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Morphological characterization of shea tree (*Vitellaria paradoxa* subsp. *paradoxa*) populations in the region of Mandoul in Chad

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Quantitative characteristics of leaf, fruit and nut collected from *Vitellaria paradoxa* subsp. *paradoxa* growing in Kol, Kemkian and Matekaga located in the region of Mandoul of Southern Chad were analyzed. Our results reveal variation of the measured morphological parameters. The smallest lamina length (15.5 cm) was found in Kol and the longest (26.3 cm) in Matekaga. The biggest lamina width (5.4 cm) and nut length (3.8 cm) were found in Kol. Using the correlation matrix data, our investigations revealed that lamina length and peduncle length were correlated with 92%. Fruit width was correlated with peduncle length and fruit length with 52 and 83%, respectively. Nut length was correlated with peduncle length, fruit length and fruit width with 55, 78 and 77%, respectively. In contrast, nut length was correlated with fruit length, fruit width and nut length with 68, 78 and 87%, respectively. The dendrograms analyses revealed the existence of four groups within and between sites instead of the six ethno-varieties described by folk classification. These findings raise the need to use molecular markers to unravel the underlying variation for use in selection and genetic improvement of shea tree.

Key words: *Vitellaria paradoxa*, shea butter tree, morphological characters, folk classification, ethno-varieties, Chad.

INTRODUCTION

The semi-domesticated shea butter tree (*Vitellaria paradoxa* (C.F. Gaertner) syn. *Butyrospermum parkii* (Kotschy), *Butyrospermum paradoxum* (C.F. Gaertner) Hepper, Family Sapotaceae) is wildly distributed in the Sudano-Sahalian region from Senegal to Uganda (Hall et al., 1996; Hemsley, 1968; Salle et al., 1991). Presently two subspecies have been identified. *V. paradoxa* subsp. *paradoxa* is found in West and Central Africa (Hall et al., 1996; Salle et al., 1991; Sanou et al., 2005; Fontaine et al., 2004; Allal et al., 2008; Nyarko et al., 2012; Kelly et al., 2004), while *V. paradoxa* subsp. *nilotica* is common in East Africa (Soudan, Ethiopia, Uganda and Republic Demotratic of Congo) (Gwali et al., 2012; Okullo et al., 2004; Byakagaba et al., 2011; Okiror et al., 2012). The tree shape is influenced by various environmental factors and they are well identified by farmers according to the folk classification. During the wet season, the tree produces fruits edible by both human and animals. The fruits contain 1 to 3 large solitary seeds, rich in fat and oil used in a variety of purposes such as cooking (Abbiw, 1990),

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medicinal, hair and skin ointments and as a base for industrial manufacture of confectioneries (Cidell and Alberts, 2006). The oil is also used in traditional and social rituals such as marriages, funerals, coronations and rainmaking (Ferris et al., 2004; Gwali et al., 2012; Hall et al., 1996; Moore, 2008). The wood of the shea butter tree is used for charcoal, furniture and construction, and the latex for glue making (Lovett and Haq, 2000a). In addition, the trees are used in agroforestry systems that play an important role in the adaptation to climate change such as contribution to soil fertility (Rao et al., 2007). For these reasons, shea butter tree generates significant incomes for households.

Because of its economic importance, genotype studies were performed based on morphological characters leading to the identification of several phenotypes including the domesticated *V. paradoxa* (Chevalier, 1943, 1948; Nafan et al., 2007; Ruyssen, 1957; Sanou et al., 2005, 2006; Ugese et al., 2010). In 1943, Chevalier identified eight varieties based on fruit and leaf variation (*cuneata*, *ferruginea*, *floccosa*, *mangifolia*, *nilotica*, *parvifolia*, *poissoni* and *serotina*). In 1957, the taxonomy was revisited by Ruyssen using tree shapes and sizes, fruits, nuts and leaves leading to the description of *V. mangifolium* as a subspecies containing two varieties (*viridis* and *rubifolia*). Further, using fruit morphology, nut color, crown shape and habitat types, phenotype variation was noticed for the shea tree in Cameroon (Nafan et al., 2007; Lamien et al., 2007). This variation was in agreement with the folk classification distinguishing ethno-varieties which was used by West African farmers to select and preserve shea tree (Gwali et al., 2011; Lovett and Haq, 2000b). Gwali et al. (2012) used morphological characters of 176 trees representing 44 ethno-varieties in Uganda to establish the congruence between the morphological variation and folk classification. Their results showed a good congruence with folk classification when they combined the qualitative traits as perceived by farmers. Recently, Mbaiguinam et al. (2007) performed studies in the population of shea tree from Mandoul region using chemical characteristics and concluded that there was no significant difference of fatty acids content within varieties. In addition, they reported that the shea butter profile was between those from Cameroon and Uganda. Nevertheless, substantial darkness points have to be addressed particularly in Chad where the level of the morphological diversity of the tree is still understood.

The objectives of this study were to discriminate the morphotypes of the shea trees growing in the region of Mandoul located in Southern Chad using leaf, fruit and nut characteristics.

