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Fatty acid and tocopherol patterns of variation within the natural range of the shea tree (*Vitellaria paradoxa*)

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Abstract The shea tree, *Vitellaria paradoxa*, is one of the most economically and culturally important indigenous tree species in the Sudano-Sahelian region. Its seeds contain a vegetable fat, internationally known as shea butter, which is widely used in edible, cosmetic and pharmaceutical sectors. Based on samples from 456 trees distributed in 17 locations across the species natural range from Senegal to Uganda, the fatty acid and tocopherol variation, and its relationship with geographic and climatic variables, was assessed in order to address the pattern and the origin of this variation across the natural range. Significant differences between Western and Eastern regions for oleic, stearic acid, saturated–unsaturated acid ratio and γ-tocopherol were identified that it is postulated maybe a result of genetic drift due to the evolutionary history of shea tree populations. Within regions the difference among stands was significant for most constituents; however the major part of the variation was observed among trees within stand (53–90 %). Relationships with climatic variables were not verified, weakening evidence for clinal variation hypotheses suggested by previous studies.

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Introduction

Commonly known as the shea tree (le karité in French), *Vitellaria paradoxa* C.F. Gaertn., is one of the most economically and culturally important tree species in the Sudano-Sahelian region (Hall et al. 1996). It is a long-lived deciduous tree species, often attaining over 20 m in height and 50 cm diameter at breast height. The dried kernel of the fruit is used to produce oil or fat (shea butter), for local consumption and commercially as an ingredient in cosmetic, pharmaceutical and edible products. It is estimated that 600,000 t of seeds are collected each year in West Africa and but only 200,000–350,000 SETs (Sheanut Equivalent Tons, inclusive of dry kernel, mechanically-extracted or hand-crafted butter and fractionated stearin) are exported annually (Lovett pers. com). Its natural range extends from the eastern part of Senegal and Gambia to the high plateau of East Africa into south-eastern Uganda forming an almost unbroken belt, 6,000 km long and averaging ~500 km wide. Within the species two subspecies have been distinguished, *V. paradoxa* subsp. *paradoxa* and *V. paradoxa* subsp. *nilotica* (Hall et al. 1996) and although most reports relate to West African collections and due to the wide variability between individuals of both subspecies, it may sometimes be difficult to differentiate between them.

This tree is traditionally wild-managed in parkland, an agroforestry system combining trees and annual crops. The fruits are collected and processed according to traditional system mainly by women. Compared to other forest tree species, the shea tree has likely been influenced by human activities for centuries; following regeneration fallow cycles, healthy trees are maintained in parklands, and Lovett and Haq (2000) proposed that unconscious selection is resulting in the semi-domestication of this species.

The economic importance of the shea tree has led to research to analyse the seed content (Akihisa et al. 2010; Davrieux et al. 2010; Di Vincenzo et al. 2005; Maranz and Wiesman 2003, 2004; Maranz et al. 2003, 2004) and its importance for nutrition (Hall et al. 1996). The seven major fatty acids present in shea butter seed (Maranz and Wiesman 2003; Maranz et al. 2004) are also the most commonly found in all plants (Somerville et al. 2000), i.e., palmitic, oleic, cis-vaccenic, linoleic linolenic, arachidic and stearic acid. However stearic and oleic fatty acids represent 85 % of the *V. paradoxa* seed fat content (Davrieux et al. 2010; Maranz and Wiesman 2003; Maranz et al. 2004). This makes *V. paradoxa* one of the few economically-viable natural sources of Stearic–Oleic–Stearic triacylglycerols (SOS-TAGs), a vegetable stearin with high demand in the confectionary sector due to the functional properties of this compound as compared to cocoa butter.

Another important type of lipid present in the seed, are the tocopherols belonging to the group more commonly known as vitamin E. In tocopherols, the degree of methylation of the benzene ring determines the four forms: alpha (5,7,8-trimethyl), beta (5,8-dimethyl), gamma (7,8-dimethyl) and delta (8-methyl) which are all present in the shea butter tree (Maranz and Wiesman 2004). Some studies have shown the role of tocopherols as non-nutritive bioactive food constituents of plants (Elmadfa and Wagner 2003). They are the key antioxidants in human cell membranes and are also important in protecting the low-density lipoprotein particles and play a role of as protecting agents against oxidative stress.

While several endogenous factors such as the plant age and the seed development stage at harvest (Bailey 1982; Dewhurst et al. 2006; Harwood and Russell 1984) have been reported influencing the proportion of these constituents in seeds, it has been demonstrated to be highly affected by selective pressures and adaptation to the environment (Linder 2000). Thus the variation of those chemical compounds is considered to be related with both genetic and environmental effects.

While many environmental factors, including the surrounding edaphic substrate, the soil microbial community composition or even the ozone concentration

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(Bailey 1982; Fangmeier et al. 1990; Harwood and Russell 1984) have been suggested slightly impacting fat composition, the most relevant environmental factors denoted in the literature inducing a variation in tree seed chemical composition are relied to precipitation and temperature (Bailey 1982; Linder 2000; Somerville et al. 2000). In particular, the fatty acid composition of seeds is reported as a determinant in drought and temperature response mechanisms, especially in maintaining the integrity of the cell wall (Casini et al. 2003; Khurana and Singh 2001; Linder 2000; Pritchard et al. 2004). Tocopherols and α-tocopherol in particular, are major factors in the plant antioxidant defense system (Brigelius-Flohé 2009). These compounds inhibit lipophilic oxidation of the membrane molecules, halting the progression of oxidative chain reaction by capturing the free electron from reactive oxygen species (ROS) (Brigelius-Flohé 2009). ROS appear with the onset of stresses such as water deficit, high and low temperatures, or excessive brightness. Thus, the antioxidant defense system is an important adaptive trait for the protection of crops, ungerminated seeds and young seedlings (Kamal-Eldin and Appelqvist 1996; Klein and Kurilich 2000; Velioglu et al. 1998; Vertucci and Leopold 1987).

Variability of shea seed chemical composition has been discussed in studies on fatty acids (Di Vincenzo et al. 2005; Maranz and Wiesman 2003; Maranz et al. 2004), phenolic compounds (Maranz et al. 2003) and tocopherols (Maranz and Wiesman 2004). The conclusions of these studies validate the higher ratio of oleic acid to stearic acid in East Africa varieties as compared with West Africa (Hall et al. 1996). Given the higher international demand for vegetable fats with high SOS content, this is a key economic finding. They have supplied original results regarding the positive correlation relationship between the relative proportion of stearic acid in sheanuts and climatic gradients between the Sahelian region of northern area of shea (low rainfall and high temperature) and the Sudan-Guinean part of southern area (high temperature more moderate rainfall) (Maranz and Wiesman 2003). Similarly, temperature gradients have been linked with tocopherol variation, with results suggesting that individuals from warmer sites have 15 fold higher α-tocopherol values than cooler ones (Maranz and Wiesman 2004).

These previous analyses on shea seeds, suggest that environmental variation has a strong influence on the profile of seed chemical constituents and acts as selective pressure, favouring certain alleles and thus creating a strong genetic determinism. However, those studies (Di Vincenzo et al. 2005; Maranz and Wiesman 2003, 2004; Maranz et al. 2004) were performed on a small and statistically unbalanced sample size. For example, in the study concerning the influence of temperature on the levels of tocopherols (Maranz and Wiesman 2004), 11 of the 21 sampled sites were represented by only one to two trees. In addition the number of seeds per tree was low (1–3 seeds). Finally, the trend and the magnitude of this variation within the natural range remain unclear. For example, it cannot be demonstrated with certainly whether this variation is influenced by the isolation of populations due to biogeographic barriers or by abiotic pressure such as climatic gradients or edaphic conditions.

