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Morphological diversity in oleaginous watermelon (*Citrullus mucosospermus*) from the Nangui Abrogoua University germplasm collection

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A hundred and seventy-one oleaginous watermelon accessions either collected from different countries or obtained from gene banks were evaluated and compared based on 11 quantitative morphological traits. Principal component analysis on 11 traits revealed 81.19% of the total variability and pointed out variations among accessions, mainly on the basis of fruit size and weight. The dendrogram and factorial discriminant analysis clustered the accessions in four groups. The multivariate analysis of variance showed a significant difference between the four groups and accessions of the group 2 had higher agronomic performances. The confusion matrix gave the details about accessions assignment and pointed out that breeders must have recourse to several multivariate analyses to have better agromorphological classification of accessions. The traits related to fruit size and weight were the most accession distinctive. Consequently, fruit morphological traits could be used for accession identification during collecting missions.

Key words: *Citrullus mucosospermus*, morphological characterization, multivariate analysis, oleaginous cucurbit.

INTRODUCTION

Cucurbits are present in both the New and Old World and are among the most important plant families that supply human with edible products and useful fibers. Cucurbits are divided into five sub-families: Fevilleae, Melothrieae, Cucurbitaceae, Sicyoideae, and Cyclanthereae.The genus *Citrullus* Schrad. ex Eckl et Zeyh placed closest to a clade formed by *Peponium* and *Lagenaria*, the latter including the bottle gourd (*Lagenaria siceraria*), confirming previous findings(Schaefer and Renner, 2011). *Citrullus*, member of the cucurbit family, includes seven species: (1) *Citrullus lanatus* (Thunb.) Matsum. et Nakai (2n = 22), found in tropical and subtropical climates

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worldwide; (2) *Citrullus amarus* Schrad. 1836 syn. *C. caffer* Schrad. 1838, also known as *C. lanatus* var. *caffrorum* (Alef.) Fosb. or *C. lanatus* var. *citroides* (L.H. Bailey) Mansf.; it is the preserving melon grown in Southern Africa and called tsamma melon (Whitaker and Bemis, 1976) is used to make jams since at least the fifteenth century (Bailey, 1930); (3) *Citrullus mucosospermus* Fursa, the so-called egusi melon, largely referred to as a subspecies of *C. lanatus* by many authors (including recently(Hammer and Gladis, 2014)), but which was earlier raised at specific rank(Fursa, 1972, 1981, 1983); (4) *Citrullus colocynthis* (L.) Schrad. (2n = 22), a perennial species growing in sandy areas throughout Northern Africa and adjacent Asia; (5) *Citrullus ecirrhosus* Cogn., another perennial wild species (Meeuse, 1962); (6) *Citrullus rehmii* De Winter, an annual wild species (de Winter, 1990), and (7) *Citrullus naudinianus* (Sond.) Hook.f., from the Namib-Kalahari region.

Watermelon (*Citrullus vulgaris*) (Cucurbitaceae) includes several other economically important species such as cucumber (*Cucumis sativus*), melon (*Cucumis melo* L), squash (*Cucurbita pepo*), calabash (*L. siceraria*), and pumpkin (*Citrullus maxima*). *C. lanatus* is an annual species, which has wild, cultivated, and feral forms.

In the finding that the watermelon and its sister species are West African plants suggests that the natural range of watermelon may have extended into Libya or into Egypt during more humid periods of the Pleistocene and Holocene(Schulz, 1987, 1991). Alternatively, watermelon seeds may have been traded from West Africa to Northern Africa. The illustrations found in Egyptian tombs of watermelon served on a tray suggest that these fruits were eaten raw, perhaps as a dessert. Seeds found in ancient Egyptian tombs, including that of Thutankhamun (Hepper, 1990) should ideally be studied using ancient DNA approaches.

Genetically, the cultivated watermelon is closest to plants from West Africa that represents the gene pool from which watermelon was domesticated (Chomicki and Renner, 2014). The sister species of watermelon is *C. mucosospermus*, the egusi melon from Nigeria to Senegal and described by Fursa (1983), Che et al. (2003), and Dane and Liu (2007). In consideration of the findings by Fursa (1983)and Chomicki and Renner (2014), the egusi-type watermelon is recognized as *C. mucosospermus* throughout this paper.

The "egusi" watermelon of West Africa is cultivated for its oleaginous and nutritious seeds that are important in the social and cultural live of several peoples (Burkill, 1985; Oyulu, 1977; van der Vossen et al., 2004; Achigan-Dako et al., 2008). For example, dried slightly toasted and ground seeds are used as soup thickener. The "egusi" seeds are reported to be rich in nutrients ~60% lipids and ~30% proteins (Loukou et al., 2007). Edible oil can also be extracted from the seeds. Commonly found in many traditional cropping systems, the plant is well adapted to extremely divergent agro-ecosystems and various cropping systems characterized by minimal inputs. *C. lanatus* thus represents an excellent plant model for which improved cropping systems implementation can insure the economic prosperity of rural women that are the main producers in tropical Africa. The west African watermelon is generally considered as allogamous, monoecious, entomophilous, and protandrous(Gusmini and Wehner, 2003). This species is named as *C. lanatus* var. *lanatus* or as *C. lanatus* subsp. *Mucosospermus* (Fursa, 1972) or *C. colocynthis*(Hutchinson and Dalziel, 1954).