MATERIALS AND METHODS

Study area and experimental design

The present study was conducted in three sites (Matekaga, Kemkian and Kol*)* located in the region of Mandoul in southern Chad, where the mean annual precipitation was 1,200 mm (Figure 1a, b). The rainy season lasts from May to October with mean temperatures of 22°C. The dry season lasts from November to April with average temperature of 32°C. In the area of our experiment, the soil was sandy, lateritic or ferralitic. Each site was swept by 4 or 8 transects crossing in its center using a GPS (Garmin, city and country). Along each transect, the quadrants were separated by 100 m. These sites were chosen because of the high density of their shea tree. It should be noted that Kemkian means the village of shea tree in the local language.

Plant material

Two hundred and forty (240) shea trees distributed in six ethno-varieties according to folk classification were investigated. The folk classification of the accessions was consistent because in the area where the materials were collected, people spoke the same language. The sampling method consisted of selecting 104 trees in Matekaga, 64 in Kemkian and 72 in Kol.

Data collection

Ten adult fresh leafy twigs and mature fruits without parasites were collected randomly from each tree. The length and the width of the leaves were measured using a vernier caliper (Shanghai, China). The total length of the leaf consisted of the length of petiole and that of lamina. The length and the width of fruit and the diameter of the nuts collected from fruits were measured for each accession (Figure 2). For accurate measurement, a mean value was calculated from ten organs. In addition, the mean value of length and width of each organ within site were calculated. The mature fruits were collected in May and September 2007. These data were used as raw material, subjected to principal component analysis (PCA) and analysis of molecular variance (AMOVA).

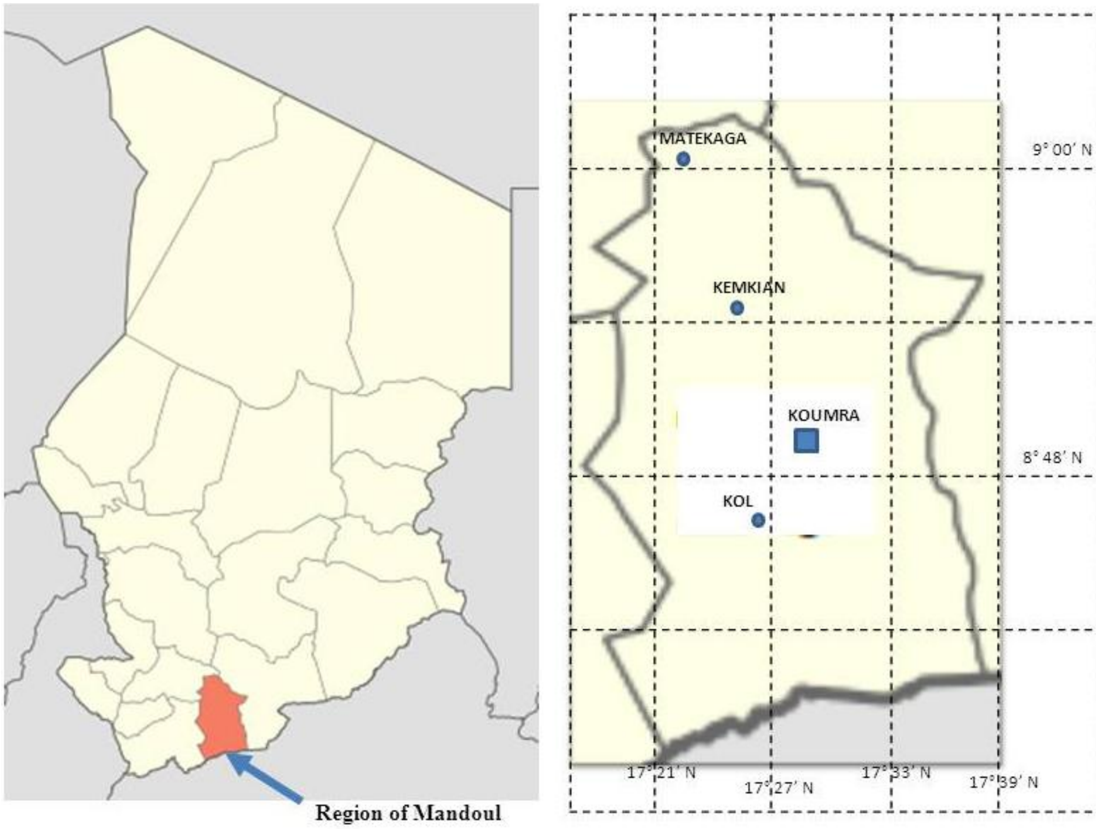
Multivariate analysis

PCA was performed using statistical package ADE-4 coupled with a hierarchical cluster ascendant (HCA) to group the accessions based on their similarities. Leaves, fruits and nuts were considered as variable but the 240 accessions were projected in a plane including the two first axes. To perform an ascending hierarchical clustering of the accessions, the coordinates of the individuals on the factorial axes as similarity matrix, the squared Euclidean distance and the Ward's method were used. The dendrogram was generated using the R (version R-2.9.0, ADE4 package) software (R Development Core Team, 2011).