To meet some of the questions above and to augment the knowledge generated by earlier results, we undertook a study from a sample covering a more extensive part of the natural range from Senegal to Uganda. This study also aimed to improve on earlier experimental designs through the inclusion of two North–South climatic gradients with statistically valid sample sizes. For this purpose, two parallel transects were established in order to cover the total climatic range of *V. paradoxa* in West Africa. Sampling procedure involving the collection of a significant number of fruits per tree (between 20 and 30) and trees per population (between 12 and 40)—larger sample sizes than previous studies. However, as with previous studies, samples were from natural populations with confounding genetic and environmental effects, preventing a complete explanation of the variation recorded.

The purpose of this study was to increase understanding with regard to the patterns of *V. paradoxa* seed variation of fatty acids and tocopherols within the natural range and their relationship with some environmental parameters. To achieve this goal we studied the chemical composition of seeds collected from 456 trees distributed in 17 locations of the Sudano-Sahelian region from Senegal to Uganda and according to a climatic gradient along two North–South transects in West Africa.

The questions addressed in this study aimed to determine the magnitude of differences between the two sub-species present in West and East Africa for the different constituents; to assess the variability and

distribution of these constituents among sites, within regions and transects, and among trees within sites; and to evaluate whether there are any significant relationships between constituent and climatic variation.

Materials and methods

Sites sampled and climatic characteristics

The sampling strategy was designed to ensure maximum coverage of shea nuts chemical variation, as suggested by previous studies (Di Vincenzo et al. 2005; Maranz and Wiesman 2003, 2004; Maranz et al. 2004). A total of 461 trees were sampled from 17 sites in 5 countries of the natural area (Table 1; Fig. 1). Within this sampling, we defined two latitudinal transects, further called "Mali" and "Ghana–Burkina", comprising 5 sites distributed on a North–South axis, maximizing climatic variation. For each site, in addition to altitude and GPS position, 20 climatic variables were collected using the database WorldClim (Hijmans et al. 2005). However, among these 20 variables only six were retained to describe our sampling due to their continuous distribution (i.e. verifying the hypothesis of Gaussian distribution) and their high variability (i.e.: exhibiting at least 15 % of coefficient of variation): the number of dry months (DRY_M) defined by the number of months with rainfall (in mm) not exceeding 2 times the average monthly temperature (in °C); the temperature seasonality (TEMP_SEAS) defined by the SD of temperature; the annual temperature range (ANN_T_RANGE); the annual precipitation (ANN_PRC); the precipitation seasonality (PRC_SEAS) defined by the coefficient of variation of annual precipitation; the precipitation of warmest quarter (PRC_WaQ).

Within each site, between 10 and 40 trees spaced at least by 50 m were harvested, selected exclusively adult trees (i.e. with diameters at breast height over 15 cm) in order to avoid the possible effect of tree immaturity. For each tree, 20–40 ripe fruits were collected with a particular care of discarding germinated/rotten/corrupted or immature fruits to avoid composition variation due to fruit development stages. Nuts were oven-dried for 3 days at 60 °C to stabilize their moisture and stored at room temperature before analysis.

Methods analytical chemistry

Unshelled shea nuts were first ground in a "Vorwerk Thermomix Robot". Raw powders were frozen at −20 °C and re-ground in a "SEB Valentin Robot" in order to obtain a final particle size between 0.5 and 0.8 mm. The resulting powder samples were then stored at −20 °C and protected from light exposure.

For each sample, moisture content (MC) was assessed by gravimetric analysis in a 103 °C oven (Chopin) for 16 h. Moisture content is not a trait of physiological interest in this study because nuts have been dried after harvest. This measure only ensured that the moisture content of seeds is below 10 % to avoid lipase activities and acid hydrolysis of triacylglycerols (TAGs) into free fatty acids (Verger and Rivière 1987). This variable was analysed in our sample and confirms that the percentage of humidity was lower than 10 % (from 1.8 to 9.3 %, with a mean value of 4.5 %, detailed results not shown) limiting the degradation process of chemical components.

Fat content (FC) was solvent-extracted (petroleum ether) from powders using a semi-automatic Soxtec 2050 extractor (FOSS) according to the manufacturers' instructions. After gravimetric quantification, extracted oils were stored at −20 °C for further chemical analyses. Fatty acid (FA) profiles were obtained according to the protocol described by Tchobo (2007) (Tchobo et al. 2007): after esterification of oil using sodium methylate, FA profiles were determined by Gas Chromatography using a Thermo Focus (Thermo Fisher Scientific Inc., Waltham, USA) GC with CP SIL 88 (highly substituted cyanopropyl phase) column (Varian, Palo Alto, USA). The chromatographic conditions were set up as follow: injection temperature 250 °C, oven temperature 150 °C (isotherm), detection temperature 250 °C and vector gas flow 1 ml/min. The injection volume was 1 µl.

On a significant subset (273 samples) of the total sampling, a "solid–liquid" fat extraction was performed at room temperature using 3 g of shea nut powder and 12 ml petroleum ether stirred magnetically for 2 h in the dark to avoid degradation of labile compounds such as tocopherols. Solvent containing fat was filtered through 0.2 µm PTFE syringe, and then helium evaporated under hood. Fat were stored at −20 °C and protected from light. The tocopherol contents of fat were analyzed by liquid chromatography (HPLC) according to ISO 9936 (AFNOR 1997).

Table 1 Characterization of the sampling

Site Id	Site	GPS position	Altitude (m)	N	Subspecies
West Africa					
Senegal					
1	Kenioto	12.57°N; 12.16°W	161	14	*paradoxa*
2	Samecouta	12.60°N; 12.13°W	126	18	*paradoxa*
3	Saraya	12.83°N; 11.75°W	180	13	*paradoxa*
Mali					
4	Nafégué	10.51°N; 5.98°W	344	40	*paradoxa*
5	Mperesso	12.28°N; 5.33°W	340	37	*paradoxa*
6	Daelan	13.25°N; 4.99°W	282	37	*paradoxa*
7	Tori	13.61°N; 3.72°W	377	35	*paradoxa*
8	Sassambourou	14.31°N; 3.51°W	392	35	*paradoxa*
Burkina Faso					
9	Titao	13.72°N; 2.16°W	336	18	*paradoxa*
10	Guibare	13.07°N; 1.61°W	303	21	*paradoxa*
Ghana					
11	Kulbia	10.83°N; 0.96°W	206	34	*paradoxa*
12	Tolon	9.43°N; 1.00°W	154	35	*paradoxa*
13	Kawampe	8.43°N; 1.56°W	125	35	*paradoxa*
Total west Africa				372	
East Africa					
Uganda					
14	Katakwi	1.82°N; 33.99°E	1100	25	*nilotica*
15	Pader	2.80°N; 33.31°E	1031	29	*nilotica*
16	Moyo	3.62°N; 31.64°E	863	16	*nilotica*
17	Uleppi-Arua	3.02°N; 30.90°E	1200	19	*nilotica*
Total east Africa				89	

N number of trees sampled

The HPLC apparatus consists of modules provided by Thermo-Finnigan (France): a quaternary pump (P1000XR), an autosampler (AS1000) and an injection valve equipped with 6-way loop of 20 ml, spectrofluorimetric detector (FL3000) and software for data processing (Chromquest). The standard samples were purchased from VWR International SAS France. The mobile phase consisted in hexane/dioxane (97:3, v/v) with a flow rate of 1 ml/min. The silica column is a "HypersilTM" of 5 microns thick, 4 mm internal diameter by 250 mm long. The wavelength of excitation is set at 290 nm and the emission at 330 nm. Three tests are performed per sample. For each type of laboratory analysis we estimated the standard error of laboratory (SEL) calculating the standard deviation obtained over 10 repetitions of a standard sample analysis of shea.