Particularly, in Côte d'Ivoire, indigenous and minor crops for local consumption have almost disappeared in favor of exotic species. Some farmers still continue to produce these crops not far from their home or on small plots cleared after harvest of the main annual crops (yams, corn, peanut, etc.) (Zoro Bi et al., 2003). Thus, some of them such as oleaginous cucurbits are still found in the market. According to Zoro Bi et al. (2003) and Djè et al. (2006), *C. mucosospermus* (but identified as *C. lanatus* by these authors in Côte d'Ivoire) is the species most widely distributed and most commercialized in Côte d'Ivoire. This plant is mainly grown in savanna areas in the North, in the pre-forest areas in Central, East, and South(Djè et al., 2006). Two distinct cultigroups have been described on the basis of the morphology of seeds. The first one (wlèwlè) is characterized by glossy seeds with a tapered proximal extremity. This morphotype is subdivided into three cultivars according to the size of the seeds(Djè et al., 2006). Fruit color of this cultigroup, is almost uniform, greenish or whitish. The second (bebu), with one cultivar has green fruit also, but with dark longitudinal bands. The seeds are heavier and have a flat ovoid shape with rugged and thick ends (Zoro Bi et al., 2003).Although the oleaginous type of *Citrullus* is an economical important crop in west Africa. Few studies were conducted on genetic resources of this plant (Ndabalishye, 1995; Djè et al., 2006) previously reported to be morphological differentiated especially in terms of fruit and seed characters.

The objectives of the present study were to survey and analyze variations in plant, fruit and seed morphology in *C. lanatus* oleaginous type collection from Côte d'Ivoire. This represents the first study on collection and characterization of *C. lanatus* oleaginous type germplasm in Nangui Abrogoua University.

MATERIALS AND METHODS

Plant and collection sites

In this study, 171 accessions of *C. lanatus* oleaginous type were used. They were provided from seven countries: Benin, Côte d'Ivoire, France, Ghana, Nigeria, Togo, and Turkey (Table 1). The samples were collected from farmers' stock, genebank or purchased from local seed markets. The selected accessions were divided into four cultivars defined on the basis of seed size and

Table 1 Passport data of accessions used for morphological analysis of Citrullus mucosospermus.*mucosospermus*.

Identification number	Country	Collection site	Cultivar	Rind feature	Seed color
CL-001	CI	Agbaou	B	UMG	Br
CL-002	CI	Agbaou	B	UMG	Br
CL-004	CI	Agbaou	B	UMG	Br
CL-005	CI	Agbaou	B	DGS	Ye
CL-006	CI	Agbaou	B	MGS	Br
CL-007	CI	Agbaou	B	MGS	Br
CL-008	CI	Agbaou	B	MGS	Br
CL-009	CI	Agbaou	B	MGS	Br
CL-010	CI	Agbaou	B	MGS	Br
CL-011	CI	Grand Alépé	B	MGS	Br
CL-012	CI	Grand Alépé	B	DGS	Ye
CL-013	CI	Assié Koumassi	B	DGS	Br
CL-014	CI	Assié Koumassi	WSS	UMG	Br
CL-015	CI	Bongouanou	WSS	MGS	Br
CL-016	CI	Bongouanou	WSS	DGS	Br
CL-017	CI	Bongouanou	WMS	DGS	Br
CL-019	CI	Assié Koyekro	WMS	DGS	Br
CL-020	CI	Assié Koumassi	WSS	MGS	Br
CL-021	CI	Assié Koyekro	WMS	UMG	Br
CL-023	CI	Danguira	B	MGIS	Br
CL-024	CI	Danguira	B	MGS	Br
CL-025	CI	Ananda	WMS	MGS	Br
CL-027	CI	Assabrikobenankro	WSS	MGS	Br
CL-029	CI	M’Bahiakro	WMS	ULG	Or
CL-030	CI	Fleguessanga	B	DGS	Ye
CL-031	CI	Fleguessanga	B	UMG	Br
CL-032	CI	Pohokaha	B	DGS	Ye
CL-033	CI	Pohokaha	B	MGS	Br
CL-034	CI	Pohokaha	B	MGS	Br
CL-035	CI	Gohitafla	WMS	MGS	Br
CL-036	CI	Manfla	WMS	MGIS	Br
CL-037	CI	Manfla	WMS	MGIS	Br
CL-040	CI	Manfla	WMS	MGIS	Br
CL-041	CI	Manfla	WMS	MGIS	Br
CL-043	CI	Manfla	WMS	MGIS	Br
CL-046	CI	Manfla	WMS	MGIS	Br
CL-047	CI	Manfla	WMS	MGIS	Br
CL-049	CI	Tibéita	WMS	UMG	Br
CL-050	CI	Tibéita	WMS	DGS	Br
CL-051	CI	Tibéita	WMS	DGS	Br
CL-052	CI	Badiéfla	WMS	DGS	Br
CL-053	CI	Badiéfla	WMS	DGS	Br
CL-054	CI	Bibikorefla	WMS	DGS	Br
CL-055	CI	Gouenfla	WMS	DGS	Br
CL-056	CI	Drikoifla 1	WMS	DGS	Br
CL-058	CI	Zambléfla	WMS	DGS	Br
CL-059	CI	Bibikorefla	WMS	DGS	Br
CL-060	CI	Botiéfla	WMS	MGIS	Br
CL-063	CI	Bohikouifla	WMS	UMG	Br
CL-064	CI	Gouafla	WMS	UMG	Br
CL-065	CI	Zraluo	WMS	UMG	Br

Table 1. Contd.