RESULTS

Morphological character analyses

The mean values of leaf, fruit and nut parameters of the samples collected from each site allowed to estimate their variation. The parameters measured from the leaves showed that the lamina length ranged from 15.8 to 23.6 cm for the trees growing in Kemkian, 15.5 to 24.2 cm in Kol, 16.5 to 26.3 cm in Matekaga. The percentage of leaves for which the length was more than 20 cm was 81% in Kemkian, 76% in Kol and 69% in Matekaga. The width of the lamina ranged from 3.2 to 5 cm in Matekaga,



a. Map of Chad b. Map of Mandoul's region

Figure 1. Map of Chad and region of Mandoul showing the localization of the sites where the samples were collected (Ministery of Interior, 2009).

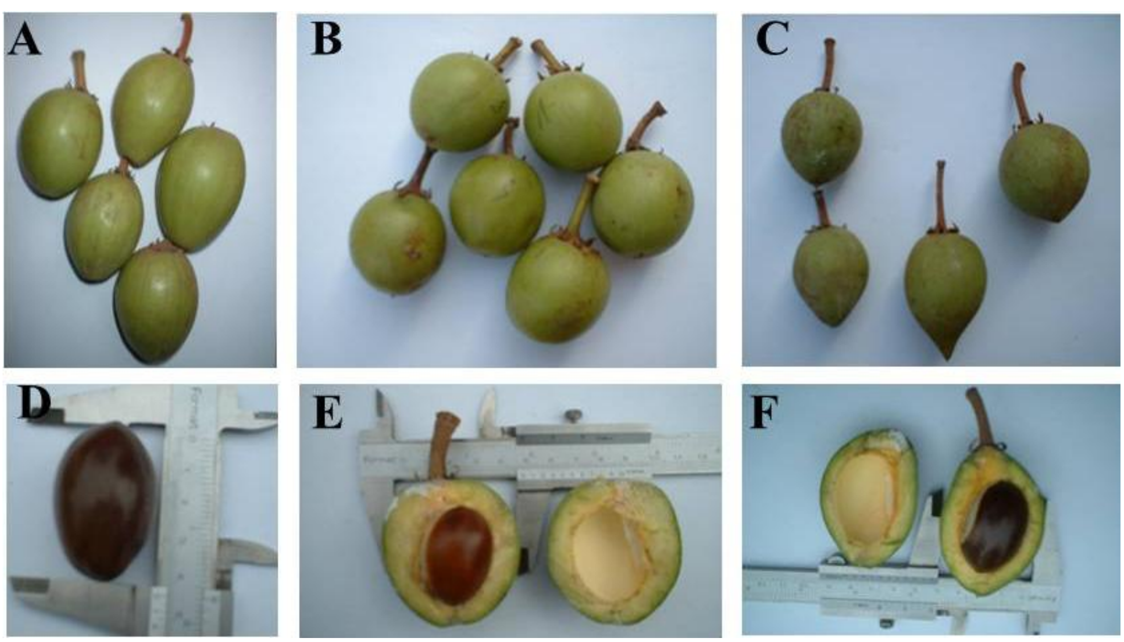


Figure 2. Morphological characteristics of fruits and nuts. (A) Ovoid fruit; (B) Spherical fruit; (C) Elliptical fruit; (D) Ovoid nut; (E) Spherical fruit; (F) Elliptical fruit.

Table 1. Correlations between morphological characters.

	PET	LL	LW	PL	FL	FW	NL
LL	0.92*						
LW	0.18	0.46					
PL	0.00	-0.02	-0.11				
FL	0.05	0.05	-0.06	0.48			
FW	0.03	0.00	-0.10	0.52*	0.83*		
NL	0.06	0.02	-0.12	0.55*	0.78*	0.77*	
NW	0.01	-0.01	-0.07	0.42	0.68*	0.78*	0.87*

PET, Petiole length; LL, lamina length; LW, lamina width; PL, peduncle length; FL, fruit length; FW, Fruit width; NL, Nut length; NW, Nut width. *The significant values are in bold.

3 to 5 cm in Kemkian, 3 to 5.4 cm in Kol. The length of the petiole ranged from 5.8 to 11.9 cm for the leaves collected in Matekaga, from 5.7 to 11.2 cm in Kemkian and 5.7 to 10.2 cm in Kol.

Fruit peduncle length variability was also reported in this study. It ranged from 1 to 3.1 cm in Kol, 1 to 3.3 cm in Kemkian and 1 to 2.7 cm in Matekaga. The fruit length varied from 2.5 to 5.5 cm in Matekaga, 2.6 to 5 cm in Kemkian and from 2.6 to 5.5 cm in Kol. Assessing the fruit width, our study founded that it varied from 2.3 to 4.3 cm in Matekaga, 2.4 to 4.4 cm in Kemkian and 2.4 to 4.3 cm in Kol. The nut length varied from 1.9 to 3.6 cm in Matekaga, 1.9 to 3.3 cm in Kemkian and 1.9 to 3.8 cm in Kol. Finally, the measure performed on the nut width showed that their values ranged from 1.5 to 2.8 cm in Matekaga, 1.4 to 2.4 cm in Kemkian and 1.5 to 2.6 cm in Kol.

