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Phenotypic Variation and Genetic Diversity of Colocynth (*Citrullus colocynthis* [L.] Schrad.) Varieties Collected in the Southeastern of Benin Republic

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Abstract: *Citrullus colocynthis* belongs to the plant family Cucurbitaceae. Despite its nutritional importance for human beings, it is less promoted. The present study aims to explore the phenotypic variation and genetic diversity existing in the crop, to examine its breeding potential and to identify useful characters to distinguish between genotypes. 40 provenances collected from farmers in the Southeastern of Benin Republic were multiplied during the growing season 2013-2014. They were surveyed in field trials at three locations (each year) and three years (2014-2017). 20 quantitative and 14 qualitative traits were then studied. Diversity within and among genotypes was analyzed by diverse statistical methods. Phenotypic variation within and between genotypes was investigated using the Shannon-Weaver diversity index (H'). Moreover, diversity between entries was analyzed by principal component analysis, multivariate analysis of variance and discriminant function analysis. Quantitative trait variation ranged from 12.44 (for seed width) to 139.35% (for limb peduncle length). Phenotypic variation was higher overall for qualitative than quantitative traits. It ranged from 3.34 (seed tegument percent) to 41.54% (seed length) and from 25.23 (leaf size) to 62.22% (primary skin color) for quantitative and qualitative traits, respectively. Shannon–Weaver diversity index (H') was in general high and over 1.00 for most of the traits. It was higher for qualitative than quantitative traits. Monomorphism (H'=0.00) was not observed. Shannon-Weaver diversity index ranged from 0.08 (time to emergence) to 2.26 (fruit width) and 1.47 (leaf pubescence density) to 2.29 (leaf color, stem pubescence density). The first five principal components explained, 99.73% of the total variation in all traits. Multivariate analysis of variance indicates significant differences between genotypes for all individual or grouped traits. Discriminant function analysis revealed that the first five canonical discriminant functions were almost significant. We conclude that the cultivated colocynth genotypes represent heterogenous groups and efficient collection, evaluation and selection are needed for great breeding success as well as promotion of the species.

Key words: "*Egusi*", Discriminate Function Analysis (DFA) · Multivariate analyses · Principal Component Analysis (PCA) · Shannon-Weaver diversity index

INTRODUCTION

From many thousands of plant species, only a hundred has been developed into crops and the major part represents the so-called neglected and underutilized crops [1]. In low-input farming systems, farmers often use a wide range of crop varieties, to provide harvest security, yield stability and the possibility to adapt to changing ecological conditions [2, 3, 4]. However, ambiguity exists about the level of genetic diversity represented by farmer crop varieties, how it develops over time and how it relates to the diversity comprised by formal varieties [2]. Colocynth ("*Egusi*") was investigated to get information on morphological variation and the level of crop genetic diversity of farmer's materials. Breeding of *Citrullus* spp. for various benefits has continuously raised interest, particularly for economically important materials [5].

Citrullus colocynthis ("*Egusi*") belongs to the plant family Cucurbitaceae. It is believed to have originated in Africa [6, 7] but is now widely spread throughout the

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tropics and the Mediterranean [6, 8]. *Citrullus colocynthis* is a native of arid soils in Africa [6]. "*Egusi*" is thought to have been domesticated in Africa at least 4000 years ago and now grown worldwide, particularly in regions with long, hot summers [6, 9]. Contrarily to the colocynth, Watermelon (*Citrullus lanatus*) is one of the most widely cultivated crops in the world [10]. The global consumption of *Citrullus lanatus* fruit is greater than that of any other cucurbit. It accounts for 6.8% of the world area devoted to vegetable production [6, 11, 12]. China is the leading country for the production of watermelon followed by Turkey, United States of America, Iran and Republic of Korea [6, 10, 13]. Several varieties of watermelon can be grown in Kenya [8]. However, "*Egusi*" production in Benin falls far below its demand. With local demand unsatisfied, its export potential cannot be realized [5]. To meet the market demand, production of colocynth in Benin and West Africa needs to be increased.

Cucurbits are very similar in aboveground development, but they have high genetic diversity for fruit shape and other fruit characteristics, resulting in a variety of uses [5, 6, 14]. It is vital for plant breeding programs to have sufficient diversity available to allow for the production of new varieties that are aimed towards the improvement of crop productivity and able to withstand damage from biotic and abiotic factors [6, 15, 16]. Colocynth contains fruit and other plant parts, that refer to different traits desired by consumers and/or growers, including such traits as fruit flesh, texture, disease resistance and appearance traits such as shape and color [6, 17]. Identification of colocynth cultivars and determination of their genetic level and relatedness depends mainly on fruit characteristics [6, 18]. Morphological markers can be an effective means to determine genetic relatedness among cultivars and among selections, which can be of interest in cucurbit breeding programs. Levi *et al.* [18] reported that extensive variation in morphological characteristics exists among farmer varieties. It is also similar in colocynth. These characteristics include rind color and thickness, fruit shape and size, flesh texture and color, sugar content, seed shape and color, days to fruit maturity and disease resistance. Most of these characteristics are qualitative traits affected by a single or a few gene mutations [18].

The factors, which result in farmers preferring local landraces are not very well understood. Significant genetic variation may exist among accessions detained by farmers. Some may be superior in certain traits but lacking in other aspects. Their morphological characteristics may also be different. There is therefore need for a detailed study of genetic variation in cultivated as well as wild colocynth accessions to generate data on local crop development, as already mentioned by Gichimu *et al.* [6]. This data will be essential to validate suggested comparative advantages and may provide new options for plant breeding. Furthermore, Zamani *et al*. [19] stressed the hyperlipidemia anti-effects of the pulp and the seeds of *Citrullus colocynthis* fruits. Medicinal plants have traditionally occupied an important position in the socio-cultural and spiritual arena of rural and tribal lives. Medicinal plants are potential renewable natural resources [20]. Seeds of African oil cucurbits such as *Citrullus colocynthis*, called "*Egusi*" are used over time in human nutrition [21, 22, 23, 24]. Moreover, it has been indicated that those seeds represent important lipids and protein sources. [25, 26, 27, 28]. The bitter pulp of *C. colocynthis* fruits have been used as medicinal parts of the plant in West Africa and other regions in the tropics and subtropics. In Benin, as mentioned for other African countries like the Ivory Coast, dried colocynth seeds are consumed in the form of pasta to harden the texture of soup [22, 29]. Those seeds are crucial income sources for poor people including mainly women, who produce those fruits in association with other crops [29]. Despite their nutritional and socioeconomic importance, African oil cucurbits remain minor crops subjected for example to diseases, which reduce yield to 40-70 % [23, 24, 29].

