genotypes (40.9%) and was characterized by highest sugar, fiber, protein and lipid content. The 22 cultivars were unique; differing from one another based on the proximate descriptors. No pairing of cultivars occurred at a similarity distance of zero. Three main clustering patterns were possible as shown in Fig. 1. The clusters with the highest membership were 1 and 3 which had nine members each; clusters 2 had four members while no cluster was a singleton. Cluster 2 and 3 had the highest inter-cluster distance. Squared Euclidian dissimilarity coefficients were calculated for all the cultivars of date palm. Dissimilarity

coefficient of the 22 cultivars ranged between 1.049 to 67.52. Based on the proximate variables considered in this study, Dantaya 2 and Saberari 1 were the closest. They tied at the similarity distance of 1.049 followed by pairs of cultivars 'Maratayi and Saberari 1' with a similarity distance of 1.469. Cultivars 'Daushenga 1 and Saberari 2 exhibited the greatest dissimilarity with similarity distance of 67.52, followed by Daushenga 1 and Hausawa with a distance of 67.039 (Table not shown).

**** HIERARCHICAL CLUSTER ANALYSIS ****

Dendrogram using Ward Method

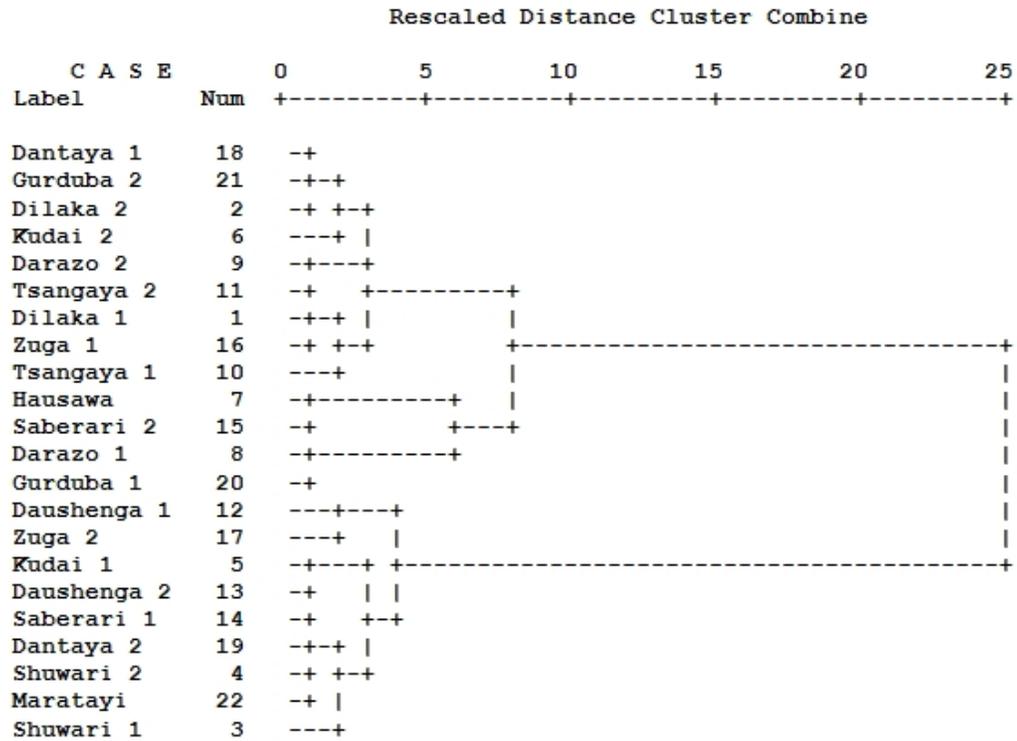


Fig. 1. Ward's dendrogram of 22 date palm cultivars using squared Euclidean distances.

Table 2. Cluster means for 6 characters of 22 date palm cultivars

Characters	Clusters		
	I	II	III
Sugar	40.27	38.08	42.83
Crude fibre	0.77	0.78	0.90
Crude protein	5.18	5.55	5.71
Moisture	23.56	22.45	22.3
Lipid	5.21	5.63	5.77
Ash	23.77	24.83	23.29

3.2 Discriminant Analysis

When discriminant function analysis was applied to group membership (the three clusters), only two of the proximate variables (sugar and crude protein content) was significant in distinguishing the cultivars. The sugar content was the most important character that discriminated the three groups from the cluster analysis followed by crude protein content. 95.5% of the original group's cases were correctly classified (Table 3).