Statistical analysis of morphological data

PCA showed that the two principal axes explained 72.95% of the variance observed. The first axis expressed 46.54% of the total variance (data not shown). The variables, nut length, fruit width, nut width and fruit length, contributed to 86.96, 83.59, 78.53 and 77.97%, respectively. The second axis expressed 26.41% of the total variance. The lamina length and petiole length represented 97.4 and 82.87% of the variance, respectively. The third axis explained 10.66% of the total variance where lamina width explained 65.71% of this value. Finally, the fourth axis expressed 8.11% of the variance where the peduncle length explained 54.84% of this value and is associated with the nut width which contributed to 5.34% of the variance.

The correlation matrix showed that lamina length and petiole length were correlated with 92%. Fruit width was correlated with peduncle length and fruit length with 52 and 83%, respectively. Nut length was correlated with peduncle length, fruit length and fruit width with 55, 78 and 77%, respectively. In contrast, nut width was correlated with fruit length, fruit width and nut length with 68, 78 and 87%, respectively (Table 1). In addition, there was significant variation of the mean of the peduncle length between the samples collected in Matekaga, Kemkian and Kol. The mean of the fruit length was similar between Kol and Matekaga but it was significantly different with the ones found in Kol (Figure 3A). Figure 3B showed that nut characteristics (means of length and width) were not different between Matekaga and Kol. These character-ristics were significantly different with the ones collected on the nuts from Kol. No significant difference was observed between the mean of the peduncle length in Matekaga, Kemkian and Kol. Similar results were observed for the means of the lamina length and lamina width taken individually (Figure 3C).

Morphological variation within sites

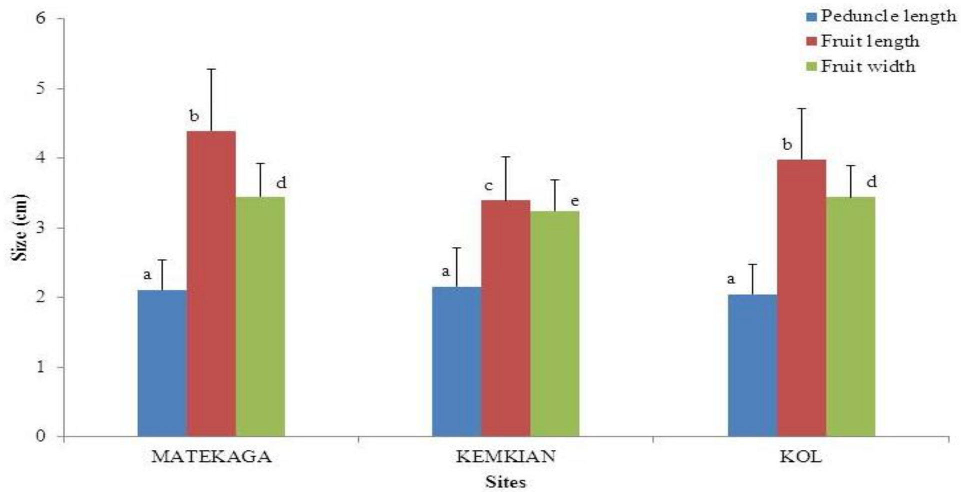
Dendrograms performed using leaf, fruit or nut parameters separately or in pair combinations failed to discriminate accurately the samples collected from each site (data not shown). In contrast, a combination of leaf, fruit and nut parameters allowed a good resolution between individual samples within each site. In Figure 4A, samples collected in Kemkian were divided into four main groups. In the group I, two sub-groups were observed. The first sub-group encompassed A121 and A141 which were clustering together while A110 and A162 were sister of A163. In the same sub-group, A120 and A142 were clustering together as A109 and A130 did. The genetic relationship among the individuals forming the second sub-group is also well resolved. The group II was also sub-divided into two sub-groups which are well resolved. In group III, the clustering was very clear except for A139 and A160 which erre linked with a short branch. The group IV was sub-divided into several numbers of sub-groups.

The material collected in Kol was divided into four groups (Figure 4B). The first group showed a high coefficient of similarity among accessions and two main sub-groups as the second group did. The third group encompassed two main sub-groups including several subdivisions each, while the fourth group was also well structured.

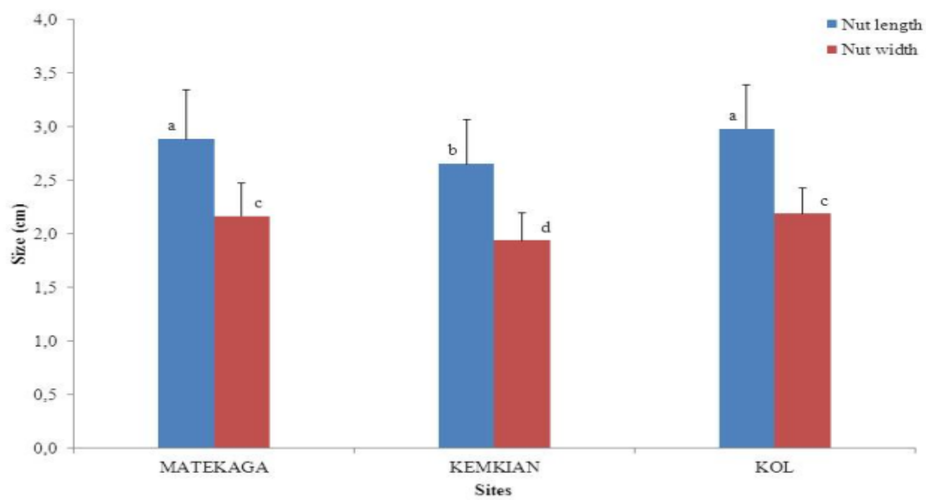
In Matekaga, based on the dendrogram, the biological material was divided into four groups (Figure 4C). The first group was divided in two main sub-groups which were well resolved. The second group encompassed several sub-groups as the third but the fourth was more diversified.