Tracking the evolutionary progress, useful for taxonomy and breeding studies, has been undertaken within the species border for ages [30]. Morphological and agronomic variables are trusted, targets of selection, detectable and easily applicable [31]. Traditionally, morphological and agronomic traits have been limited to measurements of few attributes, while geometric morphometrics allows quantifying the shape of a particular structure [30, 32]. Thus, because the quantity, as well as the quality of parameters for good statistical analyses, increase greatly, geometric morphometric dissection of traits in life beings gained importance as a powerful tool for multidimensional comparisons of morphological characters [30], as well as agronomic traits species characterization and delimitation, are often difficult tasks that lead also to difficult decisions [33]. Efforts have been made until recently to describe empirical tests of species boundaries and delimitations that contributed to more natural taxonomic [34, 35, 36]. Despite recent and rapid advances in molecular systematics, morphological and agronomic as well as quality characteristics remain amongst the fundamental basis of most diversity studies [33, 37, 93]. Even, progress in

molecular areas needs morphological and agronomic approaches for within- and between-species barrier identification. Dissecting the genetic variability or selecting parents for a crossing program is a crucial step in any breeding plan. Hence, analyzing the genetic variation of the ongoing populations and establishing well-defined groups considering several traits represent important steps during the planning of any breeding scheme [38, 93, 94]. Crop genetic diversity determines its potential for improvement efficiency and indeed its utilization for breeding purposes, which usually results in enhanced food production [39, 93]. Parents' selection is the first step in the plant-breeding program through hybridization. Some appropriate statistical methods (principal component analysis – PCA, cluster and factor analyses, multivariate analysis of variance – MANOVA and discriminant function analysis - DFA) for genetic diversity characterization, parental selection, tracing the pathway to evolution of crops, centers of origin and diversity and for studying the interactions between genotypes and environments (G × E) are currently available [39, 40, 41, 42]. Assessing genetic diversity considering a sufficient number of characters jointly is only possible by applying the multivariate statistical tools, which help to clear easier the magnitude that genotypes differed enough well whenever all quantified traits are considered together. Multivariate statistical methods were increasingly applied in genotypic variability surveys in breeding populations. Setotaw *et al.* [38] used canonical discriminant analysis to demonstrate the genetic divergence among barley accessions in Ethiopia. Genetic diversity in Ethiopian wheat landraces and Indian barley collection was dissected using multivariate techniques [43, 44]. Further multivariate techniques including MANOVA, partial least squares and DFA have been employed in crop diversity analyses [45, 46, 47] (in hairy vetch), [48] (in maize), [49] (in oilseed rape), [50, 51] (in tobacco), [52] (in cassava), [53] (in groundnut), [54] (in sesame), [55, 56, 57] (in pepper and bell pepper), [58] (in *Coffea*), [59] (in Oman barley), [60] (in tall fescue). Application of MVAs in combination with genetic distance estimates gives a clear picture of the genetic differentiation among genotypes within and between given taxa [38]. A range of distance measurement tools (e.g. Mahanalobis generalized distance D^2) has been proposed over the past decades towards the realization of specific objectives in variability study enterprises [61, 62, 95]. MANOVA performs the realness of differences among populations for a given trait and the distinctiveness is studied with several vector variables combined [63, 64]. Population or genotype discrimination can be achieved by interest in linear functions called DFA. DFA as a post-cluster analysis method was able to recognize the accuracy of clustering when used by several researchers [53]. MANOVA was used to investigate the variability existing in crisphead lettuce concerning some commercial characteristics [65]. Usually, genetic distance and relatedness among populations are estimated whenever the variables are standardized to equal importance in determining the distance. However, standardization decreases the differences between groups [39]. Results of using PCA showed a weakness and limitation of the technique when the pattern of variation is not based on a 1 and 1 scores [66] such as with molecular data. Therefore, Mellingers [67] stressed earlier the need for a combination of PCA and other techniques into appropriate groupings of genotypes or populations; the main advantage of using PCA over cluster analysis is that the genotype can be assigned to one group only [66, 68].

The main objective of the work presented here is therefore to asses genetic diversity and phenotypic variation at the crop level by comparing variety pools across several villages and not at the individual farmer variety level. Further, in this report, discrimination and ordination of *Citrullus colocynthis* genotypes have been achieved based on diverse multivariate techniques such as PCA, MANOVA and DFA, as they were not applied commonly to date for the dissection of diversity in colocynth species. The objectives of the report aim to: (i) describing, analyzing and determining the morphological and agronomic variation in the selected farmer varieties collection; (ii) identifying the most discriminating characters that sort the genotypes into different groups; (iii) analyzing whether PCA, MANOVA and DFA outputs varied across different environments in *C. colocynthis*; (iv) appraising finally the suitability of the various multivariate techniques for classification of variation in the species *C. colocynthis*.

MATERIALS AND METHODS

Plant Materials: Forty accessions of *Citrullus colocynthis* were collected from farmers in the Southeastern of Benin Republic before the growing season of colocynth in 2013. They are named CC 1 to CC 40. This material was multiplied in Ko-Anagodo (in Ifangni Commune belonging to the Department of Plateau in Benin) during the small growing season from September 2013 to February 2014.

Study Sites and Experimental Design: The field experiments were carried out between the first weeks of September to the end of February at three locations in Southeastern Benin during then the growing season of the crop. Experiments were conducted for three consecutive years (2014-2017), namely in the locations Ko-Anagodo – 02?72'E, 06?67N - (Ifangni), Késsounou - 02?55E, 06?58N - (Dangbo commune) and Idiotchè - 02? 64E, 06? 73N - (Sakété Commune), respectively in the Plateau Department for Ko-Anagodo and Idiotchè locations and the Ouémé Department for Késsounou cited above. The soils at the three stations were well-drained sandy loams at all locations during the three years of experimentations. Experiments were laid out in a random complete block design with three replications at each location and every year. The experiment plots consisted of two rows, with each comprising five plants. The within and between rows were 2m respectively. Adjacent plots were also separated by 2m. One seed was sown per hole. Weeds were removed manually during the growing season. No irrigation was applied.

Traits Recorded: In total, 34 morpho-agronomic characters were recorded (Tables 1, 2). Data were measured on a plot basis. Eight (8) plants from the central rows were used to obtain the plot mean. Characters observed, their codes and the measurement procedures are presented in Tables 1 and 2. Among the traits recorded, 20 are quantitative and 14 qualitative. The qualitative characters were treated as quantitative since they showed continuous variation between the genotypes.

Statistical Analyses

Phenotypic Variation Estimates: Statistical analyses were performed using JMP 7.0 [69]. Data were classified relative to the experimental factors: genotype (G), location (L) and year (Y). For each trait xi, the variance components were estimated according to genotypes (σ^2_G), locations (σ^2_L), years (σ^2_Y) and the errors (σ^2_E) comprising the genotype × location interactions, the genotype × year interactions and the plot error. Variance components were performed using JMP 7.0 [69] and MINITAB 19 [70] and the following statistical model was applied.

$$Y_{ijkl} = \mu_i + g_{ij} + j_{ik} + l_{il} + \varepsilon_{ijkl}$$

where Y_{ijkl} represents the observed value of the ith trait of the jth genotype for the kth location and the lth year. µi is the trial mean of a given trait; $g_{ij,}$ j_{ik} lil are respectively the effects of genotypes, years and locations; ε_{ijkl} is the error, comprising the genotype x location interactions, the genotype × year interactions and the plot error.

Phenotypic variations were estimated using the following formula as in Zanklan *et al.* [1] in yam beans:

PV = σ^2_S / V_P, where σ^2_S is the sum of variances represented by the within and between-genotypes; PV is the phenotypic variation for a given trait, comprising experimental error as well as the Genotype × Environment interactions. The environment encompasses the years and locations; V_P is the phenotypic variance.