CL-066	CI	Gohitafla	WMS	UMG	Br
CL-068	CI	Gohitafla	WMS	UMG	Br
CL-069	CI	Gohitafla	WMS	LGS	Br
CL-070	CI	Gohitafla	WMS	MGIS	Br
CL-071	CI	Bonoua	B	DGS	Ye
CL-072	CI	Agbaou	B	MGIS	Br
CL-073	CI	Agbaou	B	MGIS	Br
CL-074	CI	Ahoutoué	B	DGS	Br
CL-076	CI	Grand Alépé	B	UMG	Br
CL-077	CI	M’Bahiakro	WMS	UMG	Br
CL-079	CI	Korhogo	WBS	ULG	Or
CL-081	CI	Ananda	WMS	MGIS	Br
CL-082	CI	Dikodougou	B	MGIS	Br
CL-083	CI	Pleuro	B	MGIS	Br
CL-084	CI	Guiembé	B	MGIS	Br
CL-085	CI	Koumbala	B	MGIS	Br
CL-086	CI	Koumbala	B	MGIS	Br
CL-087	CI	Koumbala	B	DGS	Br
CL-088	CI	Koumbala	WMS	DGS	Br
CL-089	CI	Koumbala	B	DGS	Br
CL-090	CI	Ferkessedougou	B	DGS	Br
CL-091	CI	Ferkessedougou	B	ULG	Br
CL-092	CI	Katiola	B	ULG	Br
CL-093	CI	Dikodougou	B	ULG	Br
CL-094	CI	Assalékro	WMS	ULG	Br
CL-095	CI	Assalékro	WMS	ULG	Br
CL-096	CI	Assalékro	WMS	ULG	Br
CL-097	CI	Assalékro	WMS	ULG	Br
CL-098	CI	Assalékro	WMS	ULG	Br
CL-099	CI	M’Bato	WMS	ULG	Br
CL-101	CI	M’Bato	WMS	DGS	Br
CL-102	CI	Koffiagrokro	WMS	DGS	Br
CL-103	CI	Koffiagrokro	WMS	DGS	Br
CL-104	CI	Grogro	WMS	DGS	Br
CL-107	CI	Grogro	WMS	DGS	Br
CL-108	CI	Grogro	WMS	DGS	Br
CL-112	CI	N’Denou	WMS	DGS	Br
CL-113	CI	N’Denou	WMS	DGS	Br
CL-115	CI	Dossakassou	WMS	DGS	Br
CL-118	CI	Dossakassou	WMS	UMG	Br
CL-121	CI	Bediala	WMS	UMG	Br
CL-122	CI	Bediala	WMS	UMG	Br
CL-124	CI	Daloa	WMS	UDG	Br
CL-126	CI	Bediala	WMS	UDG	Br
CL-127	CI	Bediala	WMS	UDG	Br
CL-130	CI	Nanoufla	WMS	UDG	Br
CL-131	CI	Bediala	WMS	UDG	Br
CL-136	CI	Nanoufla	WMS	UDG	Br
CL-137	CI	Assie-Assasso	WMS	UDG	Br
CL-138	CI	Assie-Assasso	WSS	UDG	Br
CL-139	CI	Yoboué Blessou	WMS	UDG	Br
CL-140	CI	Abokouman	WMS	UDG	Br

Table 1. Contd.

CL-141	CI	Abokouman	WMS	UMG	Or
CL-142	CI	Abokouman	WMS	MGIS	Br
CL-146	CI	Yanvo	WMS	MGIS	Br
CL-148	CI	Iguela	B	MGIS	Br
CL-149	CI	Iguela	B	MGIS	Br
CL-150	CI	Iguela	B	MGIS	Br
CL-151	CI	Iguela	B	MGIS	Br
CL-153	CI	Korobo	WMS	MGIS	Br
CL-154	CI	Korobo	WMS	UMG	Br
CL-155	CI	N’Golokaha	B	UMG	Br
CL-156	CI	N’Golokaha	B	UMG	Br
CL-157	CI	N’Golokaha	B	UMG	Br
CL-163	CI	N’Golokaha	B	UMG	Br
CL-164	CI	N’Golokaha	B	UMG	Br
CL-167	CI	Baifla	WMS	UMG	Br
CL-168	CI	Tranhonfla	WMS	UMG	Br
CL-171	CI	Tranhonfla	WMS	UMG	Br
CL-172	CI	Tranhonfla	WMS	UMG	Br
CL-173	CI	Baifla	WMS	DGS	Br
CL-174	CI	Oufouediékro	WMS	MGIS	Br
CL-175	CI	Sakassou	WMS	DGS	Br
CL-176	CI	Yablassou	WMS	DGS	Br
CL-178	CI	Sandôh	WMS	DGS	Br
CL-179	CI	Maounou N’Zuessi	WMS	MGIS	Br
CL-181	CI	M’Bahiakro	WBS	UDG	Br
CL-182	CI	Yaouloukro	WMS	UDG	Br
CL-183	CI	Djakro	WSS	UDG	Br
CL-184	CI	Attiegouakro	WMS	UDG	Br
CL-185	CI	Yoboué Blessou	WMS	UDG	Br
CL-188	CI	Ahoulikro	WMS	MGIS	Br
CL-189	CI	Bodokro	WMS	UDG	Br
CL-190	CI	Bodokro	WMS	UDG	Br
CL-192	CI	Ouellé	WMS	UDG	Br
CL-193	CI	Bodokro	WMS	DGS	Br
CL-194	CI	Bodokro	WMS	DGS	Br
CL-195	CI	Bodokro	WMS	DGS	Br
CL-198	CI	Attiégouakro	WMS	DGS	Br
CL-199	CI	Attiégouakro	WMS	DGS	Br
CL-202	CI	N’Golokaha	B	DGS	Br
CL-203	CI	N’Golokaha	B	DGS	Br
CL-204	CI	N’Golokaha	B	DGS	Br
CL-205	CI	N’Golokaha	WMS	UDG	Br
CL-207	CI	N’Golokaha	B	UDG	Br
CL-208	CI	N’Golokaha	B	UDG	Br
CL-209	T	Kara	B	DGS	Br
CL-210	Bn	Ketou	B	MGIS	Br
CL-211	France	-	Wat	MGIS	Bl
CL-212	France	-	Wat	MGIS	Wh
CL-217	CI	Manfla	WMS	MGIS	Br
CL-218	CI	Manfla	WMS	DGS	Br
CL-219	Bn	Savin	B	DGS	Br
CL-221	Bn	Ketou	B	DGS	Br

Table 1. Contd.