Statistical analyses

To describe the chemical variability, a basic statistical analysis was performed at different scales: natural range; subspecies; transects; and populations. Mean value, variation range and SD were calculated for each chemical component using the XLSTAT 2011.1.01 software (Addinsoft, Paris, France 2009). The correlation analyses among chemical components and between climatic variables and chemicals constituents were performed using the procedure CORR (SAS Institute 1989) on the values of individuals.

For all chemical constituents, we tested the difference between groups of individuals at different scales: among regions, between sites within regions and between sites within transects. For that purpose, we tested the null hypothesis, H0, of equality of means

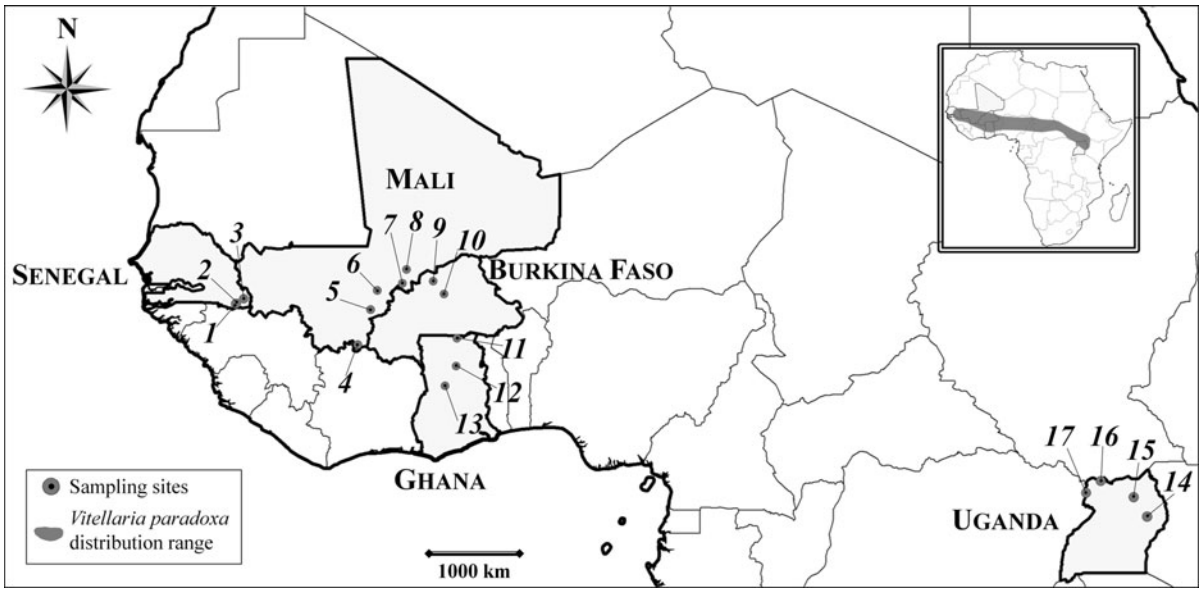

Fig. 1 Map displaying the geographic location of sampling sites and the natural distribution of shea (*Vitellaria paradoxa*)

between groups by analysis of variance using fixed model. This analysis was conducted using the GLM procedure of SAS statistical software (SAS Institute 1989) specifying the option "sum of squares type III", and testing the H0 by a Fisher's test. The comparison of group means was performed by a Bonferroni's test, the following model was used:

$$y_{ij} = \mu + group_i + tree_{j(i)}$$

where y_{ij} is the value of the dependent variable for the *j*th tree in the *i*th group (region or site); μ is the mean value of the dependent variable; $group_i$ is the fixed effect of the *i*th group (region or site); $tree_{j(i)}$ is the residual error of random effect, following $\sim N(0,\sigma_e^2)$ linked to the *j*th tree in the *i*th group.

To understand the variance structure of the chemical constituents, we estimated the variance components using a hierarchical random model. This analysis was performed using the VARCOMP procedure of SAS statistical software (SAS Institute 1989) and the restricted maximum of likelihood (REML) method. The H0 of equality of variances between groups and was tested using a Fisher test. The random model is as follows:

$$y_{ij} = \mu + site_i + tree_{j(i)}$$

where y_{ij} is the value of the dependent variable for the *j*th tree in the *i*th site, μ is the mean value of the dependent variable; $site_i$ is the random effect of the *i*th site following $\sim N(0,\sigma_\alpha^2)$; $tree_{j(i)}$ is the residual error of random effect, following $\sim N(0,\sigma_e^2)$ related to the effect of the *j*th tree within the *i*th site.

The following variance ratios were calculated for each region and each transect:

Percentage of variance due to site (within region or transect) effect in total variance:

$$\sigma_{site}^2/(\sigma_{site}^2 + \sigma_{tree}^2)$$

Percentage of variance due to tree effect in total variance:

$$\sigma_{tree}^2/(\sigma_{site}^2 + \sigma_{tree}^2)$$

In addition to analyses of variance, multivariate analyses were performed using XLSTAT 2011.1.01 software (Addinsoft, Paris, France 2009). Multivariate analyses consisted in Principal Component Analyses (PCA) and Hierarchical Cluster Analyses (HCA). Such analyses were carried out to detect eventual groups of sites presenting similar fatty acid and tocopherol content at different scales: on the natural range and within West Africa. Concerning PCA, we used average values of sites, and additional (passive) climatic variables were projected to facilitate the ecological interpretation of PCA axes. Regarding HCA, we employed the Euclidean distance as distance measurement and the Ward's as aggregation method ward (Ward 1963).

Results

Fat content

The fat content across the natural range showed significant variation with a minimum of 28.0 %, a maximum of 61.6 % and a global mean of 49.9 % (results not shown). Results exhibited normal distribution once five outliers were removed, the latter being likely to be due to immature fruits but could also be an example of human unconscious selection, e.g. maintenance of yield-volume encouraging the survival of plants having attributes not usually best for natural survival (Harlan 1975). As a result, they were discarded for subsequent analyses conducted on 456 trees. An average of 49.9 % and a coefficient of variation of 10 % were observed for all the samples.

East African origin seed (subspecies *nilotica*) fat content averaged 53.2 %, significantly higher than West African (subspecies *paradoxa*) (average: 49.1 %) (Table 2). In both regions, the average fat content varied significantly among sites (40.3 % for Saraya in Senegal to 52.8 % for Nafégué in Mali) and (51.5 % for Uleppi-Arua to 54.8 % in Moyo Uganda). The intra-site coefficients of variation were lower in East Africa (between 2 and 6 %) than in West Africa (between 5 and 17 %) (Table 2).

In the North–South Mali transect, analysis of variance showed significant differences among sites and Bonferroni test identified three groups. In the Ghana–Burkina transect, the analysis also revealed significant differences with two groups (Table 2).

In terms of variance percentage, the random model revealed that in West Africa nearly 35 % of the variance was due to differences among sites and 65 % among trees. The variance distribution was different in the East Africa: 20 % among sites and 80 % among trees. Concerning transects, in Mali, 19 % of the variance is related to the site difference and 81 % to the trees whereas in Ghana–Burkina transect, 6 % of the variance is related to the site difference and 94 % to the trees (Table S2 in supplementary material).