Moreover, phenotypic variance and average genetic diversity in the species and region studied were estimated using the Shannon-Weaver [71] diversity index (H') as applied by Zanklan *et al.* [1] and earlier Al Khanjari *et al.* [72]. The phenotypic frequency of alleles controlling each character was used. Differences among genotypes were appreciated by the Wilcoxon non-parametric test of JMP 7.0 software [69].

$$H = -\sum_{ii=1}^{n} Pi \ln Pi$$

where: n is the number of phenotypic classes for a character and P_i is the genotypic frequency or the proportion of the total number of entries in the ith class. H was further standardized by converting it to a relative phenotypic diversity index (H').

$$H' = -\frac{\sum_{i=1}^{n} Pi \ln Pi}{H \max}$$

with $H_{max} = log_e(n)$

Principal Component Analysis (PCA): PCA was performed using MINITAB 19 [70] and JMP 7.0 [69]. Spatial relationships and the importance of characters between observed traits were done by plotting the first and second principal components.

Multivariate Analysis of Variance (MANOVA): MANOVA was applied to distinguish between the genotypes. Analyses were based on different data sets consisting of the whole characters tested and the quantitative and qualitative traits separately. To test the power of discrimination of MANOVA across location and years, G × Y, G × L and G × Y × L interactions were estimated (G = genotypes, Y = years and L = locations). To determine the number of groups representing the optimal partition of genotypes, a multivariate analysis of variance was performed as in Zanklan *et al.* [1].

Table 1: Twenty observed colocynth quantitative characters, codes and measurement procedures

Characters	Codes	Measurement unit and procedures
Time to emergence	TE	No. of days from sowing to the time that plant emergence was 50% the plot
Tailspins	TT	N¤. of the days from time to emergence until 50% of initiation of tailspin on the plot
Male flowering	MF	Time counted at full flowering: on 8 plants on the plot
Female flowering	FF	Time counted at full flowering: on 8 plants on the plot
Fruit maturity	MT	N¤. of days from sowing to physiological maturity (90% of mature fruits on the plot)
Limb peduncle length	LPL	mm – measured at full flowering (6 peduncles per plant and on 8 plants)
Limb length	LLL	mm – measured at full flowering (6 leaves per plant and on 8 plants)
Limb width	LLW	mm – measured at full flowering (6 leaves per plant and on 8 plants)
Number of fruits per plant	FN	Counted at harvest: for 8 plants on the plot
Plant height	PH	m – measured at full flowering (8 plants per plot)
Internode length	IL	cm – measured at full flowering (8 plants on the plot)
Number of branches / node	NBN	Counted at full flowering for 8 plants on the plot
Fruit weight	FW	g – weighted at harvest (4 fruits per plant and on 8 plants per plot)
Fruit length	FL	cm - 4 fruits per plant and on 8 plants per plot
Fruit width	FWI	cm - 4 fruits per plant and on 8 plants per plot
Seed number / plant	SNP	Counted on 4 randomly chosen fruits per plot
Seed length	SL	mm – measured on 6 seeds per fruits and on 10 fruits per plot
Seed width	SWI	mm – measured on 6 seeds per fruits and on 10 fruits per plot
Seed tegument percentage	TP	% measured on thousand seeds weight and 10 times on plot basis
Thousand seeds weight	TSW	g – measured after harvest two times on probes of 100 seeds

Table 2: Fourteen observed colocynth qualitative characters, codes and measurement procedures

Characters	Codes	Measurement procedures
Leaf shape	LS	Scores from 1 to 3; 1=shape 1, 2=shape 2, 3=shape 3
Leaf size	LSi	Scores 5 and 7; 5=medium, 7=large
Leaf color	LC	Scores 3=light green; 5=green and 7=dark green
Leaf pubescence density	LPD	Scores 3=slight; 5=medium and 7=dense
Leaf pubescence texture	LPT	Scores 3=soft; 5=intermediate and 7=coarse
Internode length	ILq	Scores 3=short (=<5cm); 5= Intermediate (5-8cm)
Stem pubescence density	SPD	Scores 3=slight; 5=medium and 7=dense
Stem pubescence texture	SPT	Scores 3=soft; 5=intermediate and 7=coarse
Plant canopy coverage	PCC	Scores 3=poor; 5=fair and 7=good (vigorous)
Primary skin color	PSC	Scores 3=light green; 5=green; 7=dark green and 9=orange
Secondary skin color	SSC	Scores 3=no; 5=green and 7=dark green
Design produced by secondary color	DPSC	Scores 1=no; 3=stripes and 5=streaks
Female flower size	FFS	Scores 3=small (=>2cm); 5=intermediate (2-3cm) and 7=large (<3cm)
Male flower size	MFS	Scores 3=small (=>2cm); 5=intermediate (2-3cm) and 7=large (<3cm)

MANOVAs using Wilks' Lambda and Hotelling tests as well as Pillai's trace and Roys' Max root were performed with the raw data for all 37 variables studied with the MANOVA statement in JMP 7.0 [69]. As in Zanklan *et al*. [1], Lázaro-Nogal *et al.* [73] and Ukalska *et al*. [56], the following model was applied:

$$Y = 1_N m + XG + ZR + E$$

where: Y is the *(N x k)*-dimensional observation matrix with k, the number of response traits; 1_N, the *(N x 1)*-dimensional unit vector; *N*, the total number of not empty subclasses in the two-way data set; m is the *k*-dimensional vector of the general mean; X is the *(N x a)*-dimensional design matrix for genotypes; G is the *(a x k)*-dimensional matrix of the random genotypic effects; Z is the *(N x b)*-dimensional matrix for locations in years; R is the *(b x k)*-dimensional matrix of the random location (in a year) effects; and E is the *(N x k)*-dimensional matrix of residuals.

These estimates were calculated using the MANOVA statement of JMP 7.0 [69]. As in Zanklan *et al.* [1], a significant effect of a given genotype indicates genetically based phenotypic differences. The model was repeated with a mixed model applying restricted maximum likelihood (REML), testing for the fixed effects of locations and years and the random effects of genotypes and interactions.

Discriminant Function Analysis (DFA): DFA was performed in JMP 7.0 [69] to identify which variables best differentiate the genotypes. The correlation of each variable with each discriminant function based on the structure matrix was used to create the discriminant function. As in Zanklan *et al*. [1], these Pearson coefficients are structure coefficients or discriminant loadings and functions like factor loadings in factor analysis. By determining the largest loadings for each discriminant function, insights were gained into how to name each function. DFA was carried out with all 37 characters together and for the quantitative and the qualitative ones separately.

RESULTS

Phenotypic Variation: Variance component estimations (Tables 3 and 4) show, that for all characters, σ_G^2 is larger than σ_E^2 enclosing all kinds of interactions. These observations are equal for all 34 traits, both quantitative and qualitative. *C. colocynthis* farmer's varieties used in the present study appeared to be well differentiated from one another for all the 34 characters investigated, except for SWI (seed width) and TSW (thousand seeds weight), for which σ_G^2 is near 0.00%. Table 5 reports the trait variation for each quantitative attribute. Significant differences were observed between genotypes. For those quantitative characters, trait variation ranged from 12.44 (for seed width) to 139.35 (for limb peduncle length). High trait variation around or above 20% was observed in many characters such as male and female flowerings, maturity time, limb length and width, plant height, inflorescence length and fruit width and length. High differences above 50% were noted in many other traits such as time of emergence and tailspins. For many other characters, trait variation according to years and locations is over 25% (Table 5).

Phenotypic variation (PV) estimates among genotypes in quantitative characters ranged from 3.34 to 41.54% (Table 6). PV was high and above 10% for most traits (Table 6) except time to emergence, male and female flowerings, limb width, seed number per plant, seed tegument percentage and thousand seeds weight. For qualitative characters, PV was in general higher than PV for most of the quantitative traits (Table 7). It ranged from 25.23 (leaf size) to 62.22% (primary skin color). For most of the qualitative traits, PV presented values of around 30%. Nonetheless, the highest value for PV for qualitative traits was scored in primary skin color (62.22%) and the lowest in leaf size (25.23%).

