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RESEARCH ARTICLE

Phenetic characterization of *Citrullus* spp. (Cucurbitaceae) and differentiation of egusi-type (*C. mucosospermus*)

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Abstract Breeding of *Citrullus* spp. for various benefits has continuously raised interest particularly for economically important crops. However, the interspecific variations within the genus have remained obscure in many regards and the multitude of names for taxa and subtaxa eludes *Citrullus* breeders. In the absence of clear taxonomic differentiation, molecular analysis of phenotypes did not help understand the complexity of this genus until recently. In this study we carried out a phenetic characterization of a world collection of 213 accessions using 22 agro-morphological descriptors in field trials conducted in two locations and during two consecutive years. Multivariate analyses confirmed high morphological variation in *Citrullus* spp. and highlight *C. mucosospermus* as a homogenous group separated from other *Citrullus* species. This differentiation of egusi-type melon will help leverage breeding and conservation purposes as *C. lanatus* represent very important economic crops in the world. Based on our findings we conclude that our knowledge of the relationships between genetic variations and phenotypic traits and the determinism of morphological variations among and within *Citrullus* need to be further deepened.

Keywords Breeding prospects · *Citrullus* spp. · Egusi · Morphological markers · Taxonomy

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Introduction

The genus *Citrullus* Schrad. ex Eckl et Zeyh. includes seven species: (1) *C. lanatus* (Thunb.) Matsum. et Nakai (2n = 22), found in tropical and subtropical climates worldwide; (2) *C. 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); (3) *C. mucosospermus* Fursa, the so-called egusi melon, largely referred to as a subspecies of *C. lanatus* by many authors (including

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recently Hammer and Gladis (2014)) but which was earlier raised at specific rank (Fursa 1972, 1981, 1983); (4) *C. colocynthis* (L.) Schrad. (2n = 22), a perennial species growing in sandy areas throughout northern Africa and adjacent Asia; (5) *C. ecirrhosus* Cogn., another perennial wild species (Meeuse 1962); (6) *C. rehmii* De Winter, an annual wild species (de Winter 1990), and (7) *C. naudinianus* (Sond.) Hook.f., from the Namib-Kalahari region.

There is a long-standing confusion and debate about the taxonomic names of *Citrullus* species, subspecies and varieties as the main characteristics of each of these subdivisions were poorly documented (Maggs-Kölling et al. 2000; van der Vossen et al. 2004; Adjakidjè 2006). Detailed nomenclatural history and current species names in the genus *Citrullus* were provided in recent papers by Nesom (2011), Hammer and Gladis (2014), Renner et al. (2014) and Chomicki and Renner (2014). 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.

Citrullus mucosospermus, the 'egusi' melon (Burkill 1995; van der Vossen et al. 2004; Achigan Dako et al. 2008a) is grown as vegetable crop for consumption of the seeds, the only edible portion; the pulp of the fruit in these cultivars is usually too bitter for human consumption. Egusi melon thrives in West Africa where wild, semi-cultivated and cultivated forms were presumably found (Jeffrey 2001). They were indifferentiatly named as *C. lanatus* var. *lanatus* or as *C. lanatus* subsp. *mucosospermus* (Fursa 1972) or *Colocynthis citrullus* (Hutchinson and Dalziel 1954).

In spite of the description by Fursa (1981, 1983), there are still gaps in the knowledge of *C. mucosospermus* morphology. Adjakidjè (2006) in "Flore du Benin" failed to distinguish between egusi melon and watermelon. This also results from the inconsistency noticed in various Flora on West Tropical Africa editions (Hutchinson and Dalziel 1954).

Past and present attempts to establish intraspecific variation within *Citrullus lanatus* revealed interesting differences. For example rDNA internal transcribed spacer (ITS) heterogeneity revealed a TTA insertion within ITS 1 that was apparently unique to *C. lanatus* (e.g. 'New Hampshire Midget' a registered cultivar, and an egusi-type *C. lanatus* from Nigeria) (Jarret and Newman 2000). Unfortunately, as Jarret and Newman (2000) focused their studies on the interspecific relationships and the placement of *C. rehmii* in relation to other member of the genus *Citrullus*, their work involved too few accessions of *C. lanatus* to make conclusive statements regarding the intraspecific variation within this species.

Most isozymes tested for watermelon were monomorphic (Navot and Zamir 1987). This is in support to Levi et al. (2001b) who indicated low genetic diversity within the accessions of cultivated watermelon. Simple sequence repeat (SSR) analyses revealed that egusi-type watermelons from Nigeria grouped with *C. lanatus* var. *lanatus* (Jarret et al. 1997). A comparative analysis of chloroplast DNA variation revealed only two haplotypes within *C. lanatus* and these were associated with the classification as cultivated var. *lanatus* versus citron-type var. *citroides* (Dane et al. 2004).

Morphological studies usually exhibit seed traits as important discriminating factors in *C. lanatus*. Morphologic-anatomical characters and physiological properties of varieties were used to single out ten ecogeographical groups and further included into three geographical types: the Russian, the Asiatic and the Eastern types (Fursa 1972, 1981). This classification is no taxonomic classification as recognized by the author. Phenetic analysis of morphological variation for Namibian cultivars of *C. lanatus* was performed and supported the indigenous classification system based on gross morphology, ecology and usage of groups (Maggs-Kölling et al. 2000).

A wide phenetic analysis of *Citrullus* spp. including accessions from different continents is not undertaken yet. Interspecific and intraspecific classifications has remained elusive particularly with transitional genotypes resulting from gene flow between taxa. Moreover, there is obviously a lack of evidence on *C. mucosospermus* and *C. lanatus* discriminant traits with regards to their wild relative *C. colocynthis.* That is why intraspecific morphological variation needs to be further clarified.

Concurrently, there is an ongoing need to improve watermelon and egusi melon, particularly with respect to development of disease- and pest-resistant, and high yielding cultivars. Knowledge about the morphological variation of *C. lanatus* and genotypes/environment interactions is a pre-requisite for any breeding programmes oriented towards the improvement of the crop's performance.

Here, we assess the morphological differentiation of the egusi melon (*C. mucosospermus*) based on the hypotheses that *C. lanatus*, *C. mucosospermus*, *C. amarus*, and *C. colocynthis* have significant differences in the size of their leaf, flower, fruit and seed, and in the colour of their pulp and seed.

Materials and methods

Study area

The experiments were conducted at the Agricultural Research Centre of Niaouli (6°44′N, 2°07E, 81 m elevation) and at the Agricultural Research Centre of Savè (8°03′N, 2°46′E, 199 m elevation) in southern and central Benin. The two sites were located in two different phytogeographical regions (the Guinean and the Sudano-Guinean respectively). In southern Benin the rainfall is bimodal with 1,000–1,200 mm per annum for 85 raining days. The average temperature is between 27 and 29 °C. The mean relative humidity can reach 70 % with a minimum of 60 % in the dry season and a maximum of 90–100 % in the wet season. Soil type in the area is kaolinitic, known as 'terre de barre'(Akoègninou et al. 2006). In central Benin the rainfall is unimodal with 900–1,000 mm per annum. Temperature is relatively higher and the relative humidity lower. Soil types in Savè are tropical ferruginous.

Plant materials

A total of 213 accessions of *C. mucosospermus*, *C. lanatus*, *C. amarus*, and *C. colocynthis* from four continents (Africa, America, Asia and Europe) were used in these trials. This plant material consisted of 22 samples of *C. lanatus* and *C. mucosospermus* collected in 2006 and 2007 from the Sudanian and the Sudano-Guinean regions of West Africa by the first author. We obtained 100 accessions of *C. lanatus*, *C. amarus*, and *C. colocynthis* from the Genebank of the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK, Gatersleben, Germany) and 91 accessions of all four species from the United States Department of Agriculture (USDA). Accessions received from international genbanks originated from Africa, Asia, America and Europe. The list of plant materials is available as online supplementary material. For all accessions received from international genebanks, herbarium vouchers were already available in respective institutions. However, the first author deposited additional herbarium specimens at IPK Gatersleben (GAT) for other accessions collected in West Africa (Table S1).

Trial design and phenetic analysis

We used Alpha design (IPGRI 2001) with ten blocks (complete and incomplete) of 34 or 35 experimental units with two replicates in two experimental sites during two consecutive cropping seasons (2009 and 2010). As a standard, we added one egusi accession as check variety to each block. The distance between two blocks was 2 m. Each experimental unit is a line of 2 m long sown with three seedbeds separated by 1 m. Two consecutive experimental units were 2 m apart. Alpha designs are flexible enough to accommodate a large number of accessions with fewer replicates than the number of blocks, and also blocks of different sizes.