Table 2 Comparison of means between regions, sites and transects for the fat content

Sampling considered	Site Id	N	Mean (CV)	Group	F *p*
Natural area	W	367	49.1 (10)	A	**56.52**
	E	89	53.2 (5)	B	<0.01
Total nat. a.[a]		*456*	*49.9 (10)*		
West	3	10	40.3 (17)	A	**10.8**
	1	12	42.0 (9)	A	<0.01
	5	37	47.4 (11)	B	
	9	18	47.8 (9)	BC	
	2	18	47.9 (7)	BC	
	8	35	48.3 (12)	BC	
	11	34	48.8 (8)	BC	
	7	35	48.9 (5)	BC	
	10	21	49.2 (7)	BCD	
	13	35	49.4 (11)	BCD	
	6	37	51.1 (8)	CD	
	12	35	51.4 (8)	CD	
	4	40	52.8 (5)	D	
Total west		*367*	*49.1 (10)*		
East	17	19	51.5 (4)	A	**6**
	15	29	52.7 (6)	AB	<0.01
	14	25	54.1 (5)	B	
	16	16	54.8 (2)	B	
Total east		*89*	*53.2 (5)*		
Mali transect	5	37	47.4 (11)	A	**9.9**
	8	35	48.3 (12)	AB	<0.01
	7	35	48.9 (5)	AB	
	6	37	51.1 (8)	BC	
	4	40	52.8 (5)	C	
Total M. trans.[b]		*184*	*49.8 (9)*		
Ghana–Burkina transect	9	18	47.8 (9)	A	**2.7**
	11	34	48.8 (8)	AB	<0.01
	10	21	49.2 (7)	AB	
	13	35	49.4 (11)	AB	
	12	35	51.4 (8)	B	
Total G.–B. trans.[c]		*143*	*49.5 (9)*		

Bold values are significant at 5 %

N number of trees analyzed, Mean average value, *CV* coefficient of variation, *Group* grouping proposed by Bonferroni's test, *p p* value, *W* west region, *E* east region

[a] Total natural area

[b] Total Mali transect

[c] Total Ghana–Burkina transect

Fatty acid profiles

Based on sample gas chromatographic profiles, seven fatty acids, showing percentages above 0.05 % were selected for the study. We detected three saturated fatty acids (palmitic acid C16:0, stearic acid C18:0 and arachidic acid C20:0), two *cis*-mono-unsaturated fatty acids (oleic acid C18:1 *n*-9 and *cis*-vaccenic acid C18:1 *n*-7) and 2 polyunsaturated fatty acids (linoleic

acid C18:2 *n*-6 and γ-linolenic acid C18:3 *n*-6). Stearic (38.6 %) and the oleic acid (48.0 %) were the highly predominant fatty acids, accounting for 85–90 % of the total. Due to this predominance, detailed results are presented for these two constituents only and for the saturated to unsaturated ratio (SU ratio) (Table 3). Results obtained for the other fatty acid are presented in Table S1 in supplementary material.

Although significant (5 % level) correlations were observed between fatty acids, most of them were smaller than 0.6. A strong and highly significant negative correlation for the East region (−0.91) and for the West region (−0.93) was observed between stearic and oleic acids.

East African sheanuts were significantly richer in oleic acid (56.4 %) than West African (45.9 %) (Table 3). Conversely, the relative percentage of stearic acid was significantly higher in West Africa (40.8 %) than in East Africa (29.7 %). This result induced a significant difference in the SU ratio between these two regions: West (0.87) and East (0.55) (Table 3). Intra-site coefficients of variation for the two major fatty acids were relatively small (between 2 and 10 %), but higher in West Africa, stressing a higher diversity in this region. This was also true for other fatty acids (Table S1 in supplementary material).

Within the western region the differences between sites were highly significant ($p < 0.0001$) for the three variables. The grouping revealed three groups for stearic and oleic acid and four groups for the SU ratio (Table 3). Similarly, in the eastern region, the analysis of variance revealed significant differences (Table 3). Bonferroni tests showed two groups for each variable with a systematic distinction of Katakwi (the most southern site in Uganda) and Uleppi-Arua (the most Northern site in Uganda). For the other fatty acids the significant differences between sites were observed in West Africa and not systematically in East Africa (Table S1 in supplementary material).

All these results were illustrated by multivariate analyses. Figure 2a, b showed a strong dissimilarity between two main clusters, one containing eastern sites and the other one comprising West African origins. However, within the West region, the multivariate analyses did not detect a clustering correlated with either geographic distribution or climatic variation (Fig. 2c, d).

In the Ghana–Burkina transect, only significant differences were detected for some minor fatty acids (Table 3, S1 in supplementary material). Within the Mali transect, highly significant differences were observed for all fatty acids and the SU ratio (Table 3, S1). For stearic and oleic, grouping showed a clear distinction between Mperesso and the other sites. But no specific trends of these variables with latitude were detected (Fig. S1A in supplementary material).

Concerning the distribution of variability, we observed the predominance of variance between trees within sites (Table S2 in supplementary material) suggesting high variation within shea butter tree parklands. The variance between sites depends on the regions. Within the East region, the proportion of variance explained by "site" effects for oleic, stearic acids and SU ratio (39.1, 24.5 and 28.8 % respectively) was more important than within the West region (18.9, 22.2 and 20.3 % respectively).

Within transect, the proportion of variance explained by "site" was higher in the "Mali" than in the "Ghana–Burkina" transect: for stearic acid (23.1 and 4.4 % respectively), for oleic (16.0 and 1.5 % respectively) and for SU ratio (22.3 and 4.0 % respectively) (Table S2 in supplementary material).

Tocopherol content

Chromatograms obtained by HPLC on the 273 samples showed that α-tocopherol was largely predominant with an average of 112 µg/g of butter and a relatively high coefficient of variation (29 %). The γ-tocopherol was second with an average of about 13 µg/g and a high coefficient of variation (77 %). The beta and delta tocopherols were present in very small quantities, lower than 1.5 µg/g with extremely large coefficients of variation (over 110 %). In consequence only α- and γ-tocopherols results are presented. A significant but low correlation (<0.40) was observed between those two constituents. Like the other fat constituents, the coefficients of variation were larger in the West Africa region (Table 4) suggesting a greater diversity. While average levels of α-tocopherol were higher in the East region, the difference was not significant (Table 4) and the distribution of α-tocopherol was close to normal. The average levels of γ-tocopherols were significantly higher in East region (Table 4) resulting in abnormal bimodal distribution.

Table 3 Comparison of Means between regions, sites and transects for major fatty acids and saturated/unsaturated (SU) ratio