Overall, forty *C. colocynthis* accessions studied about quantitative characters (Table 8), the mean Shannon–Weaver diversity index (H') value was highest for fruit width (2.26). In general, the diversity observed was very high with a notable Shannon-Weaver index also high and around 2.00, except for time to emergence (0.08), tailspins (0.23), limb peduncle length (0.43) and thousand seeds weight (0.32). No monomorphism (H'=0.00) was noted, even if the just above-mentioned cases are all around 0.00. The Shannon–Weaver diversity index was in general high and over 2.00 for most of the qualitative traits evaluated (Table 9). They are also for most of them higher than for all the quantitative characters observed. No monomorphism was noted as was the case for quantitative traits. The genetic diversity was then very high between genotypes evaluated, as indicated by H' (Tables 8, 9).

Estimation of the Genetic Diversity in *Citrullus colocynthis* Studied by Principal Component Analysis (PCA): The first ten principal components of the analysis explained 99.96% of the total variation. The first, second, third, fourth and fifth principal components accounted for 55.28, 39.93, 2.76, 0.89 and 0.50% of the total variation, respectively. The first component was highly and positively correlated with time to maturity (MT), male flowering (MF), female flowering (FF), time to tailspins (TT) and stem pubescence density (SPD). This principal component was negatively associated with secondary skin color (SSC), design produced by secondary color (DPSC), stem pubescence texture (SPT) and primary skin color (PSC). The second component was mainly determined by stem pubescence density (SPD), leaf color (LC), leaf shape (LSV, internode length) (ILq), leaf pubescence texture (LPT), plant canopy coverage (PCC), male flower size (MFS) and female flower size (FFS), as presented on Figure 1. Tables 10 and 11 show furthermore Pearson correlation coefficients between the 34 quantitative as well as qualitative variables and the five first components. PCA showed that the 40 genotypes are well distinguishable and separate one from another.

Multivariate Analysis of Variance (MANOVA): The behavior of the genotypes was the same regardless of the environment (years and locations) used, considering all the 20 quantitative and 14 qualitative variables simultaneously. The main effect of each factor (G, J or L) was then investigated separately as done by Zanklan *et al.* [1] in yam beans. For the factor G, significant differences were noticed between all genotypes.

Table 3: Variance components estimations of genotypes (σ_G^2) and the error (σ_E^2) including the genotype x environment interactions and plot errors for 20 morphological and agronomic quantitative traits in 40 *Citrullus colocynthis* entries

	Variance component estimates			Variance components estimates	
Traits	σ_G^2	σ_E^2	Traits	σ_G^2	σ_E^2
TE	5.778	1.879	IL	560.700	141.300
TT	5.510	1.567	NBN	0.6757	0.1714
MF	94.560	42.140	FW	5681.900	1104.900
FF	63.900	36.600	FL	178.942	31.001
MT	349.000	167.000	FWI	131.834	26.094
LPL	16513.000	3206.900	SNP	129.509	55.456
LLL	2224.700	558.490	SL	0.085	0.040
LLW	587.430	204.840	SWI	0.012	0.008
FN	35.580	11.115	TP	7.230	4.340
PH	14.5370	4.5702	TSW	0.083	0.080

Table 4: Variance components estimations of genotypes (σ_G^2) and the error (σ_E^2) including the genotype x environment interactions and plot errors for 14 morphological qualitative traits in 40 *Citrullus colocynthis* entries

	Variance components estimates			Variance components estimates	
Traits	σ_G^2	σ_E^2	Traits	σ_G^2	σ_E^2
LS	30.145	1.901	SPT	36.892	1.183
LSi	7.684	0.856	PCC	15.069	1.392
LC	34.923	-1.508	PSC	77.038	1.762
LPD	23.353	1.624	SSC	34.700	0.835
LPT	18.800	1.578	DPSC	28.915	1.483
ILq	9.418	0.659	FFS	22.645	1.067
SPD	37.502	0.998	MFS	23.107	0.998

Table 5: Percentage (%) of trait variation and significance levels for 20 quantitative morphological and agronomic characters in *Citrullus colocynthis*

Trait	Percentage of trait variation	Trait	Percentage of trait variation
TE	56.07***(a)	IL	18.40***
TT	56.73***	NBN	29.43***
MF	19.21***	FW	22.70***
FF	20.43***	FL	24.93***
MT	21.87***	FWI	24.88***
LPL	139.35***	SNP	33.92***
LLL	21.27***	SL	15.42***
LLW	18.26***	SWI	12.44***
FN	38.84***	TP	28.49***
PH	26.03***	TSW	47.24***

(a) Significant at 0.01

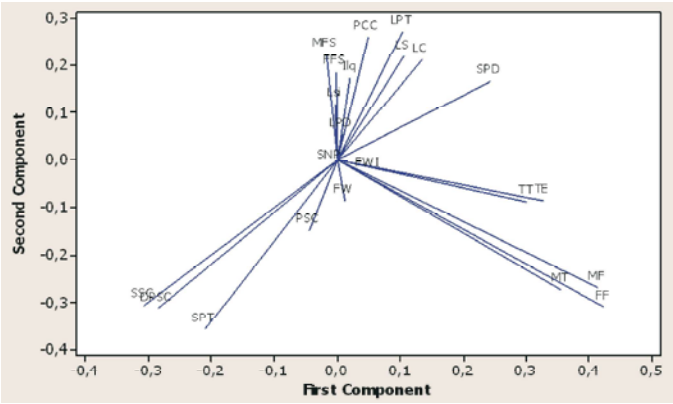

Fig. 1: Two-dimensional scatter plot for principal component analysis showing the relative contribution of each of the 34 variables to the power of components in *Citrullus colocynthis*

Table 6: Phenotypic variation (PV) between 40 *Citrullus colocynthis* genotypes evaluated at three locations during three years in Southeastern Benin for 20 morphological and agronomic quantitative traits

Traits	PV (%)	Traits	PV (%)
TE	9.31	IL	11.59
TT	10.90	NBN	12.84
MF	5.98	FW	15.91
FF	3.74	FL	17.84
MT	19.12	FWI	15.33
LPL	15.76	SNP	8.04
LLL	12.84	SL	41.54
LLW	9.62	SWI	27.00
FN	10.65	TP	3.34
PH	10.67	TSW	3.74

Table 7: Phenotypic variation (PV) between 40 *Citrullus colocynthis* genotypes evaluated at three locations during three years in Southeastern Benin for 14 morphological qualitative traits

Traits	PV (%)	Traits	PV (%)
LS	37.39	SPT	54.03
LSi	25.23	PCC	28.98
LC	46.61	PSC	62.22
LPD	35.15	SSC	61.03
LPT	30.99	DPSC	42.33
ILq	34.98	FFS	44.44
SPD	58.63	MFS	46.61

Table 8: Standardized Shannon-Weaver diversity index (H') between 40 *Citrullus colocynthis* genotypes for 20 morphological and agronomic quantitative traits

Traits	H'	Traits	H'
TE	0.08	IL	1.89
TT	0.23	NBN	1.92
MF	2.06	FW	1.91
FF	1.94	FL	1.99
MT	1.98	FWI	2.26
LPL	0.43	SNP	2.01
LLL	1.83	SL	1.90
LLW	1.60	SWI	1.91
FN	1.99	TP	1.54
PH	1.99	TSW	0.32