Trait variation between sites

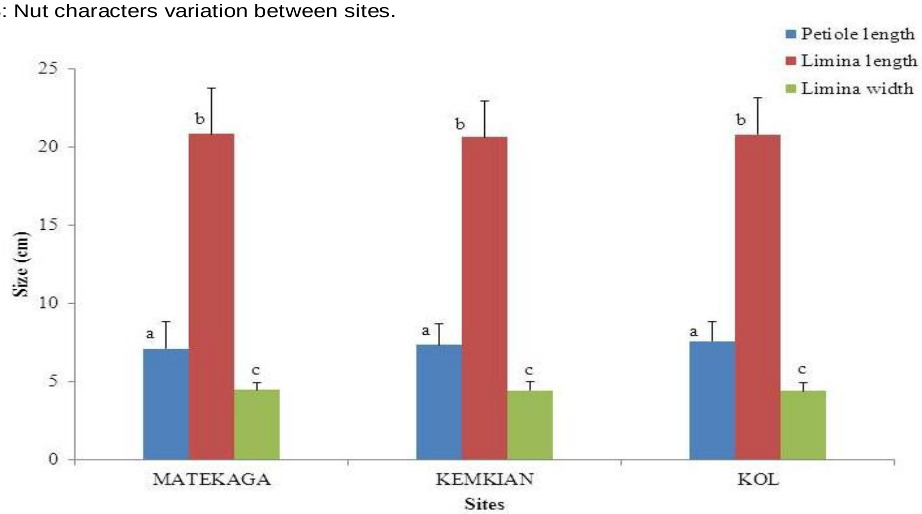
The dendrogram in Figure 5 contained the 240 individuals growing in the three sites (Kemkian, Kol and



A: Peduncle and fruit characters variation between sites.