Sampling considered	ID	N	Stearic acid			ID	N	Oleic acid			ID	N	SU ratio		
			Mean (CV)	Group	F *p*			Mean (CV)	Group	F *p*			Mean (CV)	Group	F *p*
Natural area	E	89	29.7 (7)	A	**790.8**	W	366	45.9 (7)	A	**967.4**	E	89	0.55 (9)	A	**691.1**
	W	366	40.8 (9)	B	<0.01	E	89	56.4 (3)	B	<0.01	W	366	0.87 (14)	B	<0.01
Total nat. a[a]		*455*	*38.6 (14)*				*455*	*47.9 (10)*				*455*	*0.81 (21)*		
West	5	37	36.8 (9)	A	**9.1**	11	34	44.3 (5)	A	**8.1**	5	37	0.74 (13)	A	**8.7**
	1	12	37.5 (8)	AB	<0.01	12	35	44.3 (6)	A	<0.01	1	12	0.80 (11)	AB	<0.01
	3	10	39.7 (7)	ABC		2	18	44.3 (4)	AB		7	35	0.84 (10)	BC	
	8	35	40.2 (8)	BC		6	37	45.0 (9)	AB		8	35	0.84 (14)	BC	
	7	35	40.4 (7)	BC		13	34	45.1 (7)	AB		4	40	0.85 (11)	BCD	
	13	34	40.9 (9)	BC		9	18	45.5 (6)	AB		3	10	0.88 (11)	BCD	
	4	40	41.1 (7)	BC		10	21	45.5 (6)	AB		9	18	0.88 (12)	BCD	
	9	18	41.4 (7)	BC		3	10	45.7 (4)	ABC		13	34	0.88 (14)	BCD	
	10	21	41.7 (7)	C		4	40	46.6 (6)	BC		10	21	0.89 (11)	BCD	
	6	37	41.8 (11)	C		8	35	46.8 (6)	BC		6	37	0.92 (17)	BCD	
	2	18	42.3 (6)	C		7	35	46.9 (5)	BC		12	35	0.93 (12)	CD	
	12	35	42.7 (7)	C		1	12	47.7 (4)	BC		11	34	0.94 (10)	D	
	11	34	42.7 (6)	C		5	37	48.8 (6)	C		2	18	0.96 (11)	D	
Total west		*366*	*40.8 (9)*				*366*	*45.9 (7)*				*366*	*0.87 (14)*		
East	16	16	28.6 (5)	A	**7.2**	17	19	54.5 (4)	A	**13.4**	14	25	0.53 (9)	A	**8.8**
	14	25	29.1 (7)	A	<0.01	15	29	56.3 (3)	B	<0.01	16	16	0.53 (7)	A	<0.01
	15	29	29.8 (6)	AB		14	25	57.2 (3)	B		15	29	0.55 (8)	A	
	17	19	31.1 (6)	B		16	16	57.5 (2)	B		17	19	0.59 (8)	B	
Total east		*89*	*29.7 (7)*				*89*	*56.4 (3)*				*89*	*0.55 (9)*		

Table 3 continued

Sampling considered	ID	N	Stearic acid			ID	N	Oleic acid			ID	N	SU ratio		
			Mean (CV)	Group	F *p*			Mean (CV)	Group	F *p*			Mean (CV)	Group	F *p*
Mali transect	5	37	36.8 (9)	A	**12.1**	6	37	45.0 (9)	A	**8.1**	5	37	0.74 (13)	A	**11.6**
	8	35	40.2 (8)	B	<0.01	4	40	46.6 (6)	A	<0.01	7	35	0.84 (10)	B	<0.01
	7	35	40.4 (7)	B		8	35	46.8 (6)	A		8	35	0.84 (14)	B	
	4	40	41.1 (7)	B		7	35	46.9 (5)	A		4	40	0.85 (11)	B	
	6	37	41.8 (11)	B		5	37	48.8 (6)	B		6	37	0.92 (17)	B	
Tot. M. trans.[b]		*187*	*40.1 (9)*				*187*	*46.8 (7)*				*187*	*0.84 (15)*		
Ghana–Burkina transect	13	34	40.9 (9)	A	2.3	11	34	44.3 (5)	A	1.40.22	9	18	0.88 (12)	A	2.2
	9	18	41.4 (7)	A	0.06	12	35	44.3 (6)	A		13	34	0.88 (14)	A	0.08
	10	21	41.7 (7)	A		13	34	45.1 (7)	A		10	21	0.89 (11)	A	
	12	35	42.7 (7)	A		9	18	45.5 (6)	A		12	35	0.93 (12)	A	
	11	34	42.7 (6)	A		10	21	45.5 (6)	A		11	34	0.94 (10)	A	
Tot. G.–B. trans.[c]		*142*	*42.0 (7)*				*142*	*44.8 (6)*				*142*	*0.91 (12)*		

Bold values are significant at 5 %

N number of trees analyzed, Mean average value, *CV* coefficient of variation, *Group* grouping proposed by Bonferroni's test, *p p* value, *W* West region, *E* East region

[a] Total Natural Area

[b] Total Mali transect

[c] Total Ghana–Burkina transect

Concerning the differences among sites, regions exhibited specific patterns. There were significant differences in West Africa for α-tocopherol only (Table 4). In contrast, in East Africa, significant differences among sites were only observed for γ-tocopherol.

At the natural range scale, the multivariate analysis did not reveal consistent groups with geography or climate (Fig. 3a, b). As a result, the dendrogram (Fig. 3b) displays two main clusters mixing West and East African origins.

Within the West region, no group of sites appeared clearly and no clear relationship with climatic variables was noticed (Fig. 3c). The tocopherol HCA's dendrogram (Fig. 3d) showed a confused clustering, not consistent with geographic distribution or clinal climatic variation.

While the differences among sites within both transects were significant for α-tocopherol, the result for γ-tocopherol showed no significant differences in the Ghana–Burkina transect (Table 4). This relationship between tocopherols and latitude (Fig. S2 in supplementary material) did not show specific trend.

Regarding variance components, the difference between sites explained 37.9 and 22.6 % of the total variance of α-tocopherol in West and East Africa respectively. For γ-tocopherol, 8.4 and 47.0 % were