CL-223	N	Oyo	B	UMG	Br
CL-226	N	Ogun	B	UDG	Br
CL-234	Turquie	-	WMS	UDG	Br
CL-236	CI	Katiola	WMS	UDG	Br
CL-237	CI	Katiola	WMS	UDG	Br
CL-238	CI	Petionara	WMS	UDG	Br
CL-239	CI	Katiola	WMS	UDG	Br
CL-242	Ghana	Makola City	WSS	LGS	Br
CL-244	Ghana	Makola City	WSS	UDG	Br
CL-245	Ghana	Kaneshie	WSS	MGS	Br
CL-246	CI	Petionara	WMS	MGS	Br
CL-247	CI	Doubasso	WMS	MGS	Br
CL-248	CI	Doubasso	WMS	MGS	Br

Bl: Black; Bn: Benin; Br: brown; CL: *Citrullus mucosospermus*; G: Ghana; CI: Côte d'Ivoire; N: Nigeria; T: Togo; B: bebu; WBS: wlèwlè big seed; WMS: wlèwlè medium seed; WSS: wlèwlè small seed; DGS: dark green with strips; LG: light green; LGS: Light Green with strips; MGS: medium green with strips; MGIS: medium green with intermittent strips; UDG: uniformly dark green; ULG: uniformly light green; UMG: uniformly medium green; Or: orange; Wh: white; Ye: yellowish.

Table 2. Origins and size of *Citrullus mucosospermus* samples used for morphological traits analysis.

Cultigroup	Cultivar	Sample size per country							
		Côte d'Ivoire	Benin	Ghana	Nigeria	Togo	France	Turkey	Total
	Big seeds	2	-	-	-	-	-	-	2
Wlèwlè	Medium seeds	101	-	-	-	-	-	-	101
	Small seeds	7	-	3	-	-	-	-	10
Bebu	Bebu	49	3		2	1	-	-	55
Watermelon	Watermelon	-	-	-	-	-	2	1	3
Total		159	3	3	2	1	2	1	171

designated small-, medium-, big-seeded, and thickened margin seeds. Thickened margin seeds size varies between 153 and 205 mm², big seeds from 125 to 151 mm², medium seeds from 86 to 110 mm², and small seeds from 41 to 52 mm²(Djè et al., 2006). Accessions (2 to 101) were sampled per cultivar, according to seeds availability (Table 2).

Study site

The trials were regularly monitored throughout the growing season in 2011 and 2012 (May to November). Farm experiment was conducted in the village of Manfla, located in the centre (6°49′34.38″N, 5°43′47.68″W) 400 km North Abidjan (Côte d'Ivoire). Annual rainfall varies from 800 to 1400 mm with a long-term mean of 1200 mm, and the annual mean temperature is 27°C. A complete description of study site was done by Kouassi and Zoro Bi (2010).

Experimental design

Planting was done according to a completely randomized block design, with seven replications. Each replicate consisted of a 24×27 m containing 70 holes at a depth of 3 cm, resulting in 7 holes per accession. The planting distance was 3 m between and within rows with 1.5 m of edges. A manual weeding was carried out during plant development to prevent weed invasion. Disease and pest control was carried out using a carbamate-based insecticide applied when necessary.

Traits measurement

Accessions were examined using 11 quantitative traits subdivided into four vegetative and seven yield characters selected from standard descriptors for cucurbits(Maggs-Kölling et al., 2000; Marr et al., 2007; Morimoto et al., 2005; Koffi et al., 2008). Data were measured in five plants and five fruits per accession.

Vegetative traits measured were limb length (LL), limb width (LWI), plant length (PL), and number of branches from the central taproot (BN) for each plant. Yield characters measured were weight (FWE), length (FL), width (FWI), seeds cavity diameter (SCD), seeds number (SN), and seeds weight (SW). Seed traits analyzed included 100-seeds weight (100-SWE). Measurements on 100-seeds weight were scored using five individuals randomly selected on each plant (Table 3).

Data analysis

Statistical analyses were carried out with STATISTICA software

Table 3. List of descriptors used for characterization of *Citrullus lanatus* samples used for morphological traits analysis.

Character	Codes	Type and period of observation
Vegetative character		
Limb length (cm)[a]	(LL)	Measured after formation of the first fruit
Limb width (cm)[a]	(LWI)	Measured after formation of the first fruit
Plant length (m)[b]	(PL)	Measured 120 days after planting
Number of branches[b]	(BN)	Total number of branches per plant at 120 days after planting
Yield character		
Fruit weight (g)[a]	(FWE)	Weight of the mature fruits
Fruit length (cm)[a]	(FL)	Measured on the mature fruits
Fruit diameter (cm)[a]	(FD)	Measured on the mature fruits
Seed cavity diameter (cm)[a]	(SCD)	Measured on the mature fruits
Number of seeds per fruit[a]	(NS)	Total number of seeds per fruit
Seeds weight per fruit[a]	(SW)	Weight of all seeds taken for a given individual after drying
100-seeds weight per fruit (g)	(100-SWE)	Weight of 100 seeds taken for a given individual after drying

A measurement on five organs per plant; b measurement on each plant per accession.

package version 7.1(StatSoft, 2005). Principal Component Analysis (PCA) was applied to identify most discriminant parameters followed by hierarchical cluster analysis using unweighted pair-group method using arithmetic average (UPGMA) method. Multivariate analysis of variance (MANOVA) and a factorial discriminant analysis (FDA) were performed to check difference between the variable means for each group obtained with the clustering analysis. A confusion matrix was constructed, to check the reliability of groups as defined by the hierarchical clustering.