Agro-morphological data were collected for 22 discrete and continuous traits at appropriate growth stage (flowering and maturity stages) following Maggs-Kölling et al. (2000) and Achigan Dako et al. (2008c) between March and July 2009 and 2010 in Niaouli and between September and January in Gobé. For quantitative data at least two plants were measured per accessions. Plant traits measured (Fig. 1) include for (1) leaves: lamina length (Lglb), lamina width (Lalb), central lobe width (Lglc), secondary lobe length (Lglo), distance from the vein basis to the lobe sinus (Dvls); (2) flowers: male peduncle length (Lgpm), female peduncle length (Fpdl), duration of female flowering (Dflf), duration of male flowering (Dflm); (3) fruits: fruit weight (Pdfr), fruit length (Lgfr), fruit diameter (Diafr), pericarp thickness (Eppr), flesh taste (Gopu), flesh colour (Culp), and (4) seeds: thousand seed weight (Pmgr), seed length (Lggr), seed width (Lagr), seed surface (Sfgr), seed colour (Cogr) (Table 1). Leaf traits were exclusively measured on the tenth leaf counted from the stem apex at 50 % flowering. Data on full blooming flowers were collected at the tenth leaves from top of the main stem. Metric characters were measured using a Vernier calliper (0.02 mm default) and fruits were weighted with a scale. Fruit and seed traits were measured only on mature fruits (at least three mature fruits from each

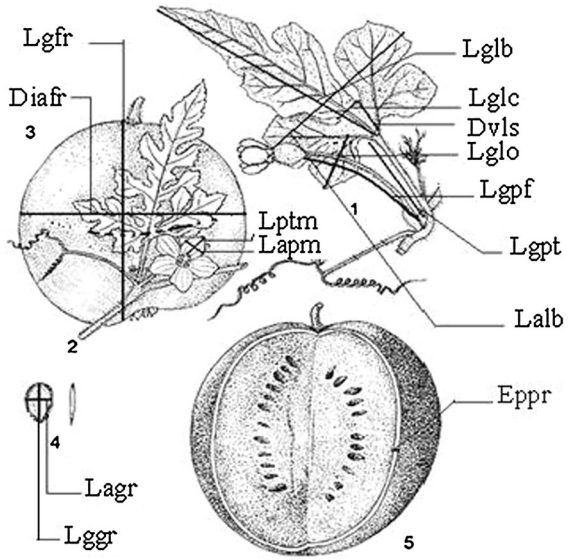

Fig. 1 Morphological traits used for phenetic analysis in *C. mucosospermus*, *Citrullus lanatus*, *C. amarus*, and *Citrullus colocynthis*. Lglb: Lamina length, Lalb: Lamina width, Lglc: Central lob width, Lgpt: Petiole length, Dvls: Distance from the base of the vein to the sinus of the secondary lobe, Lgpf: Female peduncle length, Lptm: Male petal length, Lapm: Male petal width, Lgfr: Fruit length, Diafr: Fruit diameter, Eppr: Pericarp thickness, Lggr: Seed length and Lagr: Seed width. *1* stem with a female flower; *2* stem with a male flower; *3* fruit of *C. lanatus*; *4* seed of *C. lanatus*; *5* fruit of *C. lanatus* (peel and flesh partially out). Adapted from van der Vossen et al. 2004

accession) at harvest. Colour identification was done by means of the colour chart of the Royal Horticultural Society (RHS 1995). Seed measurements were carried out at IPK Gatersleben (Germany) using the Digital Seed Analyser (GTA Sensorik GmbH, Neubrandenburg, Germany), which allowed a high counting accuracy (error <0.01). A sample of 15–50 seeds was measured per accession. Data were recorded using Marvin 4.0 software (GTA Sensorik GmbH, Neubrandenburg, Germany) (Achigan Dako et al. 2008b).

Data analysis

Data from both sites for the two cropping seasons were combined for each accession within a cultivar group. For each variable the frequency distribution was graphically examined. Multi-normality test was performed for all variables. Mean values and standard deviations were calculated, and a multivariate analysis of variance (MANOVA) was performed to test if there is any significant difference in the morphological traits of different taxa. A deviation to the homogeneity of variances and co-variances was tested using the Box's M test (Hill and Lewicki 2006). A canonical discriminant analysis (using Wilk's criterion) was performed to identify which of the 19 morphological traits (continuous variables) best discriminate the taxa.

Pearson's correlation was used to assess relationships among descriptors. The relative contribution of each variable to phenetic differentiation was examined by a principal component analysis (PCA) using the package "FactoMineR" in R (Husson et al. 2013).

Results

Variability of leaf traits

Most of the leaf traits were highly variable among group of accessions indicating that various types of leaves are observed in *Citrullus* spp. (Fig. 2). Descriptive statistics are synthesized in Table 2. The lamina length (Lglb) changed across taxa and accession groups (Fig. 3a, $p < 0.001$). In *C. mucosospermus* the lamina length can reach 203.4 mm but on average fluctuates around 150.4 ± 22.3 mm. The variation of the lamina width (Lalb), however, was not significant across groups (Fig. 3b, $p = 0.118$). In *C. mucosospermus* it can reach 197.5 mm but on average fluctuated around 128.0 ± 33.3 mm. The 'neri' type showed the smallest lamina width with 110.1 ± 22.3 mm. The petiole length showed a lot of variations ($p = 0.005$) but on average fluctuated between 60 and 75 mm in all groups (Fig. 3c). The smallest mean was found in *C. lanatus* 'neri' type (62.5 ± 15.2 mm) while the biggest mean is found in *C. amarus* (71.0 ± 17.7 mm). The secondary lobe length (Fig. 3d), the central lobe basal width (Fig. 3e), and the distance from the veins basis to the sinus (Fig. 3f) also showed significant variation across groups ($p < 0.001$).

Variability of flower traits

Inflorescences in *C. mucosospermus* and *C. colocynthis* were male and female on the same plant (monoecy). However, 12 and 18 % of accessions exhibited hermaphrodite flowers in *C. amarus*, and *C. lanatus* subsp. *vulgaris* respectively (Fig. 4; Table 3). Hermaphrodite flowers were absent or very rare in other *taxa* such as *C. colocynthis*, *C. mucosospermus*.

Table 1 Morphological traits (22) used for the phenetic analysis in *Citrullus mucosospermus*, *Citrullus lanatus*, *C. amarus*, and *Citrullus colocynthis* and significance of quantitative traits

Abbreviation	Traits	Measurement instrument	*p* value
	Leaf traits		
Lalb	Lamina width (mm)	Vernier calliper	0.118
Lglb	Lamina length (mm)	Vernier calliper	0.000
Lgpt	Petiole length (mm)	Vernier calliper	0.005
Lglc	Central lobe width (mm)	Vernier calliper	0.000
Lglo	Secondary lobe length (mm)	Vernier calliper	0.000
Dvls	Distance from the base of the vein to the sinus of the secondary lobe (mm)	Vernier calliper	0.000
	Flower traits		
Dflf	Duration of female flowering (day)	Count data	0.000
Dflm	Duration of male flowering (day)	Count data	0.000
Lgpf	Female peduncle length (mm)	Vernier calliper	0.000
Lgpm	Male peduncle length (mm)	Vernier calliper	0.000
	Fruit traits		
Gopu	Flesh taste	Direct tasting	0.000
Txsu	Total soluble solid (°Brix)	Refractometer	0.000
Colp	Flesh colour	Colour chart	0.000
Diafr	Fruit diameter (mm)	Vernier calliper	0.011
Lgfr	Fruit length (mm)	Vernier calliper	0.000
Pdfr	Fruit weight (g)	Scale	0.000
Eppr	Pericarp thickness (mm)	Vernier calliper	0.000
	Seed traits		
Cogr	Seed colour	Colour chart	0.000
Lagr	Seed width (mm)	Vernier calliper	0.000
Lggr	Seed length (mm)	Vernier calliper	0.000
Sfgr	Seed surface (mm)	Digital seed analyser	0.000
Pmgr	Thousand seed weight (g)	Digital seed analyser	0.000

Flowering takes place around 30 days after sowing for male flowers and of 45 days for female flowers (Fig. 5a, b). These flowering times varied among accession groups ($p < 0.001$). The shortest male and female flowering times were observed in *C. mucosospermus* (28.9 ± 3.7 days for male flowers and 41.7 ± 8.4 days for female flowers). The longest male flowering times were noticed in *C. amarus* (33.8 ± 7.8 days) and in subsp. *lanatus* for female flowers (54.0 ± 12.0 days).

The peduncle length is overall longer for female flowers (38.8 ± 4.5 mm) than for male flowers (29.6 ± 1.7 mm). Male peduncle length was smaller in subsp. *vulgaris* (28.4 ± 9.8 mm) and higher in *C. mucosospermus* (33.1 ± 9.9 mm). Female peduncle length was smaller in subsp. *lanatus* (34.6 ± 20.9 mm) and higher in subsp. *vulgaris* (45.8 ± 25.3 mm).

Variability of fruit traits

Various fruit sizes, shapes and colours are found in *Citrullus* spp. (Fig. 6). Fruit weight tremendously varied among species and subspecies (Fig. 7c, $p < 0.001$). Lower fruit weight were found in *C. lanatus* neri-type (0.6 ± 0.2 kg) while heavier fruits were found in *C. vulgaris* (2 ± 0.8 kg); fruit weight of more than 12 kg were also recorded in that group (Table 2). Fruit length and diameter also significantly varied among species and subspecies (Fig. 7d, e).