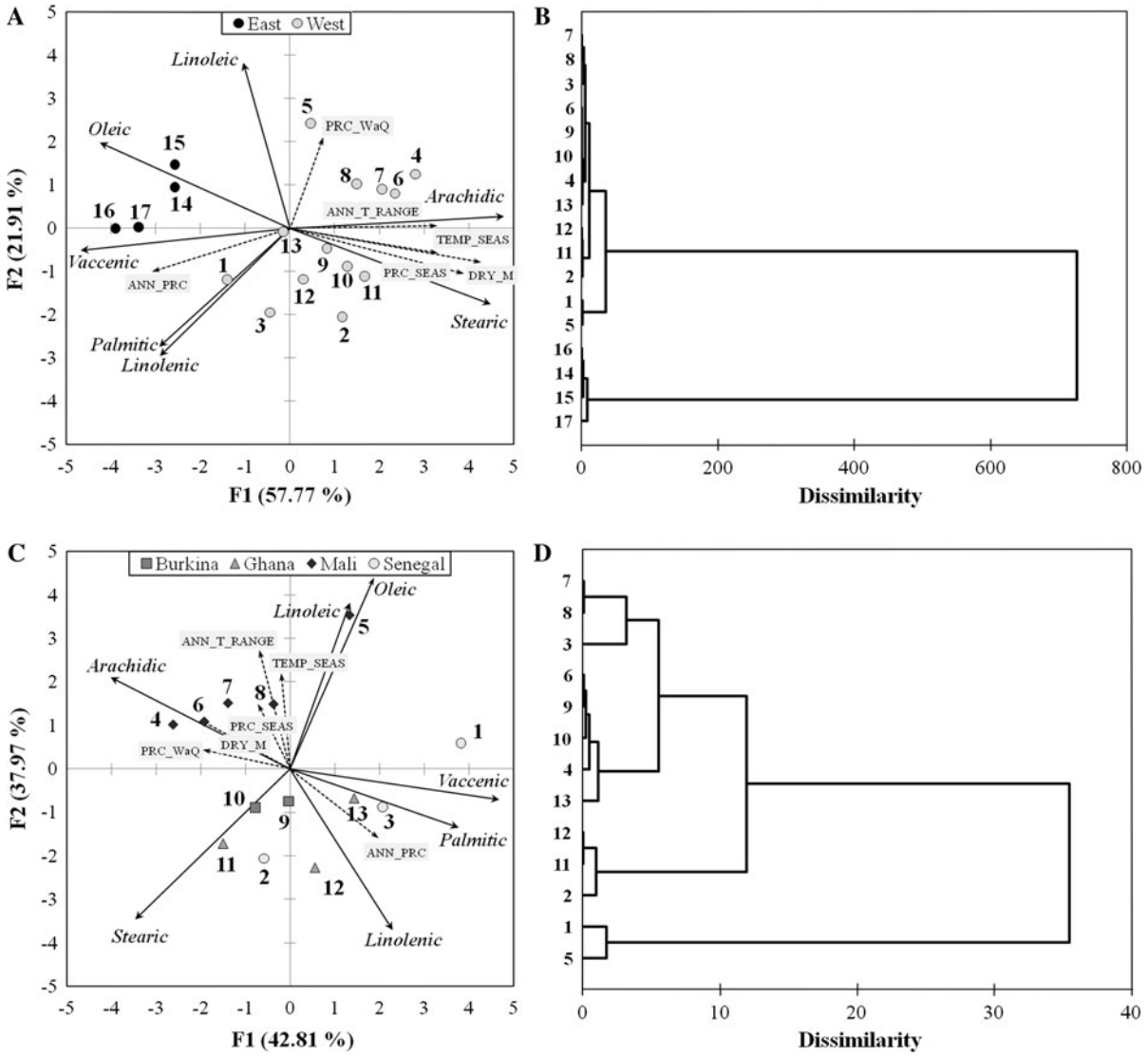

Fig. 2 PCA based on fatty acid composition for the global sampling (**a**) and West Africa (**c**). *Solid arrows* represent the contributions of the active variables (chemical constituent) and *dotted arrows* the passive climatic variables. Dendrograms obtained after hierarchical cluster analyses on fatty acid composition for the global sampling (**b**) and for West Africa (**d**). *DRY_M* number of dry months, *TEMP_SEAS* temperature seasonality, *ANN_T_RANGE* annual temperature range, *ANN_PRC* annual precipitation, *PRC_SEAS* precipitation seasonality, *PRC_WaQ* precipitation of warmest quarter

explained by the difference between site in West and East Africa respectively (Table S2 in supplementary material).

Relationship with climatic factors

A consistent relationship between seed chemical constituents and climatic variables was not displayed within West Africa transect. Correlation coefficients were low (smaller than 0.55) although some of them were significant (Table 5). The scatter points associated with these estimates (not shown) did not exhibit any linear trends and could not verify any clear relationship between chemical constituents and climatic variables in our sampling.

Correlation estimated with other minor fatty acid and other variables (altitudes, latitude and longitude) did not exhibit higher correlations (results not shown).

Table 4 Comparison of Means between regions, sites and transects for the two major forms of tocopherols

Sampling considered	ID	N	α-tocopherol			ID	N	γ-tocopherol		
			Mean (CV)	Group	F *p*			Mean (CV)	Group	F *p*
Natural area	W	246	110.6 (29)	A	3.1	W	246	10.5 (61)	B	**267.6**
	E	27	122.0 (22)	A	0.08	E	27	33.7 (32)	A	<0.01
Total nat. a.[a]		*273*	*111.7 (29)*				*273*	*12.8 (77)*		
West	1	4	54.9 (53)	A	**9.7**	6	37	6.9 (56)	A	**2.8**
	2	9	74.7 (44)	A	<0.01	11	9	7.6 (43)	A	0.02
	6	37	94.3 (27)	AB		7	35	8.1 (45)	A	
	4	40	96.6 (20)	AB		10	7	9.4 (39)	A	
	13	10	98.8 (24)	ABC		4	40	11.7 (75)	A	
	11	9	101.7 (21)	ABC		12	9	11.8 (74)	A	
	12	9	110.9 (14)	ABCD		5	37	11.8 (49)	A	
	7	35	112.7 (23)	BCD		8	35	12.0 (53)	A	
	8	35	121.4 (25)	CD		13	10	12.2 (54)	A	
	3	7	128.0 (44)	CD		2	9	12.6 (81)	A	
	10	7	130.0 (15)	CD		3	7	12.9 (36)	A	
	9	7	134.4 (17)	CD		9	7	14.4 (36)	A	
	5	37	138.5 (21)	D		1	4	14.5 (30)	A	
Total west		*246*	*110.6 (29)*				*246*	*10.5 (61)*		
East	16	4	104.1 (19)	A	2.8	15	9	24.1 (19)	A	**7**
	15	9	111.0 (30)	A	0.06	14	9	35.3 (34)	AB	<0.01
	14	9	129.4 (14)	A		17	5	41.5 (14)	B	
	17	5	143.0 (11)	A		16	4	42.0 (13)	B	
Total east		*27*	*122.0 (22)*				*27*	*33.7 (31)*		
Mali transect	6	37	94.3 (27)	A	**18.1**	6	37	6.9 (56)	A	**5.9**
	4	40	96.6 (20)	AB	<0.01	7	35	8.1 (45)	AB	<0.01
	7	35	112.7 (23)	BC		4	40	11.7 (75)	B	
	8	35	121.4 (25)	CD		5	37	11.8 (49)	B	
	5	37	138.5 (21)	D		8	35	12.0 (53)	B	
Tot. M. trans.[b]		*184*	*112.3 (27)*				*184*	*10.1 (63)*		
Ghana–Burkina transect	13	10	98.8 (24)	A	**4.9**	13	10	7.6 (54)	A	1.6
	11	9	101.7 (21)	AB	<0.01	10	7	9.4 (39)	A	0.21
	12	9	110.9 (14)	ABC		12	9	11.8 (74)	A	
	10	7	130.0 (15)	BC		11	9	12.2 (43)	A	
	9	7	134.4 (17)	C		9	7	14.4 (36)	A	
Tot. G.–B. trans.[c]		*42*	*113.1 (21)*				*42*	*11.0 (55)*		

Bold values are significant at 5 %

N number of trees analyzed, Mean average value, *CV* coefficient of variation, *Group* grouping proposed by Bonferroni's test, *p p* value, *W* West region, *E* East region