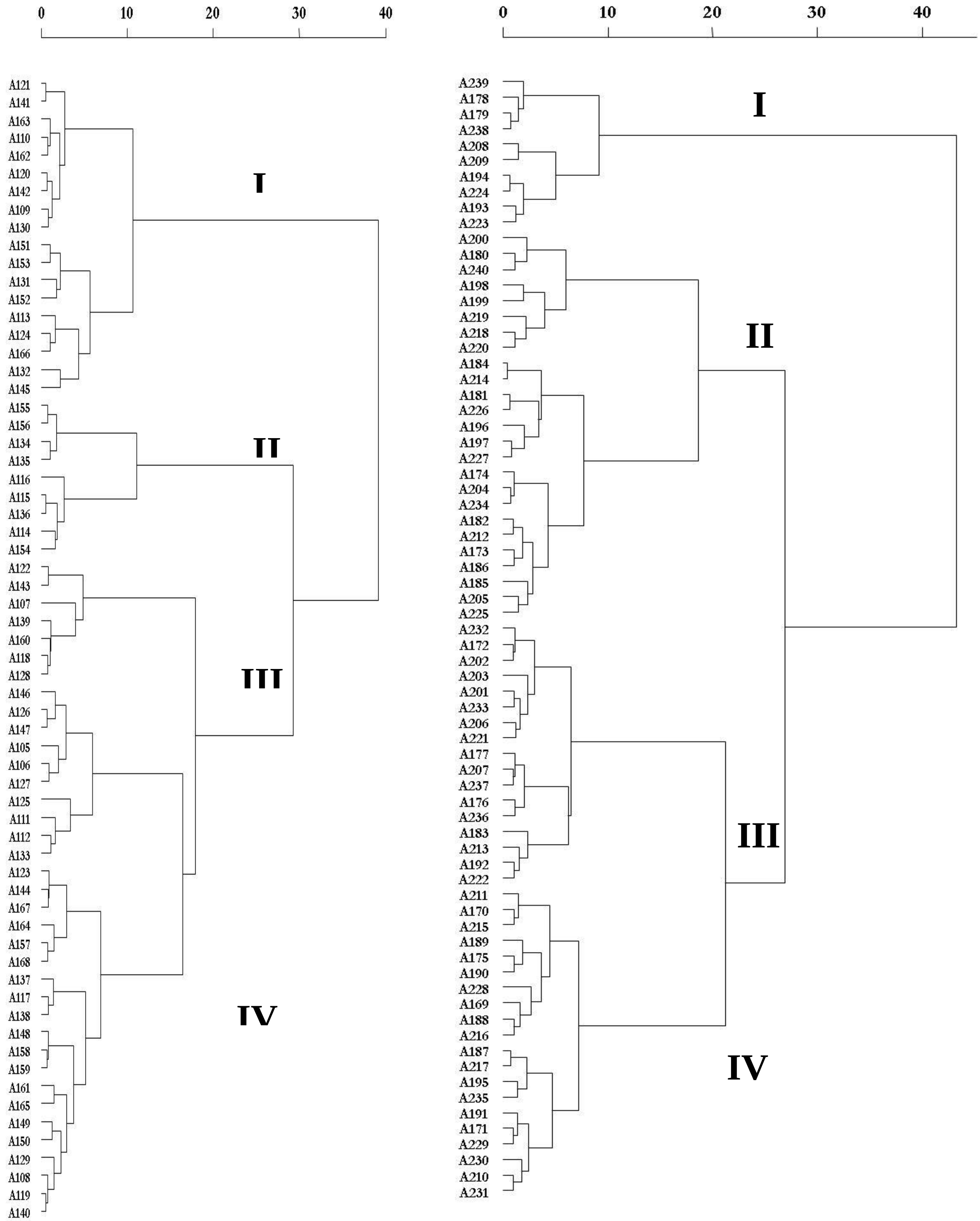


B: Nut characters variation between sites.



C: Leave characters variation between sites.

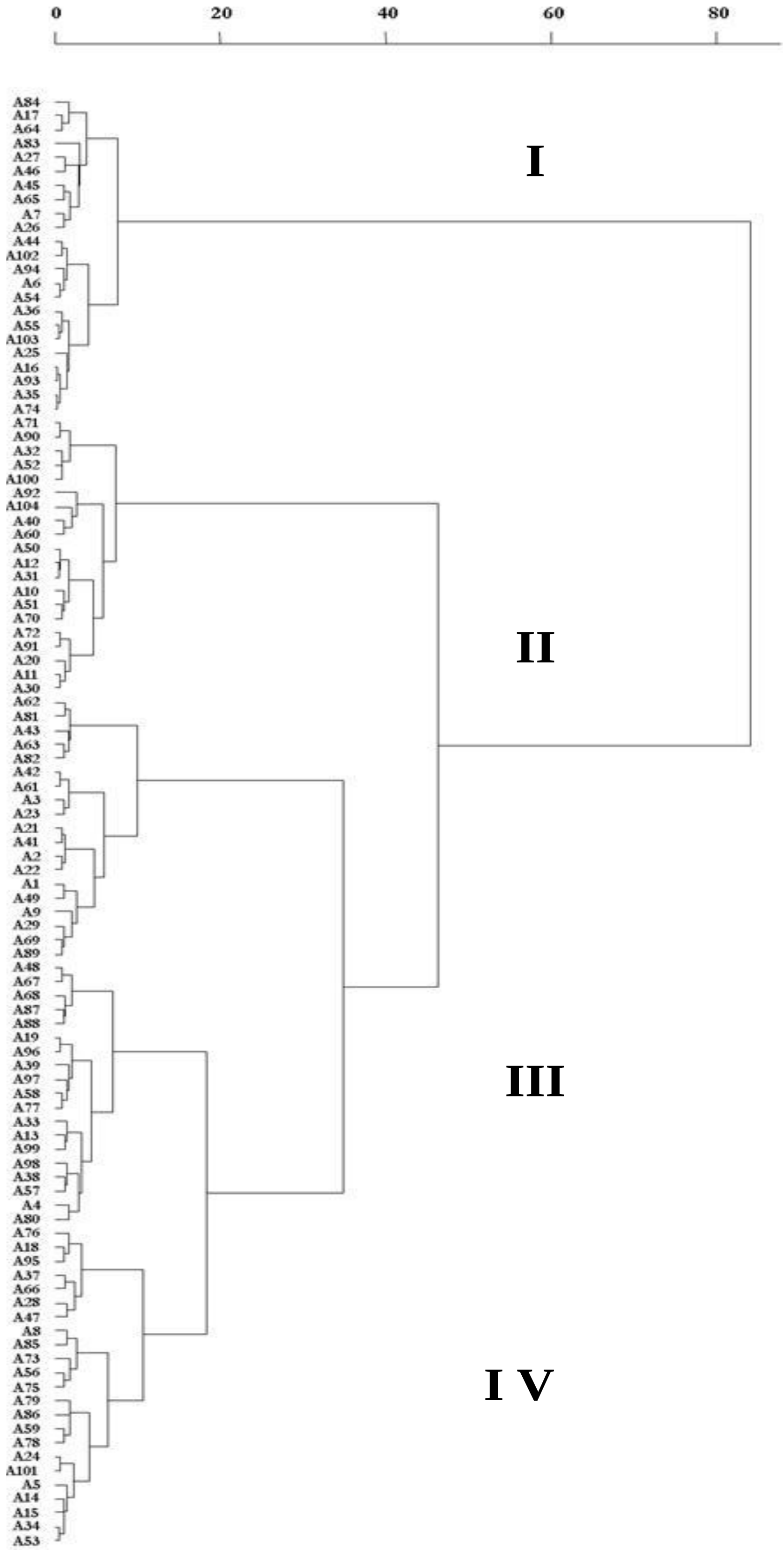
Figure 3. Variation of morphological characters of shea butter tree between sites. The characters affacted by the same letter are not statistically differents.