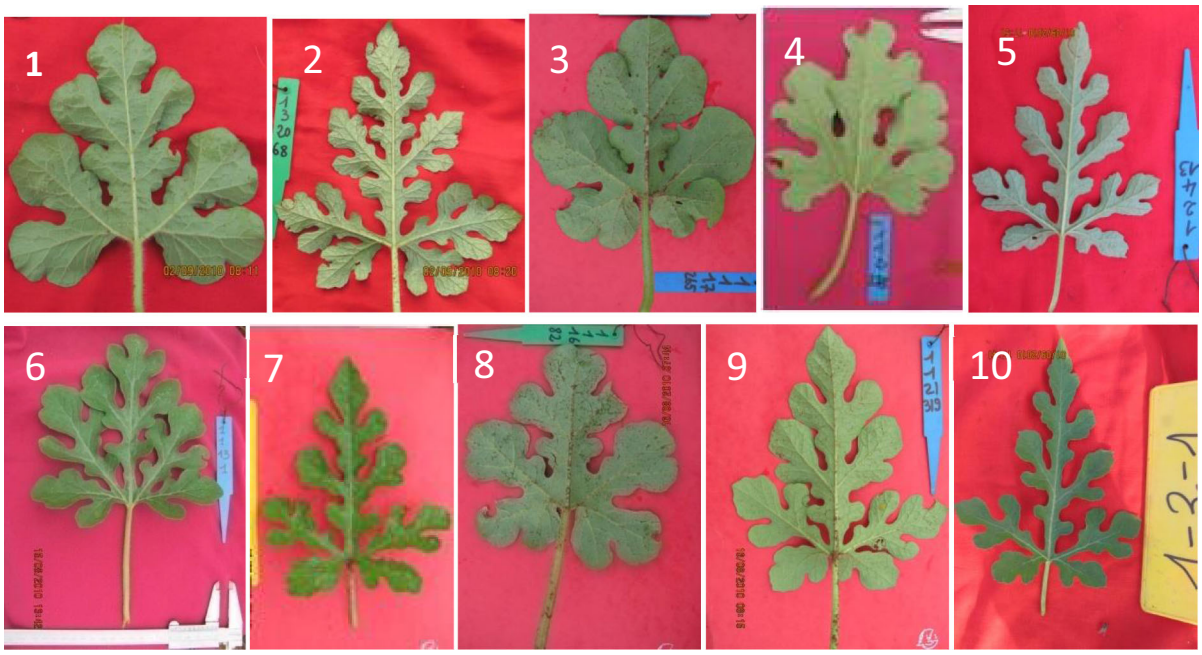

Fig. 2 Types of leaves in *C. mucosospermus*, *C. lanatus*, *C. amarus*, and *C. colocynthis*: *1*, *2 Citrullus lanatus. 3*, *4 C. lanatus* subsp. *vulgaris*; *5*, *6 C. mucosospermus*; *7*, *8 C. lanatus* subsp. *lanatus* var. *lanatus*; *9*, *10 C. colocynthis*

Bigger fruit size were found in subsp. *vulgaris* (fruit length = 151 ± 67 mm; fruit diameter = 134 ± 40 mm); fruit length reached sometimes 50 cm in that group. Smaller fruit size were found in neri-type where fruit length was on average 102 ± 41 mm and fruit diameter in the same range (104 ± 39.8 mm). Pericarp thickness overall varied between 1 and 17 mm. On average *C. mucosospermus* exhibited thicker pericarp (7.9 ± 3 mm).

Fruit flesh colour (Fig. 8) in *C. colocynthis* mainly was white colour (for 64 % of accessions) and green-white (for 21 % of accessions). In *C. mucosospermus* all accessions exhibited white flesh colour (100 %). In *C. amarus* 52 % of accessions showed white flesh colour, 24 % showed yellow flesh colour and 12 % were green-white. Red and orange-red fleshes were found in subsp. *vulgaris* (Table 4).

There were various flesh taste types in *Citrullus* spp. including acidic, bitter, plain and sweet tastes (Table 5). Most encountered tastes included bitter tasted in *C. colocynthis* (93 % of accessions), *C. mucosospermus* (79 % of accessions) *C. amarus* (88 % of accessions), and sweet taste in subsp. *vulgaris* (80 % of accessions). In subsp. *vulgaris* total soluble solid reached 6°Brix (Table 2).

Variability of seed traits

Seed colour and types varied in *Citrullus* spp. (Fig. 9). Thousand seed weight highly varied among species and subspecies (Fig. 10a). *C. mucosospermus* showed the highest thousand seed weight (138.3 ± 28.9 g) while the neri-type exhibited the smallest thousand seed weight (36.2 ± 12.3 g). Likewise, seed surface (Fig. 10b) was the biggest in *C. mucosospermus* (118.3 ± 20.8 mm^2) and the smallest in neri (35.3 ± 8.5 mm^2). This applied to seed length as well (Table 2).

Main seed colours in *Citrullus* spp. included black, grey, brown, orange, and yellow (Table 6). In *C. colocynthis* seeds were yellow-orange (50 % of accessions), grey-brown (20 % of accessions) and orange-grey (20 % of accessions). In *C. mucosospermus* seeds were yellow (35 % of accessions), orange-greyed (18 % of accessions), greyed-yellow (15 % of accessions) and sometimes black (10 % of accessions). In *C. amarus* seed colour mainly varied from Orange-greyed (22 % of accessions) to yellow-orange (38 % of accessions). *C. lanatus* subsp. *vulgaris* exhibited yellow-orange seeds (71 % of accessions) and dark seeds (11 % of accessions).

Table 2 Minimum, maximum, mean and standard deviation (SD) values of *C. mucosospermus*, *C. lanatus*, *C. amarus*, and *C. colocynthis* obtained by descriptive analysis

	Lglb (mm)	Lalb (mm)	Lgpt (mm)	Lglo (mm)	Lglc (mm)	Dvls (mm)	Lgpm (mm)	Lgpf (mm)	Dflm (day)	Dflf (day)
C. lanatus 'neri-type'										
Minimum	108.9	75.4	36.6	34.2	5.5	6.3	18.0	24.6	25.0	42.0
Maximum	171.5	130.5	90.0	74.5	8.1	17.2	38.2	83.6	36.0	59.0
Mean	142.3	110.1	62.5	57.4	7.2	15.0	30.1	40.4	30.6	51.9
SD	24.2	22.3	15.2	11.9	1.0	3.6	6.5	20.5	4.5	6.0
C. colocynthis										
Minimum	88.4	54.8	34.7	41.5	5.2	8.6	12.1	17.9	22.0	27.0
Maximum	176.3	196.0	93.0	94.0	35.4	42.1	52.0	75.3	63.0	67.0
Mean	143.9	128.7	64.4	63.1	12.1	20.6	28.6	35.7	31.0	42.6
SD	20.4	32.9	13.9	15.2	6.5	8.2	7.8	16.3	8.0	8.9
C. lanatus subsp. *lanatus*										
Minimum	84.7	78.0	47.7	44.2	4.8	20.5	11.1	13.5	31.0	48.0
Maximum	141.5	151.7	74.4	121.2	19.7	41.3	53.5	137.4	37.0	72.0
Mean	122.0	111.3	61.5	75.3	10.8	36.0	29.0	34.6	33.5	54.0
SD	24.8	33.8	12.1	32.6	5.2	8.1	8.8	20.9	2.3	12.0
C. mucosospermus (Egusi)										
Minimum	82.3	50.8	41.2	31.4	3.6	6.8	10.8	14.5	24.0	31.0
Maximum	203.4	197.5	117.2	127.3	18.4	36.6	76.8	113.2	55.0	71.0
Mean	150.4	128.0	69.5	63.7	8.3	16.2	33.1	41.6	28.9	41.7
SD	22.3	33.3	16.4	17.2	2.4	4.0	9.9	19.3	3.7	8.4
C. lanatus subsp. *vulgaris*										
Minimum	60.5	38.7	29.0	32.5	2.9	7.5	9.5	8.8	24.0	31.0
Maximum	230.0	201.4	107.8	116.1	30.8	46.9	71.6	148.0	60.0	68.0
Mean	150.3	120.0	65.0	62.2	9.2	20.9	28.4	45.8	30.9	43.9
SD	33.4	34.2	17.0	15.5	4.0	7.2	9.8	25.3	6.1	9.1
C. amarus										
Minimum	79.7	50.7	36.7	38.1	4.6	12.3	11.4	10.3	25.0	20.0
Maximum	200.9	211.6	112.3	130.4	32.5	61.8	68.8	95.8	57.0	72.0
Mean	138.3	128.0	71.0	69.7	14.3	33.5	29.4	35.0	33.8	45.0
SD	26.9	36.4	17.8	16.5	5.7	11.8	8.9	16.8	7.8	11.0

	Pdfr (kg)	Lgfr (mm)	Diafr (mm)	Eppr (mm)	Txsu (°B)	Pmgr (g)	Sfgr (mm^2)	Lagr (mm)	Lggr (mm)
C. lanatus 'neri-type'									
Minimum	0.4	11.0	11.7	3.5	1.0	25.4	29.5	4.3	8.5
Maximum	0.9	150.4	150.4	7.7	3.0	52.2	46.6	5.5	10.8
Mean	0.6	102.4	104.7	4.9	2.2	36.2	35.3	4.8	9.3
SD	0.2	41.4	39.8	1.5	0.6	12.3	8.5	0.6	1.1
C. colocynthis									
Minimum	0.5	11.2	11.0	3.1	0.5	25.0	25.4	4.5	7.1
Maximum	3.6	311.3	191.5	11.0	5.0	156.7	96.4	8.7	14.2
Mean	1.2	121.0	114.0	6.3	2.0	67.9	54.1	6.5	10.4
SD	0.8	51.8	42.0	2.5	1.1	35.3	20.5	1.3	2.1

Table 2 continued

	Pdfr (kg)	Lgfr (mm)	Diafr (mm)	Eppr (mm)	Txsu (°B)	Pmgr (g)	Sfgr (mm^2)	Lagr (mm)	Lggr (mm)
C. lanatus subsp. *lanatus*									
Minimum	0.8	131.6	127.8	4.1	1.0	35.0	34.5	5.2	8.3
Maximum	3.1	164.2	162.3	5.6	2.0	132.8	79.2	8.3	12.7
Mean	1.4	144.6	141.0	4.6	1.3	100.1	61.9	7.0	11.4
SD	0.8	14.8	14.9	0.8	0.6	37.2	15.6	1.0	1.5
C. mucosospermus (Egusi)									
Minimum	0.1	11.2	11.8	2.8	0.3	51.8	43.3	5.6	9.8
Maximum	3.7	208.8	187.1	15.1	4.5	184.2	152.6	11.2	18.0
Mean	0.9	126.0	123.2	7.9	1.8	138.3	118.3	9.8	15.7
SD	0.5	31.6	31.6	3.0	0.9	28.9	20.8	1.0	1.5
C. lanatus subsp. *vulgaris*									
Minimum	0.0	12.6	12.0	2.5	0.5	22.2	26.7	4.8	6.9
Maximum	12.5	496.0	226.1	16.8	6.0	148.4	115.1	9.5	16.0
Mean	2.0	151.4	134.7	6.1	2.6	78.8	64.7	7.4	11.3
SD	1.8	67.3	40.0	2.9	1.4	27.0	16.4	1.0	1.7
C. amarus									
Minimum	0.0	10.7	11.7	2.5	0.1	12.5	27.5	4.6	7.6
Maximum	5.3	324.3	238.8	21.7	4.5	180.4	159.6	11.3	18.5
Mean	1.2	141.3	124.8	6.9	1.6	97.3	62.4	6.9	11.4
SD	1.0	58.6	41.8	3.4	0.9	39.7	22.5	1.2	2.2