Table 9: Standardized Shannon-Weaver diversity index (H') between 40 *Citrullus colocynthis* genotypes for 14 morphological qualitative traits

Traits	H'	Traits	H'
LS	1.95	SPT	2.29
LSi	2.22	PCC	2.06
LC	2.29	PSC	2.26
LPD	1.47	SSC	2.11
LPT	2.23	DPSC	2.21
ILq	2.25	FFS	2.06
SPD	2.24	MFS	1.94

All genotypes react also in the same way since P = 0.001. Furthermore, the other multivariate contrasts were significant (Table 12). MANOVA against all 20 quantitative and 14 qualitative traits measured variables revealed then significant Wilks' Lambda (P = 0.001). MANOVA yielded also significant results with similar statistics (Pillai's trace, Hotelling-Lawley trace and Roys' Max root) (Table 12). Wilks' Lambda was transformed as an F approximation. Strong significant differences were detected among all genotypes. That fact suggested the need for discriminant analysis for centroid comparison between groups. All parallel statistical tests resulting from MANOVA (Hotelling-Lawley, Pillai's trace and Roys' Max root) were treated in the way as Wilks' Lambda for the F test.

The tested 34 morpho-agronomic traits could be efficiently utilized in further breeding programs. MANOVAs were conducted for sources of variation Year (Y), Location (L), Genotype (G), G × Y, G × L and G × Y × L in the full MANOVA, excepted for qualitative characters, where little variability is exhibited among genotypes (Table 12). In agreement with the PCA, MANOVA indicated that the main components of the total phenotypic variance were due to less than all the 34 characters evaluated across almost nine environments. A comparison of the 20 quantitative and 14 qualitative traits using MANOVA showed a significant difference between all the genotypes under investigation. The MANOVA applied to the 40 accessions studied, exhibited significant differences between the genotypes (Table 12).

Discriminant Function Analysis (DFA): Discriminant function analysis (DFA) carried out on the entire 34 morpho-agronomic characters scored with emphasis on traits recorded on different plant organs, years and locations (Fig. 2-4) showed that the cumulative variance explained by the first two canonical variates accounted for 100.00% of the total variance concerning both quantitative and qualitative traits. The first and second functions accounted for 99.62 and 0.37%, respectively. Responses of genotypes during the three years and based on the entire 34 as well as only the 20 quantitative traits are presented in Fig. 2. The variables that most contributed to canonical variates were metrical as well as visual descriptor traits as shown in Fig. 2 listing the standardized canonical discriminant function coefficients between the first two canonical scores of discriminant ordinations and 34 morphological and agronomic traits in *Citrullus colocynthis*. The first discriminant function was clearly positively correlated with 12 characters.

Table 10: Pearson correlation coefficients for the relationship between each of the first five principal components (PC) and each of 20 quantitative morphological and agronomic characters in 40 *Citrullus colocynthis* accessions

Traits	PC1	PC2	PC3	PC4	PC5
TE	0.200**	0.185**	-0.009	-0.082	0.037
TT	0.188**	0.205**	0.033	-0.064	0.008
MF	0.343**	0.067	0.015	-0.000	0.108*
FF	0.392**	0.043	0.039	-0.002	0.080
MT	0.419**	0.012	0.044	0.069	-0.000
LPL	-0.041	0.380**	0.247	0.129*	0.031
LLL	-0.015	-0.325**	-0.256**	-0.022	-0.043
LLW	0.069	-0.374**	-0.242**	-0.110	-0.015
FN	0.085	-0.068	-0.002	-0.044	0.074
PH	0.020	-0.310**	-0.132*	-0.067	0.072
IL	0, 224**	-0.298**	-0.212**	-0.103*	0.005
NBN	0.039	0.057	0.050	0.095	0.075
FW	0.078	-0.155*	-0.033	-0.078	0.030
FL	-0.047	0.092	0.036	0.193**	0.057
FWI	0.056	0.052	0.029	0.207**	-0.044
SNP	0.012	-0.029	0.001	0.067	0.047
SL	0.378**	0.003	-0.019	0.075	-0.086
SWI	0.364**	0.003	0.088	0.059	-0.064
TP	-0.345**	0.045	-0.050	-0.094	0.060
TSW	-0.035	-0.027	0.003	0.019	-0.026

*, ** Significant at p = 0.01 and 0.001, respectively

Table 11: Pearson correlation coefficients for the relationship between each of the first five principal components (PC) and each of 14 qualitative morphological characters in 40 *Citrullus colocynthis* accessions

Traits	PC1	PC2	PC3	PC4	PC5
LS	0.001	0.041	-0.275**	0.227**	0.448**
LSi	-0.025	0.067	-0.055	-0.008	0.064
LC	0.015	0.125**	-0.210**	-0.113*	-0.458**
LPD	-0.011	0.016	-0.045	0.008	-0.136*
LPT	0.009	0.084	-0.283**	0.375**	0.029
ILq	-0.020	0.037	-0.158*	0.201**	0.013
SPD	0.001	0.233	-0.185**	-0.275**	0.344**
SPT	0.002	-0.231**	0.340**	-0.107*	0.019
PCC	-0.023	0.045	-0.256**	0.229**	0.110*
PSC	-0.005	-0.070	0.124*	-0.175**	0.238**
SSC	-0.040	-0.295**	0.310**	0.364**	0.075
DPSC	-0.031	-0.246**	0.338**	0.315**	-0.013
FFS	-0.004	0.050	-0.129*	0.178*	-0.550**
MFS	-0.024	-0.015	-0.211**	0.375**	0.089

*, ** Significant at p = 0.01 and 0.001, respectively

Table 12: Results from the MANOVA analysis carried out on 40 accessions of *Citrullus colocynthis* in 34 traits observed for sources of variation (Year, Location, Genotype and their interactions)

Sources of variation	Wilks' λ test	F value	Hotelling-Lawley	F value	Pillai's trace	F value	Roy's Max root	F value
Year (Y)	6.785e-26	44.112***	1.137e-15	76.221***	2.046	21.243***	1.136e-15	18.001***
Location (L)	4.584e-28	9.789***	1.052e-14	10.566***	1.955	9.026***	7.745e-13	37.112***
Genotype (G)	3.364e-55	9.758***	2.532e-14	11.758***	4.011	9.416***	1.662e-4	40.011***
G × Y	1.057e-56	15.912***	3.342e-14	112.061***	3.990	11.600***	2.088e-14	51.411***
G (in Y1)[a]	0.742	1.334***	0.397	1.401***	0.263	1.267***	0.310	0.082***
G (in Y2)	0.175	11.565***	3.562	14.765***	1.025	8.795***	3.214	0.541***
G (in Y3)	0.347	5.795***	1.500	6.554***	0.755	5.072***	1.362	0.601***
G × L	2.962e-56	12.810***	3.836e-54	21.352***	3.986	11.638***	1.790e-14	9.439***
G (in L1)[b]	0.563	6.932***	0.666	6.930***	0.496	6.934***	0.364	7.606***
G (in L2)	0.934	0.355***	0.068	0.356***	0.066	0.354***	0.040	0.844***
G (in L3)	7.671e-8	1.259***	589.520	34.139***	8.431	0.688***	575.219	1153.644***
G × Y × L	1.431e-56	0.459***	5.165e-14	0.483***	4.004	0.438***	3.132e-14	2.391***