A: Dendrogam showing dissimilarities between Individual shea tree in Kemkian

B: Dendrogam showing dissimilarities between individual shea tree in Kol

Figure 4. Dendrograms showing dissimilarities within sites



C: Dendrogam showing dissimilarities between individual shea tree in Matekaga

Figure 4. Contd.

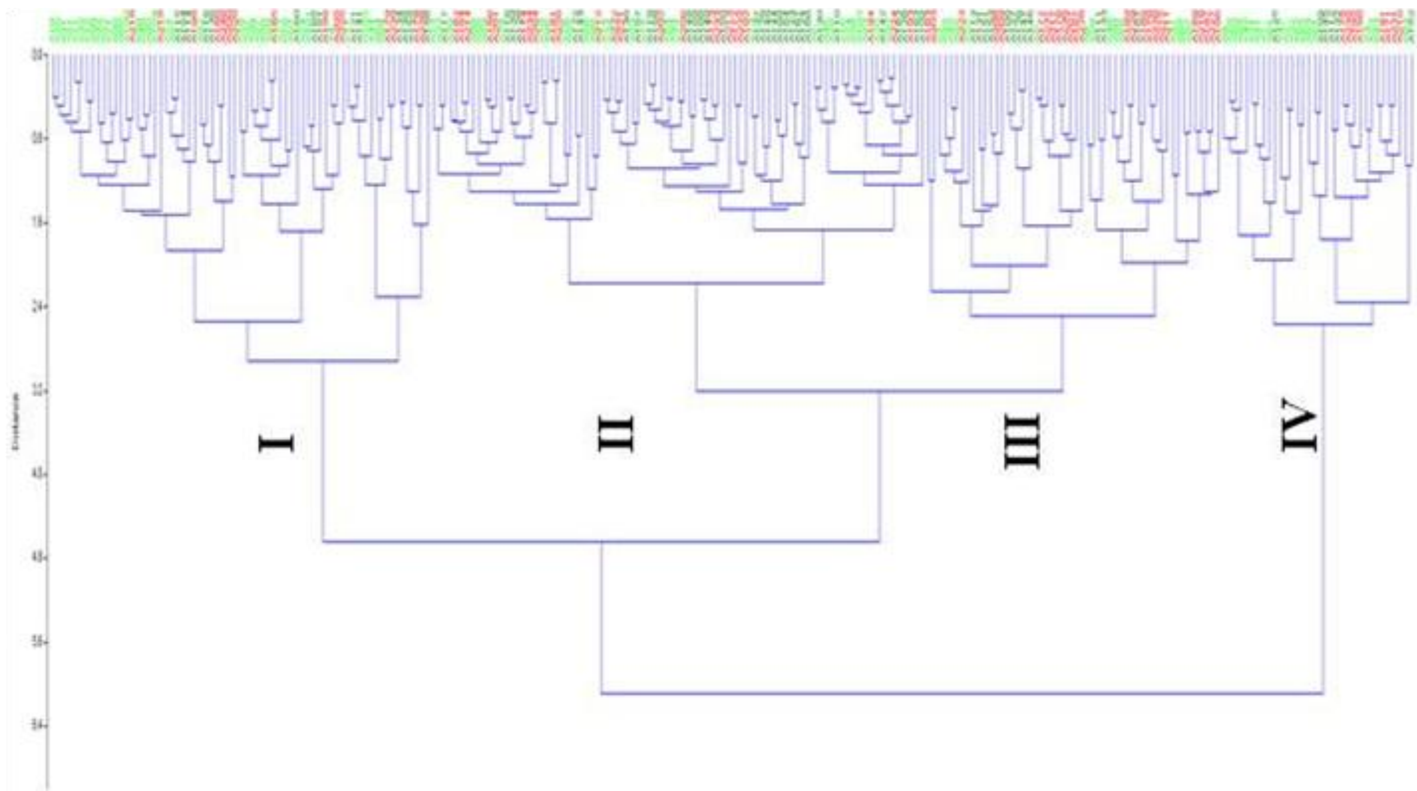


Figure 5. Dendrogam showing dissimilarities between individual shea tree among different sites (Kemkian, Kol and Matekaga)

Matekaga) and showed four groups. Group I included 36.5% of individuals from Matakaga and was divided into four sub-groups. The first sub-group included 18 (17.3%) individuals from Matekaga and only 2 from Kol. A100 from Matekaga was clustered with A218 from Kol. In the third sub-group, the majority of the individuals were from Kol while the fourth sub-group includes most of the individuals from Matekaga. The cluster A98 from Matekaga and A185 from Kemkian, A121/A141 from Kemkian and A134/A135 from Kemkian showed low dissimilarity. The group II included 69% of the individuals from Kol which were distributed in several sub-groups. The clusters formed by A39 from Matekaga /A182 from Kol, A19/A96 from Matekaga and A181/A226 from Kol showed low dissimilarity. The group III included 26 individuals (36.1%) from Kol, 11 (10.5%) from Matekaga and 12 (17.8%) from Kemkian. Most of the samples were gathered by site except the clusters A153 from Kemkian and A208 from Kol and A5 from Matekaga and A177 from Kol. The sample A107 from Kemkian which were grouped with A189 from Kol were nested inside the Kol provenances. The group IV encompassed 34 individuals among which, 20 were from Matekaga, 6 from Kemkian and 8 from Kol. A81 from Matekaga and A105 from Kemkian were grouped together, as A83 from Matekaga and A152 from Kemkian and A43 from Matekaga and A191 from Kol.