Lglb lamina length, *Lalb* lamina width, *Lgpt* petiole length, *Lglo* secondary lobe length, *Lglc* central lobe width, *Dvls* distance from the base of the vein to the sinus of the secondary lobe, *Lgpm* male peduncle length, *Lgpf* female peduncle length, *Pdfr* fruit weight, *Lgfr* fruit length, *Diafr* fruit diameter, *Eppr* pericarp thickness, *Txsu* sugar content, *Pmgr* thousand seed weight, *Sfgr* seed surface, *Lagr* seed width, *Lggr* seed length, *Dflm* duration of male flowering, *Dflf* duration of female flowering

Phenetic relationships based on morphological characters

The search for pattern and structure among variables were done using Pearson correlation analysis between pairs of variables, and Principal Components Analysis combined with hierarchical cluster analysis.

Analysis of the correlation matrix (Table 7) revealed several positive and negative correlations among leaf, fruit and seed traits. For instance, lamina length, lamina width, and secondary lobe length were highly correlated ($p < 0.001$); the central lobe width showed interesting positive correlations with male peduncle length ($r = 0.60$), female peduncle length ($r = 0.30$), and fruit weight ($r = 0.46$). Male and female peduncle lengths were positively correlated ($r = 0.64$). Both traits were positively correlated with fruit weight, fruit diameter and pericarp thickness. Fruit weight were positively correlated with flesh total soluble solid ($r = 0.62$) but negatively correlated with seed surface ($r = -0.41$), seed length ($r = -0.43$), and seed width ($r = -0.37$). In addition, flesh total soluble solid also was negatively correlated with seed traits such as thousand seed weight ($r = -0.35$), seed surface ($r = -0.51$), seed length ($r = -0.53$), and seed width ($r = -0.51$). Finally, seed traits were negatively correlated with flowering times.

The first three axes of the principal component analysis explained about 47 % of the total variability. The variable factor map of the first two axes (Fig. 11) indicated a positive correlation of the first axis (Dim 1) with seed traits such as thousand seed weight (Pmgr), seed surface (Sfgr), seed length (Lagr), and seed width (Lagr). This first axis explained 20 % of total variability. The second axis (Dim 2) which explained about 15 % of total variability showed positive correlation with leaf traits such as lamina length (Lglb), lamina width (Lalb), petiole length (Lgpt),

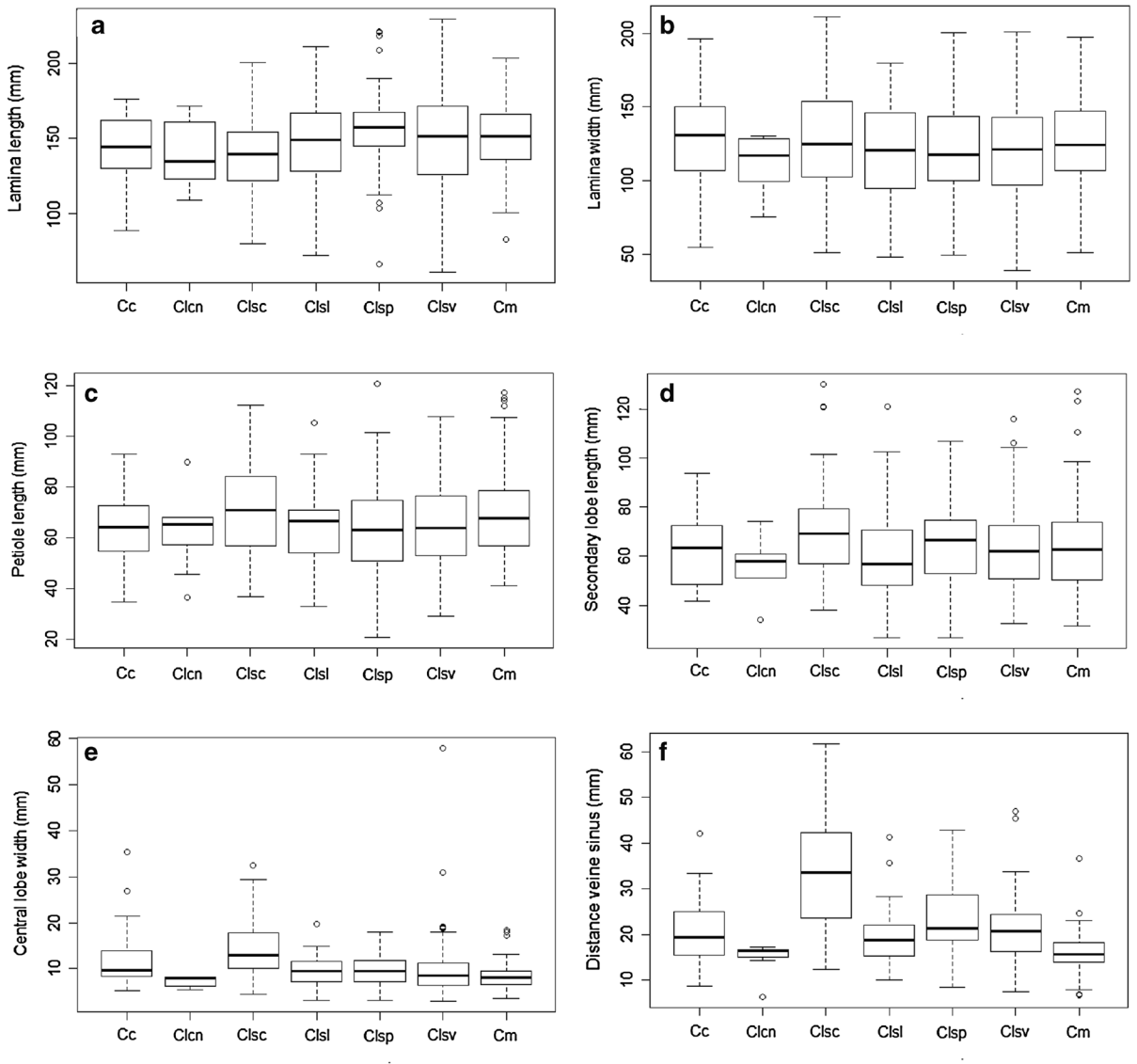

Fig. 3 Variation in leaf traits in *C. mucosospermus*, *C. lanatus*, *C. amarus*, and *C. colocynthis*; **a** lamina length ($p < 0.001$), **b** lamina width ($p = 0.1184$), **c** petiole length ($p < 0.01$), **d** secondary lobe length ($p = 0.001$), **e** central lobe width ($p < 0.001$), **f** distance to vein sinus ($p < 0.001$). The *horizontal line* is the median, the *rectangle* is a quartile and the *vertical line* is the range. Cc = *Citrullus colocynthis*, Clcn = *Citrullus lanatus* 'neri-type'; Clsc = *Citrullus amarus*, Clsl = *Citrullus lanatus* subsp. *lanatus*, Cm = *Citrullus mucosospermus*, Clsp = *Citrullus* sp. and Clsv = *Citrullus lanatus* subsp. *vulgaris*

central lobe width (Lglc), secondary lobe length (Lglo), distance to sinus (Dvls), and inflorescence traits like male peduncle length. The third axis (Dim 3) explained 12 % of total variability and showed positive correlation with fruit size and total soluble solid of fruit flesh.

The individual factor map of the two first two axes (Fig. 12) clearly isolated along the first axis accessions of *C. mucosospermus* (black dots) in a cluster. Morphological markers in this taxon were related to seed traits. The individual factor map of the first and the third axes also isolated the *C. mucosospermus*

Fig. 4 Types of flowers in *Citrullus lanatus*, *C. mucosospermus*, *C. amarus*, and *C. colocynthis:* **a** male flower; **b**, **c** females flowers; **d–f** male flower on *left* and hermaphrodite flower on *right*

Table 3 Frequency of flower types in *C. mucosospermus*, *C. lanatus*, *C. amarus*, and *C. colocynthis* (N: frequency)

	Normal sex		Abnormal sex		Total
	N	%	N	%	
C. colocynthis	14	100.0	0	0.0	14
C. mucosospermus	46	97.9	1	2.1	47
C. amarus	37	88.1	5	11.9	42
C. lanatus					
subsp. *lanatus*	27	96.4	1	3.6	28
subsp. *vulgaris*	46	82.1	10	17.9	56
'neri-type'	3	100.0	0	0.0	3
Citrullus sp.	21	91.3	2	8.7	23
Total	194	91.1	19	14.0	213

accessions in a homogenous cluster. No clear subdivision was perceptible in the other taxa.

Discussion

Morphological diversity and differentiation in *Citrullus* spp.

For the first time we carried out a phenetic analysis on a world collection of *Citrullus* spp. including *C. lanatus*, *C. mucosopermus* (two widely cultivated species), *C. amarus*, and *C. colocynthis* (wild relatives). Accessions originated from Africa, America, Asia and Europe and include US Plant Introduction accessions, IPK Gatersleben's genebank accessions and plant material collected by the first author.