Table 3. Discriminate function analysis: classification results

		GROUPS	Predicted Group Membership			Total
			1.00	2.00	3.00	
Original	Count	1.00	8	1	0	9
		2.00	0	4	0	4
		3.00	0	0	9	9
	%	1.00	88.9	11.1	.0	100.0
		2.00	.0	100.0	.0	100.0
		3.00	.0	.0	100.0	100.0

a. 95.5% of original grouped cases correctly classified.

The Eigen values, variance and pooled within correlations between discriminant variables and discriminant functions were presented in Table 4.

The analysis has revealed two functions which accounted for 100% variance for all the functions together. The first function accounted for 96.9% of the total variance whereas the second function only accounted for 3.1 % of the total variance. The first discriminant function was highly positively correlated with sugar content while ash, moisture and lipid content had a negative correlation with the first discriminant function. Crude protein and fibre content had positive correlation with the second discriminant function.

Table 4. Eigen values, total variance, cumulative variance and pooled within group correlations between discriminant variables and canonical discriminant functions

Functions	Eigen values	Variance%	Cumulative %	Pooled within group Correlations*
1	5.842	96.9	96.9	Sugar (0.828) Moisture (-0.303) Lipid (-0.138) Ash (0.117)
2	0.185	3.10	100.0	Crude protein(0.09) Crude fibre (0.08)

* Largest absolute correlation between each variable and any discriminant function

3.3 Principal Component Analysis (PCA)

The first three principal components (PCs) with eigenvalues greater than one accounted for 73.03% of the variability amongst 22 date palm cultivars (Table 5).

Table 5. Eigenvectors and variance proportion of six proximate variables in three PC-axes

Proximate composition	PC1	PC2	PC3
Sugar	0.08	-0.102	0.053
Crude fibre	0.432	0.630	0.472
Crude protein	-0.066	-0.147	0.919
Moisture	-0.229	0.640	-0.426
Lipid	0.118	-0.783	0.093
Ash	-0.852	0.056	0.033
Eigenvalues	1.868	1.434	1.079
Cumulative eigenvalues	1.868	3.302	4.381
Variance (%)	31.130	23.905	17.991
Cumulative variance (%)	31.130	55.035	73.026

The first principal component (PC1) accounted for 31.13% of total variation. The quantitative traits that loaded on the PC1 included sugar and ash content. Principal component 2 (PC2) had 23.91 % of the total variability. Crude fibre and moisture content contributed positively to PC2 whereas lipid content contributed negatively to PC2. Principal component 3 (PC3) exhibited 17.991% of the total variation in phenotypic traits and was heavily and positively associated with crude protein. The first two PCs were plotted to observe the relationship among the 22 cultivars of date palm (Fig. 2). There was no clear separation of the cultivars in the PCs into different groups. Instead the various genotypes were interspersed and had a wider spread across two principal components. This separation was based on several important nutritional differences amongst the date palm cultivars.

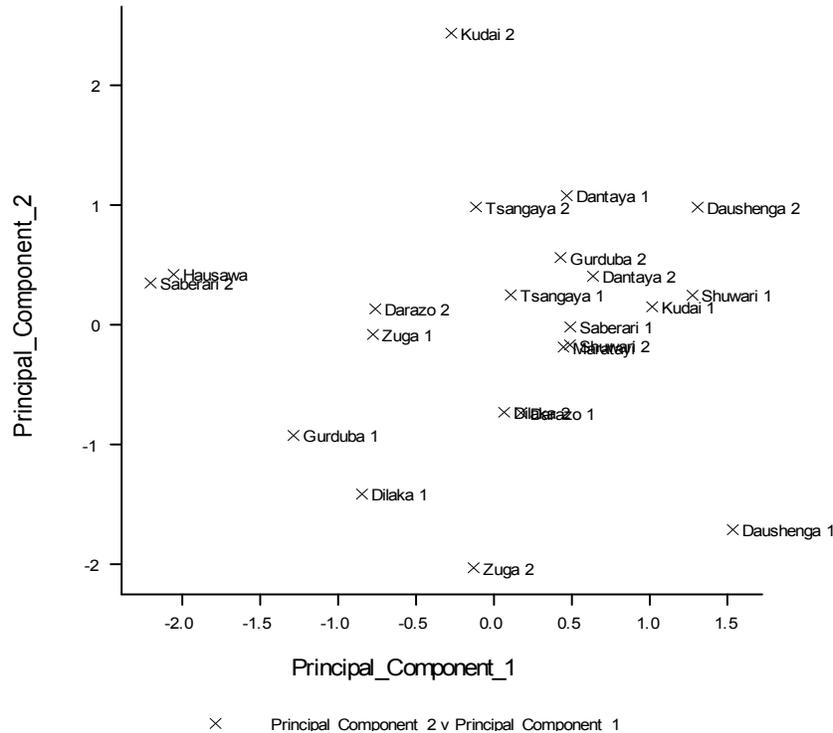


Fig. 2. Scattered diagram of first two principal components based on mean values of 6 quantitative traits in 22 cultivars of date palm