For each analysis, Wilk's ë, Hotelling-Lawley, Pillai's trace and Roy's Max root, F value and significance tests are estimated

*** Significant at the 0.001 probability level

[a] Y1 = Year 2014-2015, Y2 = Year 2015-2016, Y3= Year 2016-2017

[b] L1 = Ko-Anagodo, L2 = Késsounou, L3 = Idiotchè

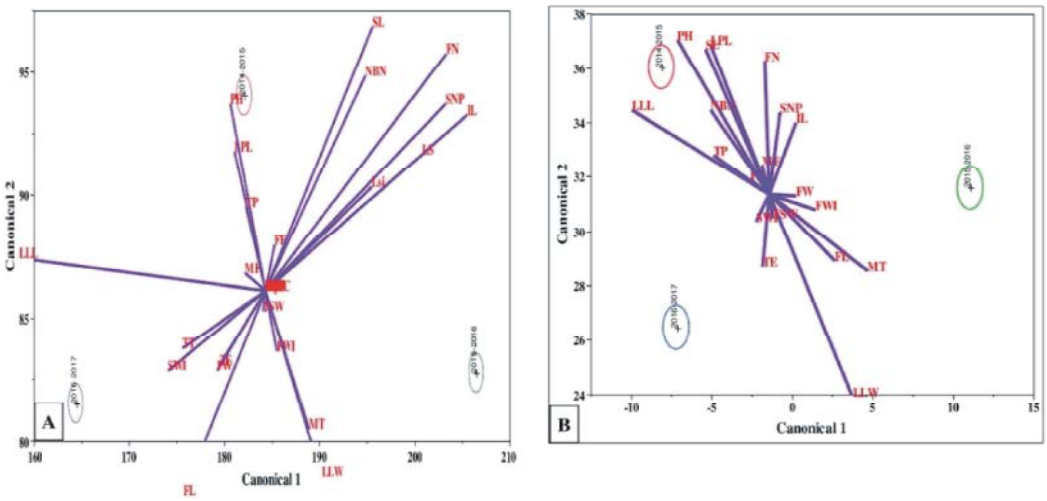

Fig. 2: Two-dimensional scatter plot for discriminant function analysis considering the factor year in *C. colocynthis*. A. On the basis of 34 quantitative and qualitative traits; B. On the basis of only 20 quantitative traits

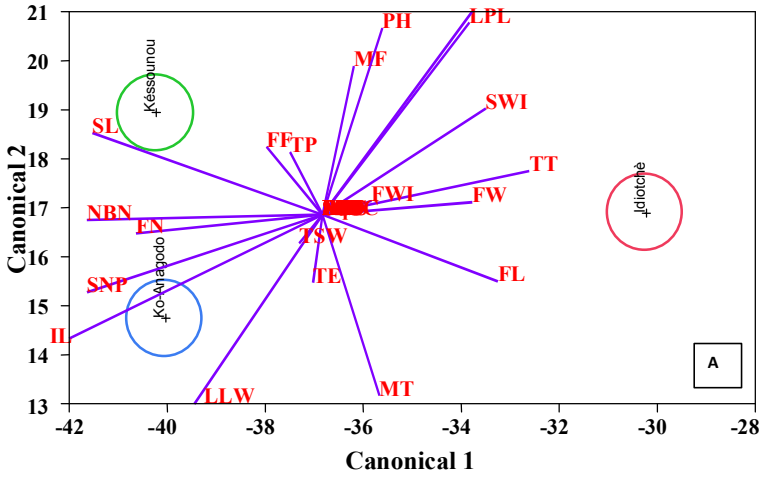

Fig. 3a: Two-dimentional scatter plot for discriminant function analysis considering the factor location in *C. colocynthis*. A: on the basis of 34 quantitative and qualitative traits recorded

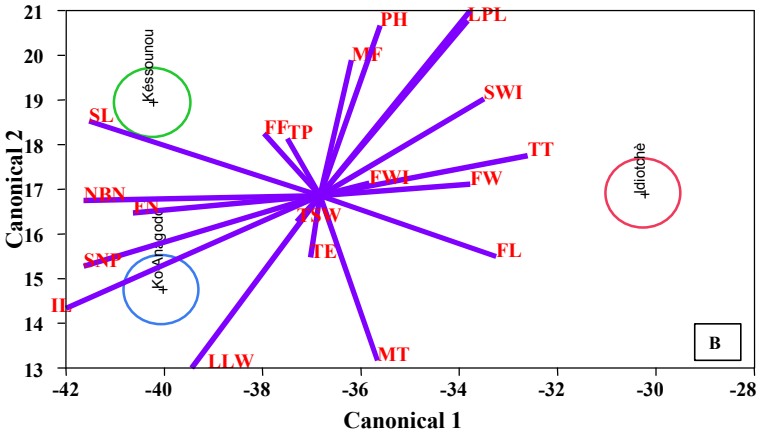

Fig. 3b: Two-dimensional scatter plot for discriminant function analysis considering the factor location in *Citrullus colocynthis*. B. On the basis of only 20 quantitative traits recorded

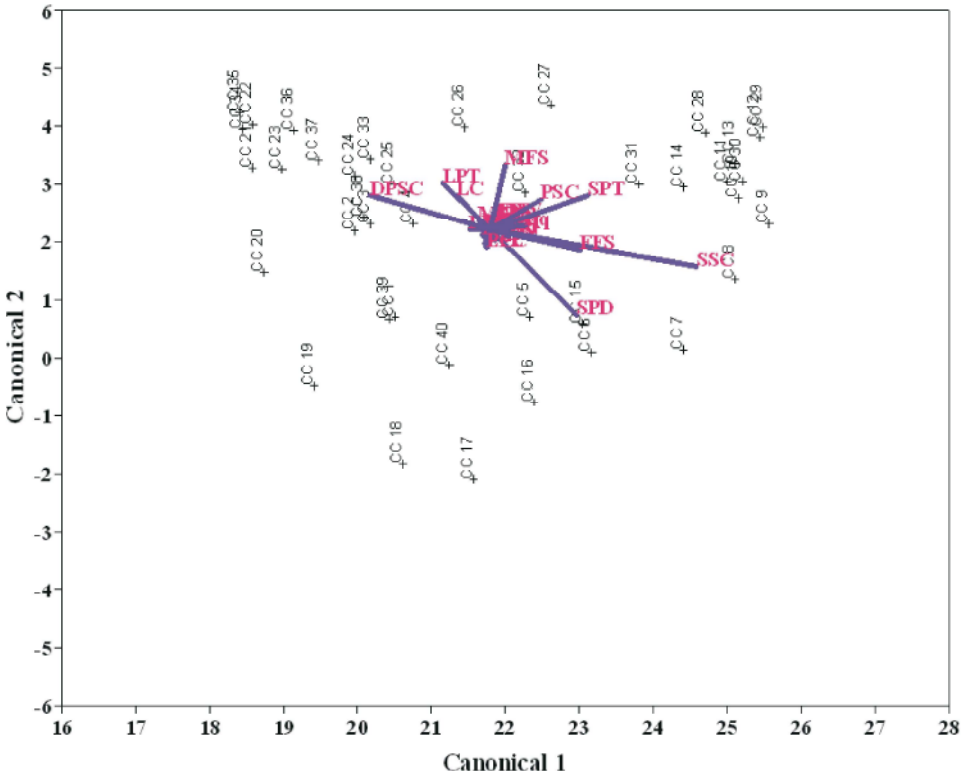

Fig. 4: Two-dimensional scatter plot for discriminant function analysis considering the factor genotype in *C. colocynthis*