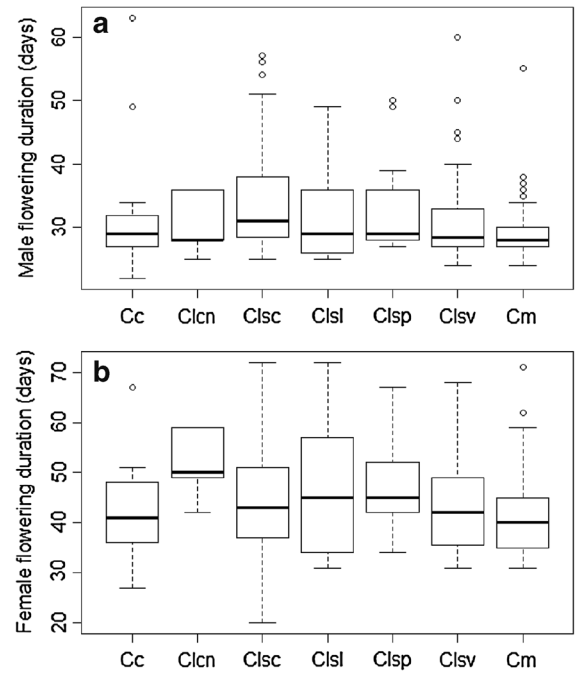

Fig. 5 Variation of male (**a**) and female (**b**) flowering time in *C. mucosospermus*, *C. lanatus*, and *C. colocynthis* ($p < 0.001$). The *horizontal line* is the median, the *rectangle* is a quartile and the *vertical line* is the range. Cc = *Citrullus colocynthis*, Clcn = *Citrullus lanatus* 'neri-type'; Clsc = *Citrullus amarus*, Clsl = *Citrullus lanatus* subsp. *lanatus*, Cm = *Citrullus mucosospermus*, Clsp = *CitAZa/rullus* sp. and Clsv = *Citrullus lanatus* subsp. *vulgaris*

Our results confirmed that *Citrullus* spp. exhibit a lot of inter- and intra-specific diversity. Diagnostic morphological markers among species include fruit and seed traits such as fruit size, flesh colour and taste, seed

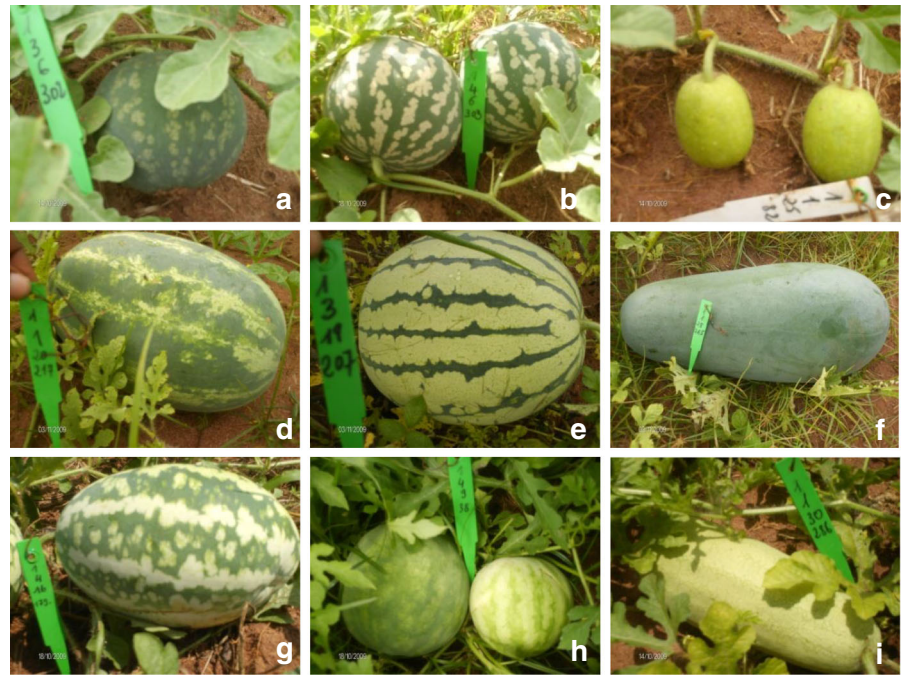

Fig. 6 Fruits types in *C. lanatus*, *C. mucosospermus*, *C. amarus*, and *C. colocynthis*: **a**, **b** *C. colocynthis*, **c** *C. lanatus* subsp. *lanatus*, **d**–**f** *C. lanatus* subsp. *vulgaris*, **g**, **h** *C. mucosospermus*, **i**: *Citrullus* sp.

size, and seed colour. For instance, large fruits were typical to *C. lanatus* subsp. *vulgaris* while *C. mucosospermus* exhibit small to medium fruit size. Fruit size reached 12 kg in subsp. *vulgaris* in our study. According to Gusmini and Wehner (2005) fruit weight in watermelon cultivars varied between 3.6 and 19.3 kg. Higher yield were found in 'Mountain Hoosier' and 'Starbrite' which were not include in our experiment. *C. colocynthis* usually exhibits small fruit size but the neri-type has smaller fruit size. Flesh taste is most of the time bitter in *C. mucosospermus* and *C. colocynthis* while *C. lanatus* rarely shows bitterness. In subsp. *vulgaris* flesh taste was sweet. Some accessions of that subspecies exhibited no flesh taste. However, those accessions equally had different flesh colours, not red. Flesh colour is most of the time red in *C. lanatus* subsp. *vulgaris*. It can turn yellow-orange and rarely white (Maggs-Kölling et al. 2000; Sari et al. 2005; Solmaz and Sarı 2009; Solmaz et al. 2010; Szamosi et al. 2009; van der Vossen et al. 2004). However, in the other species white flesh colour were dominant.

Other major differentiation traits were related to seed. Several seed sizes and colours were present in *Citrullus* spp. For instance the neri-type always exhibits small and yellow seeds. In *C. lanatus* seed size varies from small to medium and seed colour ranges from black to yellow-orange. In *C. mucosospermus*, seed are generally large with yellow tegument bordered with a blackish or whitish thick egde. The differentiation of this taxon by Fursa (1983) was based on the presence of wild and cultivated forms, polymorphic nature of the seeds, peculiarity of the karyotype, some characteristic properties of chemical composition of oil in seeds. However, the presence of wild forms of *C. mucosospermus* is difficult to establish as spontaneous plants can just represent escapes from cultivation. Several collecting missions by the first author did not yield any substantial information on wild populations of *C. mucosospermus*.

Morphological differentiation traits in *C. colocynthis* were not clear with our phenetic data which showed overlapping features. Taxonomically, the species is recognized with its large and perennial root and the scabrous and greyish leaves (Naudin 1859).

Leaf and floral traits, although variable, did not exhibit any conspicuous diagnostic traits for phenetic differentiation. However, the central lobe basal width and the distance to vein's sinus were smaller in 'neri' and *C. colocynthis* than in all other taxa. In *C. amarus*, these traits exhibited higher values. However, data showed more or less continuous values and those traits cannot be used for taxonomic differentiation.