RESULTS

Descriptors variation

The relative discriminating capacity of the PCA is shown by their Eigenvalues. The result of the PCA (Table 4) showed that five Principal Component axes (PC) had Eigenvalues greater than 1 all together accounted for over 80% of the total variability. The first five principal components accounted for 81.19% of the total variability. The first principal component (PC1) accounted for 23.85%, while the second principal component (PC2) accounted for 17.32%. The PC1 is loaded with fruit length (0.824), fruit diameter (0.919), and seed cavity diameter (0.890). These results indicated that this component was determined by individuals with big fruit. The PC2 was loaded with limb length (0.966), and the limb width (0.947). This component was determined by individuals with broad leaves. PC3, PC4, and PC5 accounted for 14.19, 13.54, and 12.29%. They were correlated to number of branches, and plant length for PC3, number of seeds per fruit and 100-seeds weight per fruit (100-SWE) for PC4 and seeds weight per fruit for PC5. These results indicated that PC3 was determined by individuals of large size plant. PC4 and PC5 were determined by most productive individuals.

Accessions structuration

The unweighted pair group method arithmetic (UPGMA) using a Euclidian distances matrix, subdivided the accessions analyzed into four major groups. These groups differ in the distance by 30 (Figure 1). All groups are mixture of accession of various origin, cultivar or cultigroup.

The highest number of accessions was located in cluster III with 53 accessions, followed by clusters II and I with 48 and 46 accessions, respectively. Cluster IV had 24 accessions. Comparison of groups using the MANOVA showed significant differences between the four groups (F= 19.91; P<0.001). This is due to all the 11 characters measured. The ANOVA showed significant difference among groups (Table 5). All parameters revealed a partial distinction of groups.

Discriminant functions resulting from variables to classify the units formed on the four groups are shown in Table 6. The two first canonical variables which had an Eigenvalue higher than 1 were retained to describe the total variability of the accessions. The two factors selected together, explained 87.54% of the total variation. The first canonical variable, *FDA1,* described 58.33% of the total variation. This component was positively correlated with the number of branches and was negatively correlated with limb width, number of seed per fruit, and 100-seeds weight. The second canonical variable, *FDA2,* explained 29.21% of the total variation and was mostly and positively constructed from fruit diameter. Figure 2 shows the position of the accessions in relation with the first two discriminant factors. Based on these analyses, accessions of group 2 were noted to have the biggest leaves. The smallest leaves were observed in accession of group 1. The most number of