There is no negative association with CAN1 (Fig. 2A). Examination of the second function suggested it was mainly associated with six characters (negative correlation) and positively linked to 13 further traits (Fig. 2A). The order in which the variables were included in the discriminant analysis indicates their relative importance in classifying genotypes within and among years of experimentation. The three years (2014-2015, 2015-2016 and 2016-2017) showed particular reactions of genotypes when compared with one another. Canonical analysis to find divergent trends of genotypes within and between years for the 20 quantitative characters resulted in two main variates that accounted together for 100.00% of the total variation in *C. colocynthis* (Fig. 2B). Reactions of genotypes were different as when all the 34 variables were used, as if qualitative characters were less necessary. Those first and second variates contributed to 99.62 and 0.37% of the variation, respectively. With regards to locations and behaviors of genotypes with emphasis on the total 34 variables (Fig. 3A), the first two canonical variates extracted from DFA were responsible for 100.00% of total variation (Fig. 3A). Canonical loadings showed that CAN1 was determined and dominated by traits presented in Fig. 3A. The first variate represented 75.61% of the total variation explained by DFA and was highly correlated to most of the original variables aforementioned ($P \leq 0.001$). The second variate explained 24.38% of the parameter variation between genotypes and was negatively correlated to most variables. DFA performed with a focus on only quantitative characters indicated that the first two variates demonstrated 100.00% of the total variation. CAN1 powered 76.05% and CAN2 explained 23.94% of the total variance. The original variables contributing to the variation observed are stressed in Fig. 3B. Canonical analysis to identify genotypic differences for the 34 characters examined resulted in ten variates that accounted together for 91.05% of the variability among genotypes inside given locations and years. The first five canonical variates described 72.41% of the total variation (Fig. 4). Distribution of genotypes through canonical axes 1 and 2 showed a conspicuous divergence between the groups formed by accessions within each location and year with high correlation to all traits (Fig. 4). Original variables with CAN1 contributed jointly to 31.64% of the variation, while CAN2 was demonstrated only 16.10% of the total variation (Figure 4).

DISCUSSION

Coefficients of variation provide a measure of diversity for quantitative traits [1, 74). Clear variability existed in the colocynth germplasm studied for all quantitative traits. Trait variation ranged from 12.34 (for SWI) to 139.35% (for LPL) considering all traits and genotypes evaluated. This indicated that genotypes belonging to *C. colocynthis* in the Southeastern of Benin Republic possess a high potential for fruit and seed yield production and their components. A remarkable diversity for most morphological and agronomic characters is shown at the intraspecific scale (Table 5). Understanding the mechanisms making some sites confer more variability to the germplasm would be desirable to plan collecting missions and to efficiently exploit the available genetic diversity in gene banks [1, 75]. Our current results indicate that there is a wide differentiation among accessions and lines within the species *C. colocynthis* under study, both for quantitative and qualitative traits that can be used to breed for higher seed yield. The grouping of similar genotypes relies on the dissimilarity among them, which can be determined by a phenotypic diversity index [1, 76, 96, 97]. The average diversity index was a lot variable among the 40 farmer's varieties evaluated, The Shannon–Weaver diversity index was calculated to compare the phenotypic diversity index (H') among traits and between groups [1]. A low H' indicates extremely unbalanced frequency classes for individual traits and a lack of diversity [1, 76]. Diversity estimates were performed for each trait and the 40 genotypes. H' was then pooled across traits and the genotypes of *C. colocynthis* (Tables 6 and 7). For qualitative traits, the 40 genotypes presented in general higher H' when compared to quantitative characters. Values of the H' index for each trait or averaged across all quantitative or qualitative characters were not correlated with environmental differences. The significant variation suggests differentiation of the species *C. colocynthis*, likely related to the selective pressures in the environments of origin, as concluded by Zanklan *et al*. [1] in yam beans. Mean adjustment of adaptive traits takes place in the long term according to the prevailing environmental conditions of the location of origin [1, 75, 77]. In the study presented here, from the relative importance of among-genotypes variation (Tables 6 and 7), such adjustment to a given environment may be realized [1]. A relatively high level of intraspecific variation, which is a primary factor of adaptation, can provide a buffering effect to the population to cope with unpredictable, seasonal climatic fluctuations [75, 96, 97]. Phenotypic variation estimated with Shannon–Weaver diversity index seemed to be unequal in importance for quantitative and qualitative characters (Tables 6, 7). This indicates the usefulness of qualitative variable types in studying genetic diversity in *C. colocynthis* and such variables should be of importance for rapid progress in breeding this crop. Our results, based on 34 morphological and agronomic characters (20 quantitative and 14 qualitative) and field trials at three locations and three years, showed a clear separation between the 40 genotypes evaluated.

The first five principal components explained about 99.73% of the total variation in all traits. The scatter plot between the first two components and the 34 variables is presented in Fig. 1. The first and second PCs explained respectively 55.28 and 39.93% of the total variation observed. This figure is high considering the 40 variables recorded in this study. Principal component analysis showed that, for the first principal component, all the entries (genotypes) had positive and negative scores with the variables. This reflects the fact that there is great genetic diversity in the material used and no selection success has been achieved by farmers for ages. There are divergences between genotypes against maturity time and yield potential. Such results were reported by Salimi *et al.* [98] in soybeans. A very large collection from farmers should be necessary to use the genetic diversity available for the breeding process in the species and the promotion of this valuable crop, a multipurpose one used traditionally by people in Africa, Asia and America. Besides the fact, that the survey presented here was conducted at three locations and three years to avoid the hard effects of the environment in the expression of traits, great correlations were shown between the genotypes as indications by the PCs (Fig. 1, Tables 10 and 11). No clear distinction among the genotypes used by PCs was observed, even though the trial was carried out in many locations and years. This indicates that there is a large amount of diversity in the crop considering the characters and then there is a need for powerful works of collection and evaluation to select high-yielding material to be utilized in breeding and agronomy.

Our results showed morpho-agronomic trait heterogeneity within the collection of 40 farmer varieties of colocynth evaluated. With MANOVA, differences among the 40 were highly significant (Table 12). The power of MANOVA declines with an increase in the number of response variables [1, 78]. To take into account these observations, we performed two sets of statistical analyses, with either with all 34 characters or the

quantitative only separately. Our results showed no significant difference with the methodology and no weakness of analysis was observed. Differences between single genotypes are based mainly on some traits (Tables 1 and 2). In all analyses involving the factors G (Genotype), L (Location) and Y (Year) including their interactions with other sources of variation, the phenotypic variance was distributed across all eigenvectors. In the full MANOVA, the primary root accounted for about 99.73% of the variance (source G) across years. Across locations, the primary root accounted for 87.35% (source G). Applying MANOVA to yam beans trial, Zanklan *et al*. [1] and tomato trial, Lounsbery *et al.* [79] reported minor rank changes among genotypes across different locations. These findings are consistent with the results presented here in colocynth. Our results indicate that genotypes of colocynth with favorable phenotypic trait expression in terms of yield (fruit and seed yields) and related other morpho-agronomic characters exist.