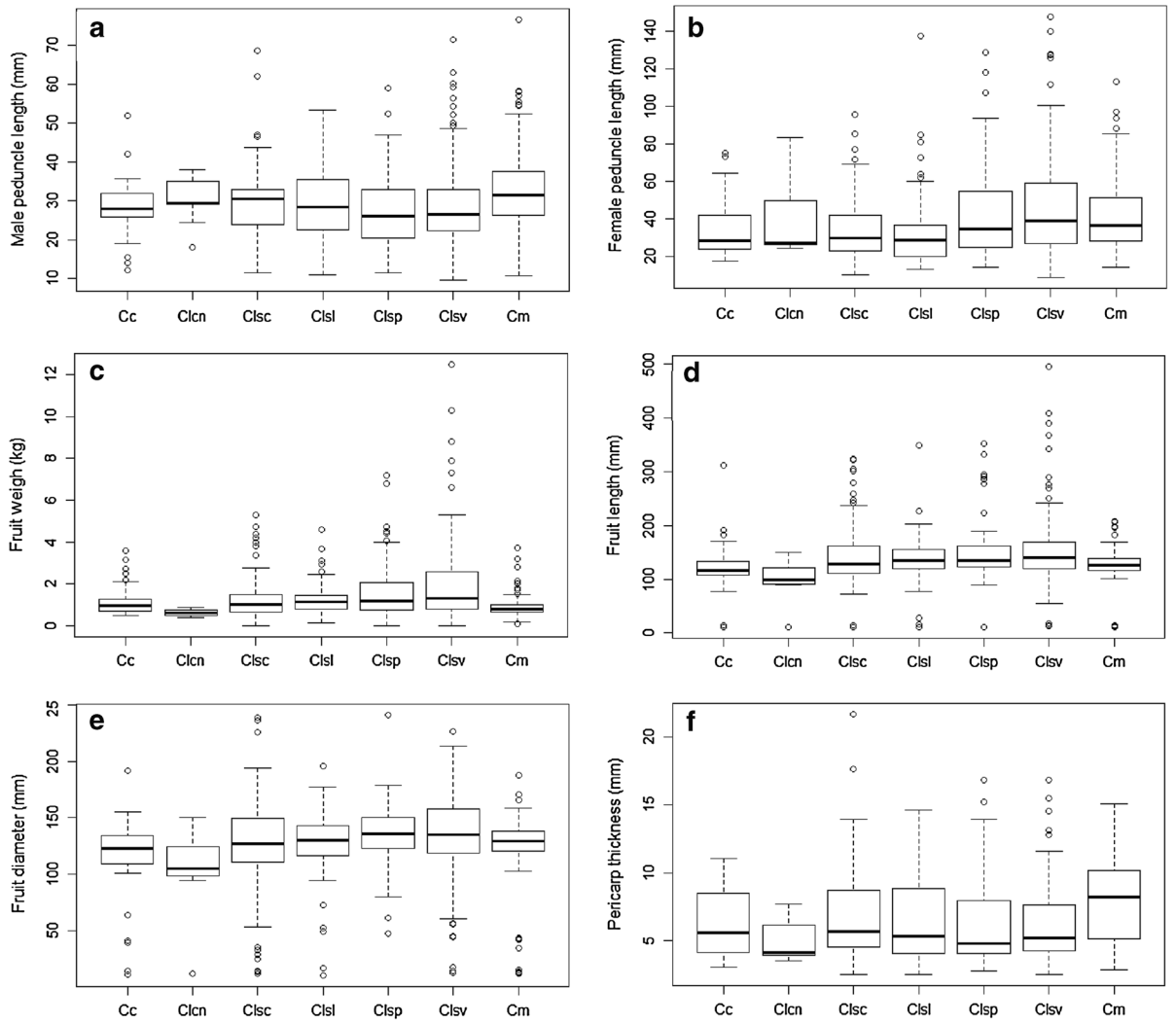

Fig. 7 Variation in fruit traits in *C. mucosospermus*, *C. lanatus*, *C. amarus*, and *C. colocynthis*; **a** male peduncle length ($p < 0.001$), **b** female peduncle length ($p < 0.001$), **c** fruit weight ($p < 0.001$), **d** fruit length ($p < 0.001$), **e** fruit diameter ($p < 0.001$), **f** pericarp thickness ($p < 0.001$). The *horizontal line* is the median, the *rectangle* is a quartile and the *vertical line* is the range. Cc = *Citrullus colocynthis*, Clcn = *Citrullus lanatus* 'neri-type'; Clsc = *Citrullus amarus*, Clsl = *Citrullus lanatus* subsp. *lanatus*, Cm = *Citrullus mucosospermus*, Clsp = *Citrullus* sp. and Clsv = *Citrullus lanatus* subsp. *vulgaris*

Morphological and genetic characterization of Hungarian and Turkish accessions of watermelon (including *C. lanatus* and *C. colocynthis*) revealed that germaplasm resources present a wide range of diversity (Sari et al. 2005; Solmaz and Sarı 2009; Solmaz et al. 2010; Szamosi et al. 2009). However, accessions of the two countries show many similarities and therefore cannot be separated clearly (Szamosi et al. 2009). Our study did not clearly separate *C. lanatus* and *C. colocynthis*. These species share several morphological features and are not always easy to differentiate by non-experts. The overlaps and discrepancies among and within species and subspecies make closer comparisons inconsequential. Also, the fact that all *Citrullus* species are compatible renders taxonomic differentiation compounded (Maggs-Kölling et al. 2000).

Intraspecific variations were perceptible in all four species but some clarifications are needed between *C. lanatus* and *C. amarus* (syn. *C. caffer* or

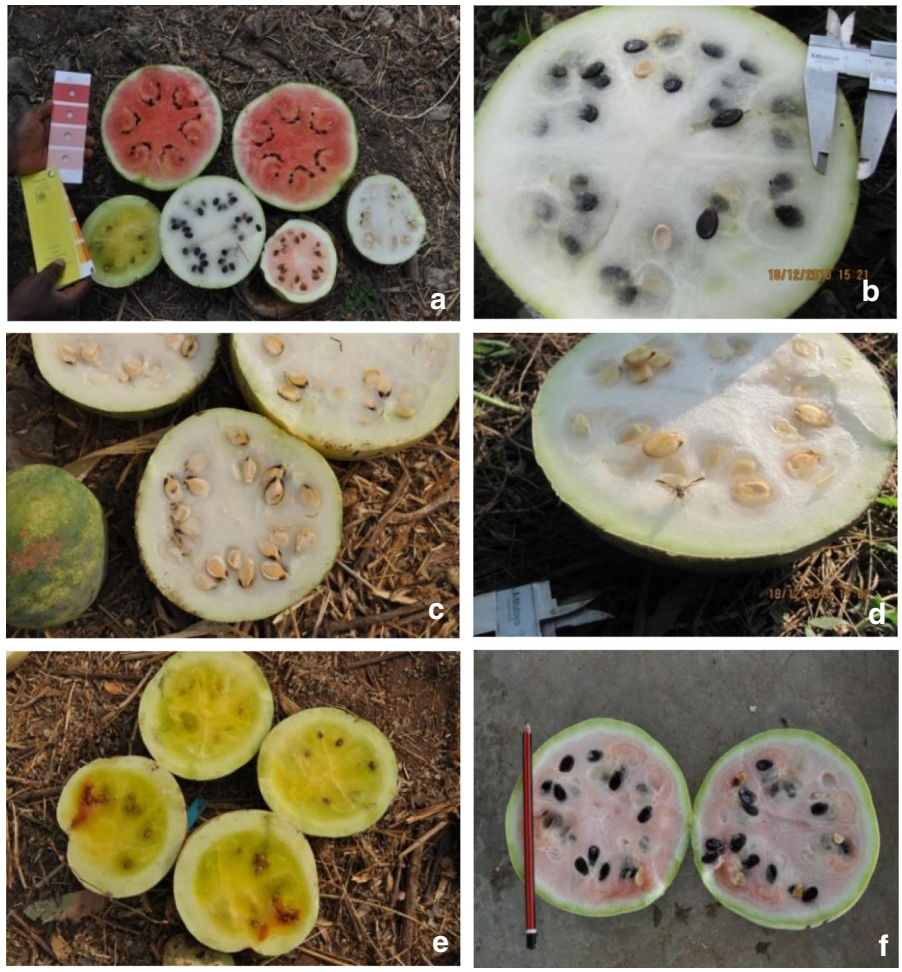

Fig. 8 Fruit flesh colour in *C. lanatus*, *C. mucosospermus*, *C. amarus*, and *C. colocynthis*: **a**, **b** *C. lanatus* subsp. *vulgaris*, **c**, **d** *C. mucosospermus*, **e** *C. amarus*, **f** *C. lanatus*

var. *citroides*). For instance, in *C. amarus* flesh colours were green-white, white, yellow, and yellow-green while seed colours were black, brown, orange-greyed, and yellow. These flesh colours were also observed in the Hungarian and Turkish accessions (Szamosi et al. 2009). In *C. lanatus* we had green-white, orange-red, red, white, and yellow flesh with orange-greyed or yellow-orange seed colour. Morphological differentiation between *C. amarus* and *C. lanatus* also justifies the reconsideration of the taxon at specific rank as *C. caffer* (Nesom 2011) or *C. amarus*, which is the oldest name available (Chomicki and Renner 2014; Renner et al. 2014). This taxon has also been regularly differentiated with molecular tools (Laghetti and Hammer 2007; Levi et al. 2013; Mujaju et al. 2010, 2011, 2013) and the recent phylogenetic data (Chomicki and Renner 2014; Renner et al. 2014) provide new insights into future collections and use of genetic variations within the genus. It is, however, worth mentioning that Hammer and Gladis (2014) suggest downgrading *C. amarus* to a varietal level as *C. caffrorum* together with var. *lanatus* (for which the typification is still under discussion according to Nesom (2011) and var. *mucosospermus* (Fursa) K. Hammer, stat. nov. which was previously elevated at specific rank by Fursa (1983). Molecular phylogenetics of *Citrullus* spp. including Linnean material (Chomicki and Renner 2014) clearly separated common watermelon (*C. lanatus* subsp. *vulgaris*) and egusi melon (*C. mucosospermus*), which is in agreement with our findings.

Table 4 Main fruit flesh colour in *C. mucosospermus*, *C. lanatus*, *C. amarus*, and *C. colocynthis* (N: frequency)

	Green-white		Orange		Orange-red		Red		Red-purple		White		Yellow		Yellow-green		Yellow-orange		Total
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N
C. colocynthis	3	21.4	0	0.0	1	7.1	0	0.0	0	0.0	9	64.3	0	0.0	1	7.1	0	0.0	14
C. mucosospermus	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	47	100	0	0.0	0	0.0	0	0.0	47
C. amarus	5	11.9	1	2.4	0	0.0	0	0.0	0	0.0	22	52.4	10	23.8	4	9.5	0	0.0	42
C. lanatus																			
subsp. *lanatus*	4	14.3	0	0.0	4	14.3	4	14.3	0	0.0	15	53.6	1	3.6	0	0.0	0	0.0	28
subsp. *vulgaris*	0	0.0	4	7.1	28	50.0	13	23.2	2	3.6	1	1.8	1	1.8	1	1.8	6	10.7	56
'neri-type'	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	3	100.0	0	0.0	0	0.0	0	0.0	3
Citrullus sp.	0	0.0	2	8.7	1	4.3	2	8.7	0	0.0	13	56.5	3	13.0	2	8.7	0	0.0	23
Total	12	5.6	7	3.3	34	16.0	19	9.0	2	1.0	110	2.0	15	7.0	8	4.0	6	3.0	213

Table 5 Frequency of flesh taste types in *C. mucosospermus*, *C. lanatus*, *C. amarus*, and *C. colocynthis* (N: frequency)

Species	Flesh taste								
	Acid		Bitter		Plain		Sweet		Total
	N	%	N	%	N	%	N	%	N
C. colocynthis	0	0	13	92.9	1	7.1	0	0.0	14
C. mucosospermus	1	2	37	78.7	5	10.6	4	8.5	47
C. amarus	0	0	37	88.1	4	9.5	1	2.4	42
C. lanatus									
subsp. *lanatus*	0	0	14	50.0	2	7.1	12	42.9	28
subsp. *vulgaris*	1	2	8	14.3	2	3.6	45	80.4	56
'neri-type'	0	0	2	66.7	0	0.0	1	33.3	3
Citrullus sp.	0	0	19	82.6	2	8.7	2	8.7	23
Total	2	0.9	130	61.0	16	7.5	65	30.5	213

In *C. mucosospermus* intraspecific variations were mainly related to seed traits with two types of seeds: the first type bordered with white rugged edge and the second type bordered with black rugged edge. Morphological variation analysis in landraces of *C. mucosospermus* (but identified as *C. lanatus* by authors in Ivory Coast) revealed two groups based on fruit and seed traits (Adjoumani et al. 2012). These two groups encompassed not only *C. mucosospermus* but certainly also neri-type [Fig. 1 in (Minsart et al. 2011)]. In our analysis the two groups were deemed different taxa.