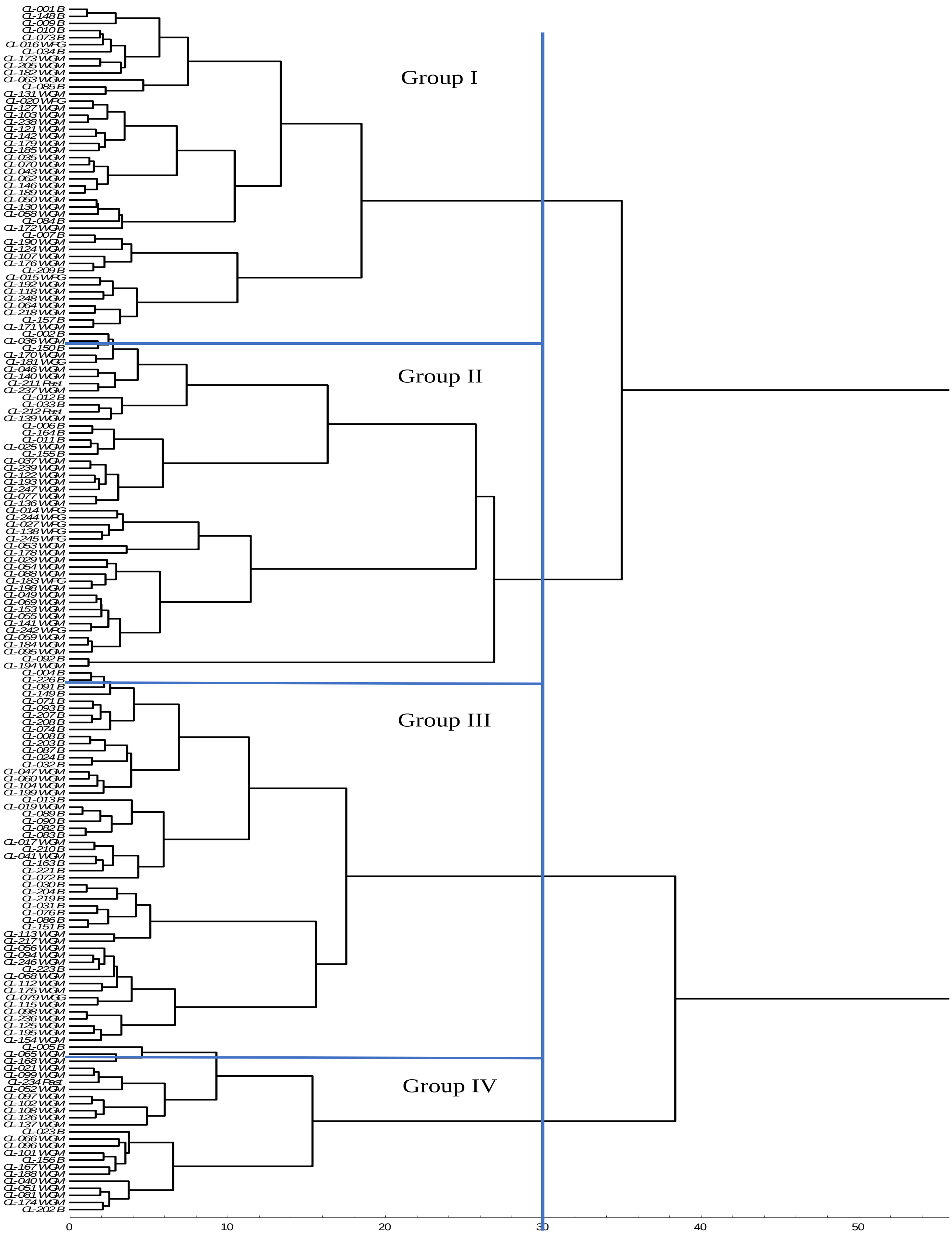

Figure 1. Dendrogram of *Citrullus lanatus* oleaginous type accessions in four group generated by using 11 quantitative morphological characters. CL: *Citrullus lanatus*; W: 'Wlèwlè '; B: 'Bebu'; PG: small seeds; GM: medium seeds; GG: big seeds; Past Watermelon; Group I (46 accessions), Group II (48 accessions), Group III (53 accessions), and Group IV (24 accessions).

Table 4. Correlation coefficients explained by the first five principal components (PCs) for 11 traits analysed in 171 accessions of *Citrullus mucosospermus*.

Eigenvector	PC 1	PC 2	PC 3	PC 4	PC 5
Eigenvalues	2.623	1.905	1.562	1.490	1.352
% of variance explained	23.846	17.319	14.197	13.543	12.294
Cumulative contribution (%)	23.846	41.165	55.362	68.905	81.199
LL	0.051	0.966*	-0.041	0.015	0.016
LWI	0.056	0.947*	-0.137	-0.039	0.033
BN	-0.013	-0.171	0.843*	0.015	-0.119
PL	-0.044	-0.002	0.885*	-0.021	0.102
FWE	0.409	0.141	-0.129	0.080	-0.469
FL	0.824*	0.061	0.102	-0.018	-0.112
FD	0.919*	0.026	-0.077	0.017	0.163
SCD	0.890*	0.015	-0.088	-0.046	0.194
NS	0.206	0.078	-0.004	-0.785*	0.467
SW	0.274	0.125	-0.088	0.080	0.861*
100-SWE	0.118	0.030	-0.010	0.925*	0.265

*****Significant correlation values. LL: Limb length; LWI: limb width; PL: plant length; BN: number of branches; FWE: fruit weight; FL: fruit length; FD: fruit diameter; SCD: seed cavity diameter; NS: number of seeds per fruit; SW: seeds weight per fruit; 100-SWE: 100-seeds weight per fruit.

Table 5. Agro-morphological characteristic of 11 traits analyzed in the four groups provided by the dendrogram with UPGMA methods.

Variable	G1 (N=46)	G2 (N=48)	G3 (N=53)	G4 (N=24)	*F*	*P*
LL	10.95±1.02^{b}	13.00±1.31^{a}	12.5±1.26^{a}	11.17±1.02^{b}	30.27	<0.001
LWI	9.19±0.95^{c}	11.53±1.32^{a}	10.67±1.24^{b}	9.17±0.99^{c}	41.04	<0.001
BN	3.95±1.58^{b}	3.28±0.91^{b}	3.41±1.2^{b}	8.39±3.45^{a}	55.04	<0.001
PL	1.97±0.69^{b}	1.90±0.70^{b}	2.04±0.6^{b}	3.26±0.68^{a}	25.91	<0.001
FWE	0.66±0.13^{ab}	0.74±0.59^{a}	0.51±0.12^{b}	0.54±0.12^{b}	4.83	0.003
FL	10.01±1.3^{a}	9.92±0.82^{a}	9.01±0.86^{b}	9.38±1.34^{b}	9.59	<0.001
FD	10.28±0.63^{a}	10.14±0.54^{a}	9.25±0.48^{b}	9.36±1.84^{b}	34.00	<0.001
SCD	8.22±0.54^{a}	8.11±0.49^{a}	7.27±0.45^{b}	7.36±0.75^{b}	36.32	<0.001
NS	159.91±45.87^{b}	212.12±68.12^{a}	129.56±30.40^{c}	145.58±30.93^{bc}	26.57	<0.001
SW	10.35±2.71^{b}	12.25±3.34^{a}	10.01±2.68^{b}	8.58±1.71^{c}	10.66	<0.001
100-SWE	7.79±2.52^{ab}	6.98±2.42^{b}	8.86±2.70^{a}	6.52±1.75^{b}	7.12	<0.001

LL: Limb length; LWI: limb width; PL: plant length; BN: number of branches; few: fruit weight; FL: fruit length; FD: fruit diameter; SCD: seed cavity diameter; NS: number of seeds per fruit; SW: seeds weight per fruit; 100-SWE: 100-seeds weight per fruit; G1: group 1; G2: group 2; G3: group 3; G4: group 4.

the longest plants were observed in group 4. Groups 1 and 2 have the larger and heavier fruit. The best seed yield is obtained with accessions of group 2, but 100 seed-weight was higher in groups 1 and 3.