[a] Total natural area

[b] Total Mali transect

[c] Total Ghana–Burkina transect

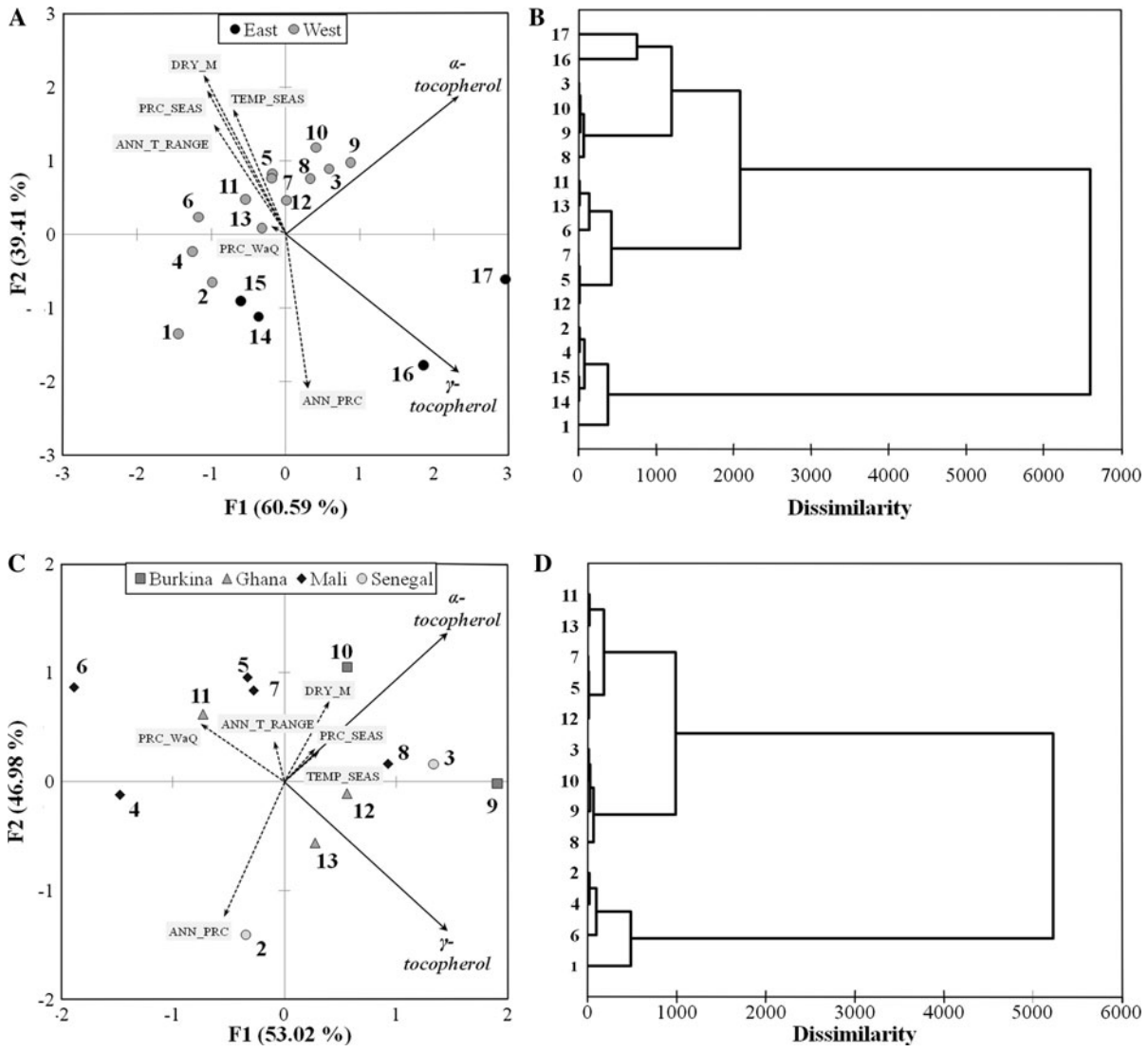

Fig. 3 PCA based on tocopherol content for the global sampling (**a**) and West Africa (**c**). *Solid arrows* represent the contributions of the active variables (chemical constituent) and *dotted arrows* the passive climatic variables. Dendrograms obtained after hierarchical cluster analyses on tocopherol content for the global sampling (**b**) and for West Africa (**d**). *DRY_M* number of dry months, *TEMP_SEAS* temperature seasonality, *ANN_T_RANGE* annual temperature range, *ANN_PRC* annual precipitation, *PRC_SEAS* precipitation seasonality, *PRC_WaQ* precipitation of warmest quarter

Discussion

Fat content and constituents of shea butter

In the present study, we observed highly variable chemical compounds. As endogenous factors such as the seed maturity states may have an impact on this apparent variation, we previously have in insured that each seed displayed a correct ripeness state with no signature of germination or any corruption. Moreover, no significant correlations between chemical constituents and age-link traits of trees (diameter at breast height, trees height) were observed (data not shown), concluding in no apparent relationship between tree development stages and fat profiles. We observed the very high levels of fat content, representing between 30 and 60 % of the total nut dry matter. Although our sampling was not exhaustive, it covers a significant

Table 5 Correlation analysis between seed chemical constituents and bioclimatic variables

Variable	Fat content		Stearic Acid		Oleic Acid		SU Ratio		Alpha Tocopherol		Gamma Tocopherol	
	Mali transect	Ghana–Burkina transect	Mali transect	Ghana–Burkina transect	Mali transect	Ghana–Burkina transect	Mali transect	Ghana–Burkina transect	Mali transect	Ghana–Burkina transect	Mali transect	Ghana–Burkina transect
DRY_M	−0.13	−0.21	0.16	0.02	−0.16	0.07	**0.19**	−0.01	0	0.39	−0.07	−0.04
TEMP_SEAS	**−0.29**	−0.15	−0.02	0	−0.04	0.1	0.04	−0.05	0.19	**0.54**	−0.12	0.07
ANN_T_RANGE	**−0.24**	−0.16	0	0	−0.1	0.11	0.09	−0.05	0.12	**0.53**	−0.18	0.03
ANN_PRC	**0.24**	0.16	−0.05	0.01	0.09	−0.11	−0.1	0.06	−0.11	**−0.53**	0.14	−0.05
PRC_SEAS	**−0.21**	−0.16	0.09	0.03	−0.12	0.08	0.14	−0.01	0.07	**0.48**	−0.15	−0.03
PRC_WaQ	**0.4**	0.03	**0.23**	−0.08	−0.16	−0.06	**0.19**	−0.02	**−0.41**	**−0.51**	−0.04	0.04

Bold values are significant at 5 % level

DRY_M number of dry months, *TEMP_SEAS* temperature seasonality, *ANN_T_RANGE* annual temperature range, *ANN_PRC* annual precipitation, *PRC_SEAS* precipitation seasonality, *PRC_WaQ* precipitation of warmest quarter

part of the total distribution allowing the comparison with previous studies (Di Vincenzo et al. 2005; Maranz and Wiesman 2003, 2004; Maranz et al. 2004). We confirm the predominance of stearic (from 26 to 48 %) and oleic acids (from 38 to 60 %) representing between 85 and 90 % of the total fatty acids. This result can be explained by the nature of the biosynthetic pathway of these two 18 carbons-chained interdependent fatty acids. Indeed, the oleyl-ACP (precursor of the oleic acid) is produced by a single desaturation of the stearoyl-ACP (precursor of the stearic acid) under the control of a lonely soluble enzyme of the plastid stroma, called Δ9-stearoyl-acyl-carrier-protein desaturase (SAD) (Lindqvist et al. 1996; Somerville et al. 2000). This process explains the strong correlation observed between these two fatty acids (r = 0.91 and 0.93 in East and West regions). The melting temperature of stearic acid is 69.6 °C while that of oleic acid is only 16.3 °C (Bailey 1982); therefore their relative proportion in nuts modifies the physicochemical properties of the extracted oil. The more the shea "butters" are rich in stearic acid the higher their melting points. As for the fat profile, the fatty acid composition makes shea butter an important source of SOS-TAGs for use in formulation of cocoa butter equivalent vegeTable fats (Ming 2008).

Concerning the tocopherols, levels of γ-tocopherol were smaller than those in the Maranz and Wiesman (2004). This difference may be explained by the sampling strategy. In the present study the priority was given to the number of individuals per site (on average 3.5 times more), a more balanced and statistically reliable sampling. Another possible explanation is the low repeatability of the tocopherol measurement stressed by different studies. Particularly, in walnut (*Nux regia* L.), variation in laboratory measurement was estimated between 5 and 10 % (Lavedrine et al. 1997). In our study we estimated the errors of laboratory measurement at 9 % for α-tocopherol and 10 % for γ-tocopherol. Therefore, each sample was independently analyzed three times to limit the impact of uncertainty. An ultimate possible explanation comes from the function of these compounds. These antioxidant molecules are subject to degradation in particular of reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2) (Brigelius-Flohé 2009). It is then possible that the lower levels measured in our study were due to post-harvest degradation of tocopherols.