Another differentiation traits can include inflorescence types. For instance, our results also revealed that hermaphrodite flowers prevailed in *C. amarus* and *C. lanatus* subsp. *vulgaris* as also observed in the Turkish and Hungarian accessions (Szamosi et al. 2009). However, in *C. mucosospermus* and *C. colocynthis* this phenomenon was rare or absent. Hermaphrodism was revealed in other Cucurbitaceae such as *Cucumis sativus* in which sexual differentiation is controlled by genotypic and environmental factors (Malepszy and Niemirowicz-Szczytt 1991; Pike and Mulkey 1970). In watermelon sex expression is also influenced by environmental conditions and by its production of fewer pistillate flowers than other cucurbit crops (Sugiyama et al. 2004). This trait in watermelon can be used to serve breeding purposes particularly to control inbreeding depression.

Molecular variations in *Citrullus* spp.

Molocular markers can be useful in assessing genetic diversity and in classifying *Citrullus* accessions (Levi

Fig. 9 Seed types of in *C. lanatus*, *C. mucosospermus*, *C. amarus*, and *C. colocynthis*: **a** *C. colocynthis*; **b**, **c** *C. mucosospermus*, **d**, **e** *Citrullus lanatus* var. *citroides*, **f** *C. lanatus* 'neri-type', **g** *C. lanatus* subsp. *lanatus* var. *lanatus*; **h** *Citrullus lanatus* subsp. *vulgaris*

et al. 2001a). Molecular characterization among and within *Citrullus* spp. usually results in low genetic variability contrary to the remarkable phenotypic variation. Cultivated watermelon exhibited narrow genetic base (Levi et al. 2001b) as a results of many years cultivation and selection for specific qualities. For instance, RAPD analysis among 303 Turkish accessions of *Citrullus* species (including wild relatives) indicated low level of genetic variation (Solmaz et al. 2010). ISSR analysis in four accessions of *C.*

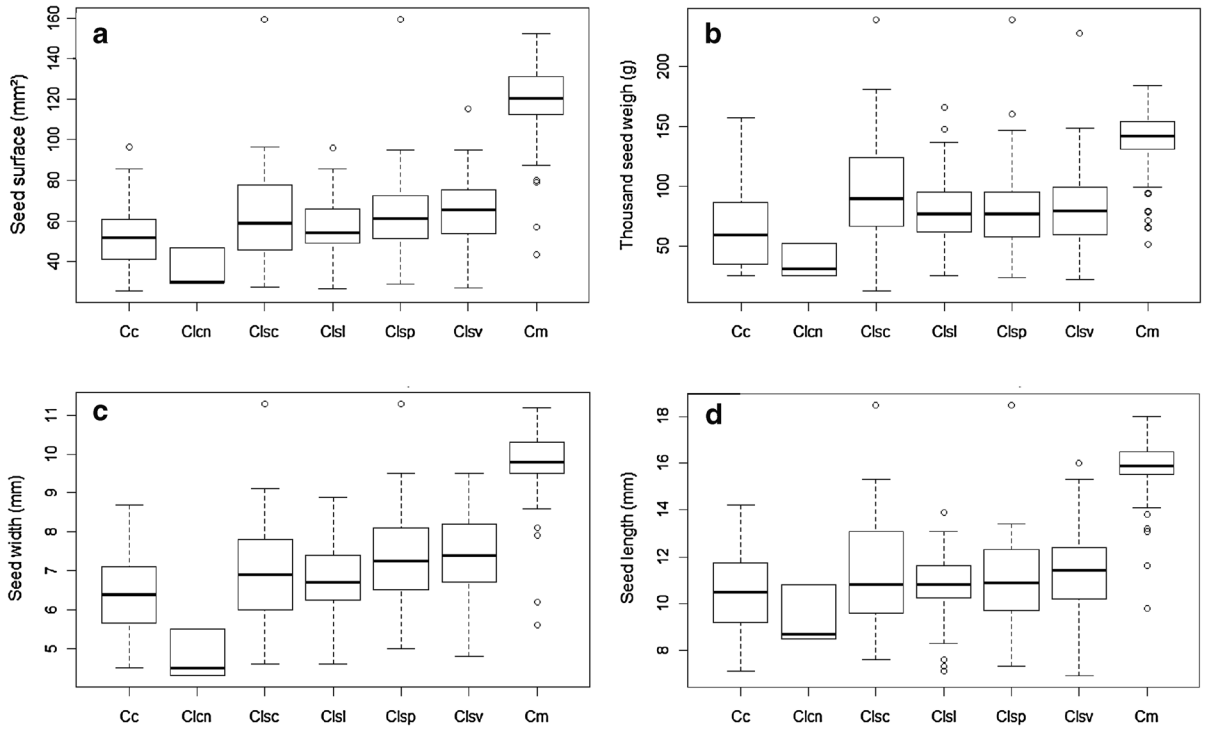

Fig. 10 Variation of seed traits in *C. mucosospermus*, *C. lanatus*, *C. amarus*, and *C. colocynthis*; **a** seed surface ($p < 0.001$), **b** thousand seed weight ($p < 0.001$), **c** seed width ($p < 0.001$), **d** seed length ($p < 0.001$). The *horizontal line* is the median, the *rectangle* is a quartile and the *vertical line* is the range. Cc = *Citrullus colocynthis*, Clcn = *Citrullus lanatus* 'neri-type'; Clsc = *Citrullus amarus*, Clsl = *Citrullus lanatus* subsp. *lanatus*, Cm = *Citrullus mucosospermus*, Clsp = *Citrullus* sp. and Clsv = *Citrullus lanatus* subsp. *vulgaris*

Table 6 Main seed colour in *C. mucosospermus*, *C. lanatus*, and *C. colocynthis* (N: frequency)

	Main seed color																	
	Black		Grey-brown		Greyed-purple		Greyed-yellow		Orange-greyed		Orange-red		Yellow		Yellow-green		Yellow-orange		Total
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N
C. colocynthis	1	10.0	2	20.0	0	0.0	0	0.0	2	20.0	0	0.0	0	0.0	0	0.0	5	50.0	10
C. mucosospermus	4	10.0	3	7.5	2	5.0	6	15.0	7	17.5	1	2.5	14	35.0	1	2.5	2	5.0	40
C. amarus	1	3.1	2	6.3	1	3.1	0	0.0	7	21.9	0	0.0	9	28.1	0	0.0	12	37.5	32
C. lanatus																			
Subsp. *lanatus*	0	0.0	0	0.0	0	0.0	0	0.0	3	50.0	0	0.0	0	0.0	0	0.0	3	50.0	6
Subsp. *vulgaris*	4	10.5	2	5.3	0	0.0	0	0.0	3	7.9	0	0.0	2	5.3	0	0.0	27	71.1	38
'neri-type'	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	3	100.0	3
Citrullus sp.	0	0.0	1	6.3	0	0.0	1	6.3	1	6.3	2	12.5	4	25.0	0	0.0	7	43.8	16
Total	10	6.9	10	6.9	3	2.1	7	4.8	23	15.9	3	2.1	29	20.0	1	0.7	59	40.7	145

Only 145 accessions were assessed for this trait

mucosospermus (referred to as *C. lanatus* seeded type by the authors) from Ivory Coast revealed high polymorphism and variation among accessions; however, the genetic structure observed did not correspond to accessions analysed (Dje et al. 2010). SSR analysis of the diversity of *C. mucosospermus*

Table 7 Correlation matrix of leaf, fruit and seed traits of accessions of *C. lanatus*, *C. mucosospermus*, *C. amarus*, and *C. colocynthis*

	Lglb	Lalb	Lgpt	Lglo	Lglc	Lgpm	Lgpf	Pdfr	Lgfr	Diafr	Eppr	Txsu	Pmgr	Sfgr	Lagr	Lggr	Dflm	Dflf	Dflh	Dvls
Lglb																				
Lalb	0.71**																			
Lgpt	0.60**	0.23																		
Lglo	0.33*	0.54**	0.12																	
Lglc	−0.13	−0.11	−0.08	0.11																
Lgpm	0.19	0.06	0.09	0.24	0.60**															
Lgpf	−0.26	−0.40**	−0.12	0.01	0.30*	0.64**														
Pdfr	0.06	−0.24	0.14	0.16	0.46**	0.49**	0.35**													
Lgfr	0.05	−0.13	0.10	0.21	0.24	0.50**	0.45**	0.38**												
Diafr	0.14	−0.10	0.16	0.14	0.16	0.44**	0.42**	0.43**	0.93**											
Eppr	−0.23	−0.23	−0.18	0.16	0.10	0.23	0.33*	0.27	0.08	0.04										
Txsu	0.01	−0.41**	0.19	0.02	0.22	0.14	0.24	0.62**	0.28	0.43**	0.15									
Pmgr	0.18	0.22	0.18	−0.08	−0.18	0.01	0.17	−0.33	−0.16	−0.21	0.10	−0.35								
Sfgr	0.01	0.25	−0.07	−0.22	−0.20	−0.15	0.05	−0.41**	−0.21	−0.16	0.07	−0.51**	0.72**							
Lagr	0.01	0.23	−0.04	−0.31*	−0.18	−0.11	0.07	−0.37**	−0.26	−0.21	0.09	−0.51**	0.71**	0.97**						
Lggr	0.05	0.32*	−0.08	−0.12	−0.19	−0.15	0.03	−0.43**	−0.15	−0.13	0.04	−0.53**	0.7**	0.98**	0.93**					
Dflm	−0.24	−0.22	−0.13	0.15	0.11	−0.10	0	0.22	−0.26	−0.24	0.21	0.21	−0.03	−0.31*	−0.27	−0.37*				
Dflf	−0.06	−0.07	−0.21	0.10	−0.04	0.02	0	0.19	−0.25	−0.22	0.09	0.03	−0.04	−0.22	−0.18	−0.28	0.8**			
Dvls	−0.20	0.04	−0.01	0.37*	0.22	−0.01	0.04	−0.02	0.19	0.13	0.06	0.05	−0.16	−0.26	−0.28	−0.21	0.21	−0.17	−0.11	