According to confusion matrix, the composition of groups as defined by the hierarchical clustering is different. Group 1 contains 41 accessions instead of 46. Accessions CL-146 and CL-172 have been reclassified in group 2. Similarly, accessions CL-142 and CL-085 have been reclassified in group 3 and accession CL-131 has been reclassified in group 4. Group 2 consists of 44 instead of 48 accessions. An enrollment of 50 accessions belongs to group 3 instead of 53. Group 4 includes 23 accessions instead of 24 (Table 7).

DISCUSSION

To maintain, evaluate and utilize germplasm efficiently, it is important to investigate the extent of genetic diversity available. Morphological characterization is an important step in the description and classification of crop germplasm, because a breeding program mainly depends upon the magnitude of morpho-phenological variability (Smith

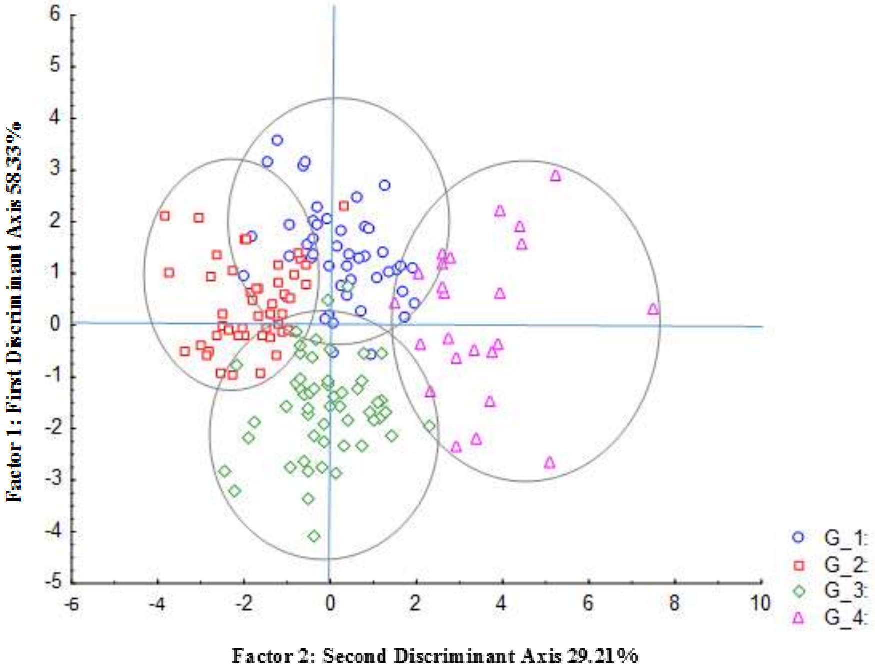

Figure 2. Representation on the discriminant plane of accessions in relation with the two discriminant factors, 1 and 2. G_1: group 1; G_2: group 2; G_3: group 3, G_4: group 4.

Table 6. Eigenvectors, eigenvalues, and inertia percentage explained by the two first canonical variables for 11 traits analysed in 171 accessions of *Citrullus mucosospermus*.

Eigenvector	F1	F2
Eigenvalue	2.67	1.33
Inertia percentage	58.33	29.21
Cumulative inertia percentage	58.33	87.54
Vegetative character		
LL	-0.03	-0.40
LWI	-0.51*	-0.19
BN	0.63*	0.16
PL	0.47	-0.07
Yield character		
FWE	-0.14	0.3
FL	-0.01	-0.05
FD	0.17	0.62*
SCD	-0.26	0.35
NS	-0.77*	0.15
SW	0.05	0.03
100-SWE	-0.54*	-0.47

*Significant correlation higher than 0.5. LL: Limb length; LWI: limb width; PL: plant length; BN: number of branches; few: fruit weight; FL: fruit length; FD: fruit diameter; SCD: seed cavity diameter; NS: number of seeds per fruit; SW: seeds weight per fruit; 100-SWE: 100-seeds weight per fruit; F1: discriminant factor 1; F2: discriminant factor 2.

Table 7. Classification matrix based on the groups obtained by agromorphological characters.

Group	Classification(%)	G 1	G 2	G 3	G 4
G 1	89.13	41	2	2	1
G 2	91.66	3	44	1	0
G 3	94.33	2	1	50	0
G 4	95.83	1	0	0	23
Total	92.39	47	47	53	24

G1: Group 1; G2: group 2; G3: group 3; G4: group 4.

et al., 1991).

In this study, 171 accessions of *Citrullus* of Nangui University collection were characterized based on morphological traits. In the analysis made to estimate the relative contributions of the different traits studied towards the overall phenotypic variation among the 171 accessions, a total of five principal components (PCs), explained 81.2% of variability. Fruit-related parameters were the most discriminant. In fact, fruit length, fruit diameter, and seed cavity diameter were parameters which contributed more to the first principal component (24%), implying that 24% of the variability was explained by these parameters. These results suggest that fruit size is a useful criterion for separating *Citrullus* accessions. In the cucurbit family, the significant contribution of the fruit traits to morphological variability has been reported for watermelon (Gusmini and Wehner, 2003; Maggs-Kölling et al., 2000). According to Achigan-Dako (2015) diagnostic morphological markers among species include fruit such as fruit size.

The least discriminant parameters were numbers of seeds and seeds weight. This finding is due to the fact that medium-sized seeds represented 60% of sample analyzed. Contrary to these results, Achigan-Dako et al. (2015) demonstrated that other major differentiation traits is related to seed.