DISCUSSION

Understanding population genetic structure is relevant to phytogenetic resources management because it is the first step before implementing any selection process. Phytogenetic resources management was applied to a wide range of economically important plants including shea tree. In its area of distribution particularly in West Africa, shea tree resource management has been mainly based on folk classification for centuries aiming at conservation, domestication and selection of superior individuals (Lovett and Haq, 2000b). On the other hand, in Chad, few studies were reported aiming to enhance our understanding of shea tree genetic variation (Mbaiguinam et al., 2007).

Morphological variations of the shea tree

In this study, a variation in lamina length was observed within and between sites. The smallest lamina length (15.5 cm) was found in Kol, while the longest (26.3 cm) in Matekaga. In addition, the biggest lamina width was found in Kol with 5.4 cm. This morphological variation suggested that a single morphotype was not growing in these areas. Variation of the length of petiole was also observed within site and between sites. The longest petiole was reported in the population from Matekaga while the smallest was observed in Kol. These findings are agreement with the results of Nyarko et al. (2012) who reported petiole length variation in shea tree from Ghana. In the same manner, as the variation of the peduncle length within sites and between sites, the longest peduncle length (3.3 cm) was observed in Kemkian while the longest fruit was found in Matekaga and Kol. The morphological parameters collected from fruits and nuts showed variation within sites but significant differences were not observed between sites. The variations of the parameters from Chad observed in this study were close to those estimated from shea trees in Mali, Ghana, Guinea, Sudano-Sahelain and Uganda. These findings suggested the same amplitude of morphological variation between Central Africa, East and West accessions (Gwali et al., 2012; Sanou et al., 2006; Nyarko et al., 2012). Variation of fruit morphological characters has been reported for the tropical species such as *Balanites aegyptiaca* and *Tamarindus indica* (Soloviev et al., 2004). These variations can be explained by natural and/or human selection, gene flow mediated from genetic drift (Irwin, 2000; Tremblay et al., 2010; Darwin, 1869; Vaughan et al., 2007; Abasse et al., 2011). In addition, rainfall regimes and soil characteristics might be involved in the morphological variations as it was reported in West African provenances (Sanou et al., 2006).

Statistical analysis

Statistical analysis using morphological characters showed high variability of *V. paradoxa* subsp. *paradoxa* growing in the region of Mandoul located in the South of Chad. Individually, these morphological characters were allowed classifying the samples in different morphotypes. Similar results are reported by several authors (Lovett and Haq, 2000a; Nafan et al., 2007). In this study, significant correlations between lamina length and petiole length or between fruit and nut characteristics were observed and it is in agreement with results obtained in provenances from Mali, Côte d'Ivoire and Ghana (Sanou et al*.,* 2006; Lovett and Haq, 2000a; Nafan et al., 2007). Therefore, four main characters as fruit length, fruit width, nut length and nut width were useful to discriminate morphotypes. This assertion confirms the work of Chevalier (1943) who used morphological characters (leave and fruit) to identify eight varieties (*cuneata*, *ferruginea*, *floccosa*, *Mangifolia*, *nilotica*, *parvifolia*, *poissoni*et*serotina*) within *V. paradoxa* subsp. *paradoxa.*

Genetic relationship between shea trees

HCA showed that the use of a single morphological character was not efficient to differentiate the accessions but combining leaf, fruit and nut parameters allowed a good resolution. In this study, each site showed four groups as the dendrogram including all the sites did. These findings were not congruent with the folk classification which identified 6 varieties in the same sites as that of the present study (Mbaiguinam et al., 2007). This incongruence might result from allogamous nature of shea tree which induces phenotypic variation. Phenotypic variation can be influenced by environmental factors or result from genetic variation (Tremblay et al., 2010). Therefore, it is difficult to identify shea tree based on morphological characters alone. The grouping of A11/A30 both from Matekaga and A98 (Matekaga)/A185 (Kol) for example suggested a hybridization by insects pollination or diverse forms of gene flow within or between sites. Hybrids can be problematic for butter quality production because previous studies showed that the morphotypes growing in this area do not produce the same amount of chemical compound (Mbaiguinam et al., 2007). On the other hand, hybridization can be beneficial because high genetic variation induces variability in the population.

Conclusion

Using morphological characters, our study pointed out a high variation of *V. paradoxa* subsp. *paradoxa* populations within and between sites in the region of Mandoul in Southern Chad. A high resolution of the variation was obtained when several morphological characters were combined but a lack of congruence with the folk classification was noticed. The present study can be extended to others Chadian regions were the shea trees are endemic for comparing the local knowledge and for better identification of the morphotypes growing in Chad. Molecular approach can also be used to test if the morphological variation resulted from the DNA polymorphism.

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