Lglb lamina length, *Lalb* Lamina width, *Lglc* central lob width, *Lgpt* petiole length, Dvls: distance from the base of the vein to the sinus of the secondary lobe, *Lgpf* female peduncle length, *Lgfr* fruit length, *Diafr* fruit diameter, *Eppr* pericarp thickness, *Txsu* total soluble solid, *Lggr* seed length, *Lagr* seeds width

* $p < 0.05$; ** $p < 0.01$

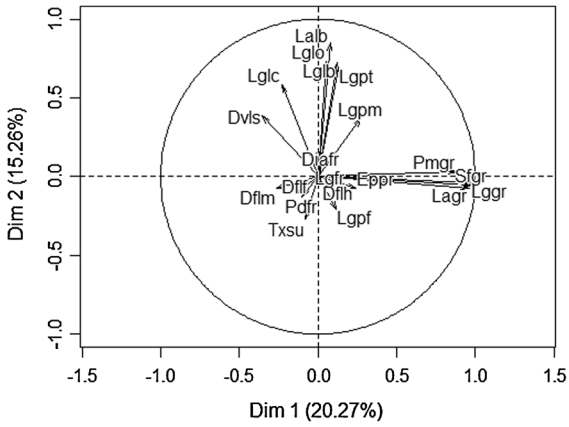

Fig. 11 Principal components analysis (PCA) variable factor map. Lglb: Lamina length, Lalb: Lamina width, Lglc: Central lob width, Lgpt: Petiole length, Dvls: Distance from the base of the vein to the sinus of the secondary lobe, Lgpf: Female peduncle length, Lgfr: Fruit length, Diafr: Fruit diameter, Eppr: Pericarp thickness, Lggr: Seed length and Lagr: Seeds width

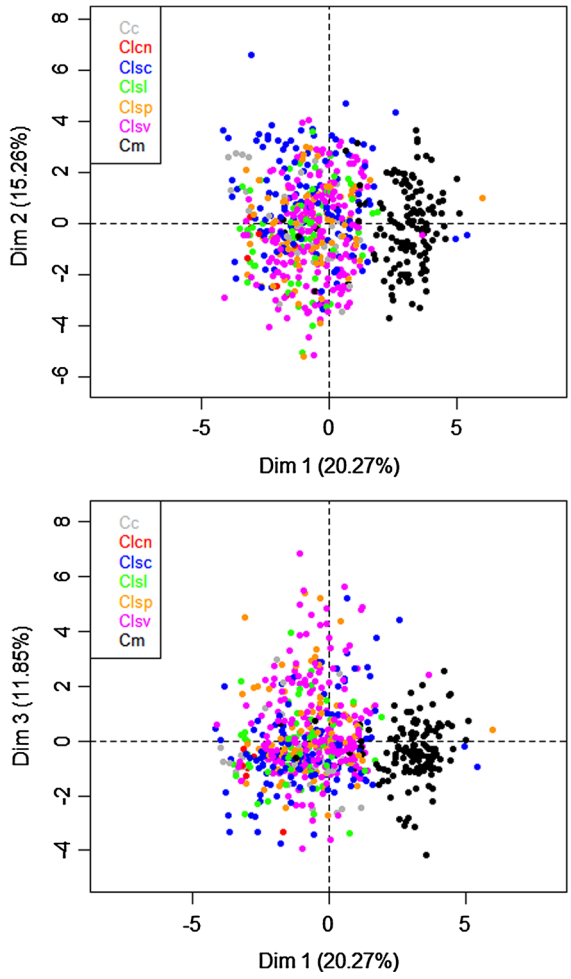

Fig. 12 Individuals factor maps of the principal component analysis *C. lanatus*, *C. mucosospermus* and *C. colocynthis* of morphological traits. Cc = *Citrullus colocynthis*, Clcn = *Citrullus lanatus* 'neri-type'; Clsc = *Citrullus amarus*, Clsl = *Citrullus lanatus* subsp. *lanatus*, Cm = *Citrullus mucosospermus*, Clsp = *Citrullus* sp. and Clsv = *Citrullus lanatus* subsp. *vulgaris*

and neri-type (referred to as *C. lanatus* subsp. *vulgaris* oleaginous type by the authors) from Ivory Coast showed evidence that morphological variability does not match genetic variation pattern in *C. lanatus* (Minsart et al. 2011). Moreover, SSR alleles number in individuals was small as a consequence of low polymorphism in *C. mucosospermus* compared to other cucurbit species [e.g. *Cucumis melo* L. (Ritschel et al. 2004)]. This low polymorphism was reported mainly on US Plant Introductions accessions associated with disease resistance. However, new data revealed broad genetic diversity among the *Citrullus* spp. accessions useful for enhancing disease or pest resistance in watermelon cultivars (Levi et al. 2013).

The phenotypic variation in *C. mucosospermus*, specifically the egusi seed type, was attributed to a single recessive gene (e.g.) after the study of the inheritance of this phenotype in crosses including subsp. *vulgaris* (Gusmini et al. 2004). In *C. lanatus*, the phenotypic diversity among cultivars was rather attributed to point mutation in genes controlling fruit colour, which may not be readily detected by dominant inherited markers (Levi et al. 2010). Expressed sequence tag (EST)-derived simple sequence repeats (EST-SSR) investigation in 35 watermelon accessions from Zimbabwe (Mujaju et al. 2013) clearly separated sweet watermelon (var. *lanatus*) and cow-melons (var. *citroides*, now *C. amarus*); moreover, it revealed somewhat higher within-accession variation in sweet watermelon compared to cow-melons; this completes a previous report which indicated that sweet watermelon accessions apparently contain diversity of the same magnitude as the cow melon (Mujaju et al. 2010). Genetic differentiation of watermelon landraces in Mali using microsatellite markers revealed two groups related to flesh colours (Nantoumé et al. 2013). This gives an insight into possibilities to unraveal the interplay between morphological classification and genetic groups.

Phenetic analysis hardly differentiated *C. colocynthis* from the other species. However, indels at the *ndh*A, *trn*S–*trn*fM and *trn*C–*trn*D chloroplast regions

can be used to separate *C. colocynthis* from the other *Citrullus* species (Dane et al. 2004) although higher similarity exists between chloroplast and mitochondrial genomes of *C. colocynthis* and *C. amarus* than with those of the subsp. *vulgaris* (Levi and Thomas 2005). Probably the level of gene flow and compatibility among species are high. Very few reports exist on the magnitude of the gene flow among species and accessions of *Citrullus* and how often it happens. Similarly no incompatibility was raised so far. Several Plant Introduction accessions analysed using high frequency oligonucleotides targeting active gene (HFO-TAG) showed transitional positions, indicating that those accessions were the results of gene flow between the major *Citrullus* groups or subgroups (Levi et al. 2013). However, the cross between Charleston Gray (subsp. *vulgaris*) × PI 560006 (*C. mucosospermus*) resulted in high levels of sterility (Gusmini et al. 2004). Earlier, Fursa (1983) found one-sided compatibility in crosses between subsp. *vulgaris* and *C. mucosospermus*.

Previous and current results indicate that we still need to deepen our knowledge of associations between genetic variation and phenotypic traits and the determinism of morphological variation among and within *Citrullus* as also recognized by Minsart et al. (2011), Nimmakayala et al. (2011), and Mujaju et al. (2013). It has been useful to look into the phylogenetic relationships among *Citrullus* species taking into account all species and herbarium specimens (Chomicki and Renner 2014). An extensive investigation that would include PIs accessions, the egusi type found in West Africa (Levi et al. 2013), and wild relatives should help clarify within and among landrace diversity and assess variability within and between species so as to exhibit associations with utility values, geographical differentiation and origin (Mujaju et al. 2010).

Conclusion

Taxonomic differentiation in *Citrullus* spp. has remained elusive for long time and did not help full use of genetic resources for maintenance and breeding purposes. In the absence of the correct name of plants/accessions many authors referred to egusi type as *C. lanatus* (Adjoumani et al. 2012; Dje et al. 2010; Minsart et al. 2011) although valid taxonomic nomenclature were provided (Fursa 1983). Our study provides valid diagnostic morphological characters for *C. mucosospermus* (egusi) in comparison with other *Citrullus* species found in West Africa where the taxon thrives. These characters include fruit size, fruit flesh taste and colour, seed type, size and colour. Our results can serve for practical decision in taxonomic revision, further germplasm collection and *ex situ* conservation, and breeding.

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Conflict of interest The authors declare that they have no conflict of interest.

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