4. DISCUSSION

All the 22 studied cultivars differed from one to another for all the proximate variables. The range of values for most traits was high with the various cultivars having superior performance for each variable. In human diet, protein quality and quantity are of major concerns. WHO/FAO suggests a daily intake of 0.88 g of protein per kg body weight for children in the age range of 1-10 years [23]. The crude protein contents of dates in contrast to the work of [24] was however, lower than that recorded for protein rich foods including cowpea seeds (24.7%), lentil (26.1%), *Mucuna flagellipes* (24.9%), green pea (24.9%), *Tamarindus indica* and *Parkia biblobosa* (20.9%) [25]. Consumption of 100 g of these date cultivars may be capable of providing 4.83 - 6.3 g of protein which satisfies recommended daily allowance of protein for children. Fiber cannot be neglected as it decreases serum cholesterol levels, risk of coronary heart diseases, hypertension, diabetes, colon and breast cancer [26]. The high coefficient of variability in lipid and fibre is an indication of availability of opportunity for further selection of these traits for improvement of date palm. The cell uses carbohydrates as its main source of energy. To maintain a constant supply of energy for the cells, the level of glucose in the blood must be kept fairly constant. Aside from providing energy, the cell also uses carbohydrates for its various activities and processes. Carbohydrates located on the cell surfaces regulate communication between cells and other molecules. This communication helps the body recognize and remove harmful bacteria and pathogens and cancerous cells and bring about immune responses against allergy-causing substances. The carbohydrate values obtained for the date cultivars are comparable with the values given by [27] for peas, mangoes, potatoes [26]. The high soluble carbohydrate content indeed gives an indication that it compares favourably with other fast energy giving food stuffs. Hence it can be added to some food content as an additive [28]. Since carbohydrates have oxygen and hydrogen atoms as constituent elements and in solution, the production of charges such as oxygen and hydrogen ions. The formation of oxygen ion which is negatively charge can attract metallic ions and possibly remove them from aqueous solution [29]. It appears that, the date palm cultivars have high carbohydrate content (37.3 – 44.2%), which may be due to their low fat content. Minerals play many vital roles, working synergistically with vitamins, enzymes, hormones and other nutrient cofactors to regulate literally thousands of the body's biological functions. Proper blood formation, energy production, nerve transmission and regulation of healthy acid-alkaline balance are among these essential functions. Minerals also support healthy bones and teeth and are required for proper support of the body's overall structure and function. A key role of minerals is in healthy cell function; they are critical for proper cell regeneration as cells progress through their normal life cycle and are replaced by new, healthy cells. If the body's mineral supply is deficient, there is an increased likelihood that both existing cells and the cells that replace them can be compromised, setting the stage for various chronic and degenerative diseases. The moderately high ash contents in dates indicate it could be used as a good source of inorganic minerals. The ash content is also an indication of the presence of carbon compounds and inorganic components in the form of salts and oxides in date palm [30]. Carbon plays a vital role in the adsorption of substances due to its porous nature which is an indication that date palm fruit in its granular or ash form can effectively serve as good adsorbent in the removal of metallic ions, odour, colours and other particulate matter from aqueous medium of water and waste water and as such can remove smell or odour from water and other solution. Consuming too many fats, no matter what kind, can lead to obesity. Fats contain more than twice as many calories per gram as carbohydrates and protein. The surgeon general lists diabetes, high blood pressure, sleep apnea, cancer, arthritis and several other diseases as health risks associated with obesity. The fat content in the studied date palm cultivars were low (3.7 – 6.3%) which indicate a low health risk to consumers.