When comparing our levels of α-tocopherols (110.6 and 122.0 μg/g in West and East regions respectively) with other plants, we notice lower levels than in oil palm (*Elaeis guineensis*; α-tocopherol = 300–500 μg/g) or sunflower (*Helianthus annuus*; α-tocopherol = 500–700 μg/g) (Grusak and DellaPenna 1999) but close to those of other tropical species known for their anti-oxydant properties such as *Apium graveolens*, a local celery (α-tocopherol = 136.4 μg/g), or the black tea (*Camelia chinensis*; α-tocopherols = 183.3 μg/g) (Ching and Mohamed 2001). These levels of tocopherols confer to sheanuts interesting potential for food as advised by the "Recommended Dietary Allowance" (more than 7 mg of E vitamin per day are suggested to reduce cancer risks) (Agudo et al. 1997). These levels are also attractive for the cosmetics operating its antioxidant properties in anti-aging skin cares (Alvarez and Rodríguez 2000).

Marked difference between Eastern and Western origins

We observed huge differences between regions for the different constituents independently (Tables 2, 3 and 4). For oleic, stearic acid, SU ratio and γ-tocopherol, a strong regional East–West structure explaining up to 87 % of the total variation, was noticed (detailed result not shown). Fat composition of nuts is thus clearly distinguishable between West and East African origins (Fig. 2a, b), such as demonstrated for fatty acid in recent studies (Davrieux et al. 2010; Maranz et al. 2004). For tocopherol, our results based on reliable sampling, bring new evidence to assist with distinguishing East and West varieties of *V. paradoxa*.

Several factors may explain the difference between regions such as climatic variations, as hypothesized in previous studies (Maranz and Wiesman 2003, 2004). However, it is impossible to conclude on this hypothesis because the climatic variables are confounded in those experiments as well as in the present study, with other ecological variables (geology, altitude or soil for example) and human practices, potentially also have an influence on chemical composition of shea seeds. In particular, while no domestication syndrome has been clearly described yet for the shea tree, Lovett and Haq suggest the possible impact of an anthropic selection in the semi-arid parklands of sub-Saharan West Africa as local farmers eliminate unwanted woody species on farmland, leaving only those Sheanut trees that meet criteria based on spacing, size, growth, health, age and yield (Lovett and Haq 2000). This traditional Shea farming practice in West Africa associated to the aged traces of shea usage in this region (1000 YBP) (Neumann et al. 1998) may have lead to an unconscious selection of stearic-rich shea butters in West Africa explaining the observed differentiation (Lovett and Haq 2000).

Another possible explanation comes from the distinction in the natural range of two subspecies: *V. paradoxa* ssp. *nilotica* distributed in the eastern region and *V. paradoxa* ssp. *paradoxa* in the western region (Hall et al. 1996). While this distinction remains uncertain (Hall et al. 1996), a recent study shows from molecular genetic markers the role of past climatic change in shaping genetic diversity. The last glacial maximum (20,000 years BC) together with other periods of climatic variation, could have induced a diverging evolution of ssp. *nilotica* and ssp. *paradoxa* on both sides of Adamawa mountains between Cameroon and Nigeria (Allal et al. 2011). This range fragmentation could have resulted in genetic drift and led to specific chemical composition of seeds in biogeographically segregated populations.

Moreover, if this genetic hypothesis is not verifiable in our study due to missing intermediate populations between Uganda and West Africa, results provided by previous studies (Di Vincenzo et al. 2005; Maranz and Wiesman 2003; Maranz et al. 2004) do support this theory. Indeed, shea nuts with origins to the east of the Adamawa mountains (longitude ≥10° East) showed "East"-type fatty acid profiles, while those with origins to the west (longitude ≤10° East) typically showed "West"-type fatty acid profiles. In order to verify the relative historical importance of the Adamawa mountains versus the high-speed winds that form the Bodélé Depression—Dahomey gap causal agents—on the paleogeographic distribution of *V. paradoxa* (Koren et al. 2006), additional analysis of samples from Nigeria through Chad and Cameron is a research priority.

Trends in fat composition within regions

In this study, we noticed significant differences between sites within region and as a result, a noticeable proportion of variance (Tables 2–5). Various factors could explain this pattern. It could be attributed to genetic differences between populations.

If our experimental design (sampling in the wild) does not allow to calculate the heritability of traits, because genetic and environment effects are confounded, it has been shown in the shea tree (Sanou et al. 2005) a high heritability for some agro-morphological characters, particularly related to early growth, resulting from local adaptation mechanism. Thus, inter-site variation could result from genetic difference due to natural selection for local adaptation. This hypothesis is consistent with the confused HCA (Figs. 2d, 3d) and the ACP (Figs. 2c, 3c) that prevented recovering groups in adequacy with geographic position or climate variation. This assumption is supported by the literature showing that the variability of chemical seed constituents, including fatty acids and tocopherols can be explained genetically. For example, the fatty acid compositions of seeds is subject to a strong genetic control with heritability up to 88 % in soybean (Hou et al. 2006; Primomo et al. 2002; Rebetzke et al. 1998) peanut (Amaral et al. 2004) and walnut (Isleib et al. 2006).

Absence of clear relationship with climatic variables

The hypothesis raised by Maranz and Wiesman (2003, 2004) was that the composition of shea nuts in fatty acids and tocopherols follows environmental gradients. In particular, this hypothesis suggested that the oleic acid relative proportion increases with rainfall (conversely for stearic acid) (Maranz and Wiesman 2003; Maranz et al. 2004), and that level of α-tocopherol increases with temperature of tree origin (Maranz and Wiesman 2004). In the present study we tested these hypotheses using a sampling strategy within two climatic gradients ("Mali" and "Ghana–Burkina" transects) in the western region. However, our results showed very low and often not significant correlations between chemical constituents and climate variables (Table 5). Thus, based on an extensive and robust sampling within transects, covering the range of climatic variation in West Africa, many of the assumptions made by Maranz and Weisman (2003, 2004) has not been verified, notably the virtual lack of correlation of stearic and oleic acid variation with climate. Our results rather suggest an ecotypic variation not simply governed by climatic conditions. They are in agreement with previous relationship analysis between rainfall and shea tree agro-morphological traits showing an absence of correlation (Sanou et al. 2006). In addition, as stressed earlier, the among site variation results from confounded effects (climate, soil conditions, genetic origin, agro-forestry practises, etc.) and our conclusion should be verified with future experiments designed to incorporate and test for some of these additional variables.

Impact of domestication on the chemical variability

In this study we observed a greater variability of chemical constituents in the "Mali" than in the "Ghana–Burkina" transect (for example for stearic acid, difference between sites explains 23.1 % in Mali and 4.4 % in "Ghana–Burkina" and Table S2 in supplementary material for details). These observations are consistent with those reported by Lovett and Haq (2000) and Maranz and Wiesman (2003) on both morphological and chemical traits which indicated a smaller diversity in the region of Northern Ghana and the Mossi plateau. This low diversity on the western edge of the Dahomey gap, may well be an artefact of recent re-colonisation by shea trees in this area, following a dry period 4,500–3,400 years BP (Salzmann and Hoelzmann 2005). The existence of shea parklands north of this transect was evident by c. 1,000 years BP in northern Burkina Faso (Ballouche and Neumann 1995) in an area that has since reverted to desert sand dunes again, supporting evidence for the return of drier conditions and a potential recent expansion of this species into the more southern Ghana–Burkina transect sites.

In Uganda, low variation may result from the same climatic shifts and recent southern colonisation of shea trees into central-north Uganda—an area where Baobab, the ubiquitous savannah tree species, *Adansonia digitata*, is still strangely absent from. Contrary to this, the high diversity recorded in Mali, would be expected for an area with Tables savannah conditions, coupled with semi-domestication resulting from protection afforded by the ancient practice of wild tree management in the traditional parkland farming system previously described by Lovett and Haq (2000) from observations made in an area colonised by people purportedly coming from southern Mali only c. 600 years BP.

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