Although our accessions originated from seven different countries, they did not cluster according to geographical distribution. This may be suggestive of the single ancestry of the analyzed plants (Dane and Liu, 2007). This may be because genetically, the cultivated watermelon is closest to plants from West Africa that represents the gene pool from which watermelon was domesticated (Chomicki and Renner, 2014). The sister species of watermelon is *C. mucosospermus*, the egusi melon, ranging from Nigeria to Senegal (Renner et al., 2014). Plants from Benin closely resemble the collection type of the name *C. mucosospermus* (which is from Ghana). In order to explain the low genetic diversity of *C. mucosospermus* (but identified as *C. lanatus*), Dane and Liu (2007) demonstrated that the watermelon varieties have diverged into small populations. Moreover, the domestication and the resulting selection may have reduced the genetic diversity from the original watermelon crops. Levi et al. (2001) also revealed that cultivated watermelon exhibited narrow genetic base as a results of many years cultivation and selection for specific qualities.

Morphological and genetic characterization of Hungarian and Turkish accessions of watermelon (including *C. lanatus* and *C. colocynthis*) revealed that germplasm resources present a wide range of diversity (Sari et al., 2005; Solmaz and Sari, 2009; Solmaz et al., 2010). However, accessions of the two countries show many similarities and therefore cannot be separated clearly (Szamosi et al., 2009). In the present study, comparison of the four groups using a MANOVA combined with factorial discriminant analysis, allowed the classification of accessions according to agro-morphological characters. Accessions of group 2 had the biggest leaves. The smallest leaves were observed in accession of group 1. The largest number of ramification and longest plants were observed in group 4.

Groups 1 and 2 have the largest and heaviest fruits. The best seed yield is obtained with accessions of group 2, but 100 seed-weight was higher in groups 1 and 3 than in the two other groups. Morphological variation analysis in landraces of *C. mucosospermus* (but identified as *C. lanatus* by authors in Côte d'Ivoire) also allowed to classify accessions based on fruit and seed traits (Adjoumani et al., 2012).

Although, the Hierarchical Cluster Analysis gave a clear separation of accessions in distinct groups, the confusion matrix displayed a reclassification of some accessions. Accessions CL-146 and CL-172 has been reclassified in group 2. Accessions CL-142 and CL-085 has been reclassified in group 3 and accession CL-131 has been reclassified in group 4.

Traditionally, watermelon breeders focus on fruit quality traits. These traits include sugar content, flesh color, fruit size, and rind patterns (Hashizume et al., 2003). Investigations of the inheritance of fruit morphology and quality traits date back as far at the 1930's (Porter, 1933, 1937). Since then, many efforts have been made to better understand traits associated with watermelon fruit quality and morphology. Many genes have been described that control the internal fruit quality and morphology in watermelon (Guner and Wehner, 2004). An internal fruit characteristic that has received attention is the Brix value.

A linkage map was developed and a QTL that accounts for 19% of the variation in Brix was mapped on linkage group (LG) 8 (Prothro, 2010). Fruit shape and weight are important external characteristics that breeders must consider when developing watermelon cultivars.

Watermelon shape can be classified as being either round, oval, blocky or elongate (Wehner et al., 2001). The only gene described that controls fruit shape is the elongate fruit gene (O). Fruit weight has recently become an important consideration for breeders due to increased consumer preference for smaller sized watermelons (Gusmini and Wehner, 2007). Watermelons have traditionally been classified into five categories based on fruit weight. These are icebox (less than 5.5 kg), small (5.5 to 8.0 kg), medium (8.1 to 11.0 kg), large (11.1 to 14.5 kg), giant (greater than 14.5 kg), and recently mini fruit size (1.5 to 4.0 kg) was added (Gusmini and Wehner, 2007; Maynard, 2001). No genes have been identified that are responsible for fruit weight inwatermelon (Gusmini and Wehner, 2007; Maynard, 2001). Fruit quality and morphological traits, such as the ones described earlier, gain the most attention from watermelon breeders. There are many other important traits in watermelon that receive little to no attention. The egusi seed trait is one of these traits. The egusi seed trait has been shown to be inherited as a single gene (Gusmini et al., 2004).

A single QTL was identified for rind thickness (Prothro, 2010). The objective when breeding for this trait should be to have a rind thickness that is a small percentage of the fruit diameter. Three QTL were identified for fruit length and two QTL were identified for fruit width, in the same region on LG5. Another QTL for fruit length was identified on LG3. But it is not known if they are associated with the elongate fruit gene. Kumar et al. (2009) produced a population segregating for fruit shape and found that his data did not fit the single gene theory for fruit shape inheritance. Watermelons varieties that produce fruit that fit into the weight category most preferred by consumers must be available for growers(Gusmini and Wehner, 2007). The QTL identified for fruit length and fruit width should be useful for fruit weight selection in a breeding program. Two QTL were identified for this trait on LG2. Another QTL was identified for seed oil percentage. This QTL falls between the locations of the two QTL identified for the egusi seed trait on LG2 (Prothro et al., 2012).

Conclusion

A great deal of diversity morphological traits has been observed in *C. mucosospermus* oleaginous type accessions from the Nangui Abrogoua University collection. From the present investigation, it was concluded that the indigenous oleaginous cucurbit *C. mucosospermus* displayed a wide range of diversity for most of the morphological traits studied. Traits related to fruit size and weight were the most accession distinctive. Consequently, fruit morphological traits could be used for accession identification during collecting missions. Accessions had been divided into four groups in which group 2 had the highest seed yield. Therefore, accessions of this group must be used to enhance yield. These findings are important to plan future germplasm sampling and evaluation studies.

Conflict of Interests

The authors have not declared any conflict of interests.

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