Exploitation of the proximate constituents of date palm cultivars with respect to crude protein, carbohydrates and ash contents becomes necessary since the percentage composition of these compounds are needed to be compared to some crops like legumes whose nutritional demands are highly competitive between humans and livestock. Generally, the ash, moisture and fat content were higher than that reported by [23,31] in date palm. High value of moisture contents showed that these dates cannot be stored for a long period of time without spoilage (short shelf-life) and it will be susceptible to microbial growth. Ward's hierarchical cluster analysis based on proximate compositions resulted in three clusters. Crosses between individuals from different clusters may result in high heterosis. Even though, the genetic mechanisms that explain heterosis are not fully understood, it is well documented that crosses between unrelated and consequently genetically distant parents, show greater hybrid vigor than crosses between closely related parents [32] since it is expected to produce new recombinants with desired traits. According to the cluster means, Cluster II showed better performance in case of ash content, indicating that genotype of this cluster could be used for parent in future hybridization program for higher mineral content. Moreover, Cluster III was found to have higher mean values for sugar, fibre, protein and lipid content indicating higher potential for the use of the cultivars of this cluster for genetic improvement of nutritional characters. The cultivars Daushenga 1 and Saberari 2 had the highest genetic distance and they were grouped in different clusters followed by Daushenga 1 and Hausawa which were also grouped in different clusters. Odewale *et al.* [33] also reported the separation of most widely divergent genotypes into different clusters in date palm. Therefore, it can be expected that crosses between these biochemically distant cultivars will result to a high degree of heterosis [34-36]. Hence the use of these parents for hybridization should be given greater emphasis for the production of transgressive segregates with high nutritional potential. On the contrary, Dantaya 2 and Saberari 1 had the least genetic distance and hence crosses between these closely related varieties should be avoided. Mean values of characters were more or less continuous across clusters hence no sharp distinction between clusters was observed. This was an indication that clusters were under polygenic control [37]. Cultivars from clusters II and III which show high mean for important nutritional characters can be used in the future breeding program to recombine these traits. Discriminant analysis identified sugar content as the most important discriminating traits among the date palm cultivars. Crude protein content was the other important trait identified by the discriminant analysis. As compared to other techniques, discriminant analysis explained a high value of 96.9% of the within entries variance in the same number of axes. Similar result was reported by [37] in groundnut. In the principal component analysis (PCA), the first component (PC1), was positively correlated with sugar content and negatively correlated with ash content. This means that the cultivars with high values of PC1 have high sugar content and low ash content and vice versa. Accordingly, cultivars with high PC2 values will have high crude fibres, moisture and low lipid content while cultivars with high PC3 values will have high crude protein content. Sugar and crude protein content were among the identified characters that distinguishes the date palm cultivars. In the present study, principal component analysis captured most of the variation within the cultivars in higher number of axes compared to discriminant analysis. However both techniques did not show much difference in the characters considered most important as they identified sugar and crude protein content as important characters with the highest positive loadings in their various axes of ordinations. Thus, a combination of PCA and discriminant analysis would be appropriate for describing the variation in date palm germplasm. In spite of the reduction of the characters to only three principal components, it was possible to account for over 70% of the total variations among the date palm cultivars. Thus the capacity of PCA in data reduction without loss of information was confirmed [38].

5. CONCLUSION

In conclusion, this research work shows that from the proximate composition; carbohydrates have the highest percentage value, followed by ash and moisture content. Date palm fruit is a good source of minerals, fast energy giving food as well as an effective material precursor in water and waste water treatment among other uses. Studied cultivars showed high coefficient of variation for fibre content as well as moderate variation for lipid content. The cultivars based on studied traits were classified into three groups with cluster 2 and 3 having the highest inter-cluster distance indicating the use of cultivars from these clusters for hybridization in order to produce transgressive segregants with high nutritional potential. Principal component analysis (PCA) revealed that the first three principal components accounted for 73.026% of the total variation. Among the studied traits, sugar content showed the highest positive correlation with the first component (PC1). Crude protein content showed the highest positive correlation with the second component (PC2). Ash and lipid content presented negative correlation with PC1 and PC2 respectively. The variables that are strongly associated in the same group may share some underlying biological relationship, and these associations are often useful for generating hypothesis for better understanding of behaviour of complex traits that would allow breeders to maximize their knowledge. According to breeding goal, breeders can chose cultivars by considering appropriate PCs values. However, increasing the studied characters to 10-12 will increase the resolution of cultivars identification and discrimination power as well. Molecular studies will be useful to confirm the genetic diversity based on proximate composition and to characterize these cultivars for more detailed examination. This may help to emphasize the availability of these genetic resources for future breeding programmes. The differences among the studied cultivars are also to be attributed to soil and climatic condition and so to genotype-environment interaction.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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