To classify accessions, a discriminant function analysis (DFA) was conducted using the entire set of 34 morpho-agronomic traits including 20 quantitative and 14 qualitative characters. Variables that have relatively high positive regression weights on a variate are positively inter-correlated as a group [1]. Similarly, those having high negative weights are also positively inter-correlated, but negatively with those showing positive weights. The magnitude of the weights indicates the relative contribution of the original variables to each canonical variate. The total amount of variability was explained by two to five canonical variates considering the factor levels genotype, year, location and their interactions. DFA revealed a clear separation between genotypes within the species. The discriminant function analysis based on the entire 34 traits explored and on the 20 quantitative characters identified correctly nearly 100% of genotypes within *C. colocynthis*. The two-dimensional plots (Fig. 2-4) obtained from the first two variates indicated the formation of distinct groups represented by each genotype of the species. We found no earlier morphometric studies in *C. colocynthis* conducted under field conditions (during three years and three locations per year) into that scale for comparison with the data analyzed in this report. Floral and other phenological traits exhibited clear differences among genotypes, but narrow variation within the species. Similar observations were made for leaf, stem and fruit characters. Among and within genotypes, floral and fruit variability was confirmed by DFA completing MANOVA results. Genotypes were separated into distinct groups by first canonical variate with almost all the variable sets investigated as shown in all graphics presented (Fig. 4). Results indicate that all traits made with greater contribution to the genetic diversity. DFA results demonstrate further that genotypes varied in their phenotype dependent upon the environment and the magnitude of that variation was very diverse depending on the trait, indicating the need for further research on stability analysis upon the most important agronomic traits since the results presented here are highlighting significant G × Y, G × L and G × Y × L interactions. Results from DFA in combination with those from MANOVA were more useful and powerful statistical tools than simple ANOVA because considering variables in combination as described earlier by Zanklan *et al.* [1] in yam beans and Lounsbery *et al.* [79] in tomato. With the DFA following the MANOVA, the complex interrelationships among dependent traits could not only be revealed but could also be taken into account in statistical inference, which is not done in a simple ANOVA

Phenotypic evaluation of germplasm is a fundamentally important step for the management of collection and determining genetic variation within accessions, which is crucial to the choice strategy to incorporate useful diversity into breeding programs and to facilitate the introgression of valuable genes into allied gene pools and finally, but not at least to understand the evolutionary relationships among accessions [1, 80, 81, 96, 97]. A more comprehensive assessment of genetic diversity would allow the better management of germplasm collection as well as better use in improvement.

Investigation on intra- and interspecific variation in colocynth for many traits at this scale using MVAs has not been undertaken to date. Zanklan *et al.* [1] reported for yam beans 76.4% of variability dissection from the first ten principal components resulted in PCA. The present study indicates the existence of genetic diversity with superior characteristics that could be used in diverse breeding programs. The results presented here demonstrate the congruity between the patterns of morpho-agronomic and quality characters along with genetic variation among genotypes of *Citrullus colocynthis*. In the study, all MVAs (PCA, MANOVA and DFA) separated the three species from one another. High genetic morphological variability has been described for many species or species complexes in crops [1, 82, 96, 97]. Results presented here using 20 scored quantitative and 14 qualitative traits with application of MVAs remain similar with earlier findings indicating clear divergence

($p \leq 0.001$) between yam bean genotypes regardless of their taxonomical inference [83, 84]. The extent and patterns of variation in morphology, agronomy, quality traits and the degree of overlap were quantitatively studied. Wide variability has been found in different quantitative and qualitative traits of the evaluated colocynth germplasm. A lot of genotypes possess high fruit and seed yields with quality suitable for use in diverse nutritional purposes. The information on diversity provides breeders with the ability to develop desirable types having high yields as well as better nutritional profiles. The reduction in the number of variables makes it easy to evaluate the performance of individuals or G, or treatments since it is often difficult to consider properly each response variable in one general index [85, 99]. Thus, it is preferable to have a few variables to be scored rather than a high number. Surprisingly, only one canonical variable accounted for nearly 90% of the total variation in all the intergenotypic levels. A good visualization of discrimination between species and genotypes (accessions) within and among the taxa examined is presented in scatter plots (Figs 1-4). The MANOVA and DFA were important in the study of morpho-agronomic and quality characteristics of the yam bean species [1]. They allowed the simultaneous analysis of the most important attributes of the crop. Moreover, they facilitated the distinctiveness of genotypes regardless of their taxonomic origin. Utilization of the multivariate techniques is therefore recommended in further studies in Cucurbitaceae breeding. The standardized canonical correlations with variables scored indicated similar trends with little changes when considering separately quantitative and qualitative traits.

Likewise, PCA, Cluster analysis as in Zanklan *et al*. [1], Olowe *et al.* [99] and Biswas *et al.* [100], MANOVA and DFA permitted a clear differentiation of accessions at the intraspecific level. Results from PCA, MANOVA and DFA indicated that all those techniques have excellent predictive power for distinguishing among genotypes whichever taxa they belong to. DFA still performed markedly better. However, though we cannot conclude that one method is better than the other since a judgment of these classification methods depends on two conditions: the completeness of the data and the objectives of the study. Our results showed that DFA was slightly better than the others at classifying and discriminating the 40 genotypes evaluated based on their morpho-agronomic expression at nine different environments in Benin, West Africa. Since our objective is to assign to those genotypes' specific variables, which are the most challenging to discriminate, DFA seems the more appropriate method, so far a clear separation of genotypes both within species was achieved with the application of that methodology regardless of the variables and variable sets used for performing analyses. Results from DFA also showed a range of possibilities to use diverse types of traits to discriminate between genotypes. Furthermore, it lets us forecast an easier implementation of future trials aimed at evaluation and discriminating and/or selecting faster parents as well as offspring from their hybridization using simple recordable characters such as qualitative ones, which are often well correlated to more complex attributes. As both human and natural selection factors affect morphological traits related to adaptation of populations [79, 86, 87], classification of genotypes in a given taxa using multiple agronomic traits identifies a special genotype and would improve its evaluation for potential adaptation [1, 86, 88]. In general, all parameters that we used stressed invariably the closer grouping of genotypes. Categorizing germplasm into morpho-agronomically similar and likely also genetically similar groups is useful for selecting parents for crossing programs [86, 88]. Crossing accessions belonging to different colocynth groups based on DFA could maximize opportunities for the introgression of valuable attributes. Hence, there is a high probability that distantly related genotypes would contribute unique desirable alleles at different loci [15, 89, 90, 91, 92].

Compared to PCA (99.73%), the discriminant function analysis accounted for nearly 100% of the within and among variance in the same number of axes (five axes). The discriminant analysis identified more clearly several variables to be used in subsequent studies. Albeit, a combination of all techniques would be appropriate for describing the variation in colocynth germplasm. Then, the categorization of the diversity among the genotypes into groups with similar characteristics can be used to design a collection strategy. Furthermore, the high level of variability exhibited by the genotypes, indicates that heterosis could be utilized to produce superior hybrids, which can be used to enhance crop production.

However, other MVAs should be also suitable alternatives to DFA whenever data sets are incomplete. Overall, DFA in combination with PCA, cluster and regression analysis, MANOVA as indicated can be a very powerful approach for classification analysis for two reasons. Firstly, all other MVAs apart from DFA can be used as a quantitative method for screening for candidate variables and complex interactions among variables before

parametric analysis. Secondly, cases can be classified according to the strengths of each method. In our analysis, DFA was slightly better at classifying colocynth genotypes within species, while the other approaches were slightly less good at classifying the variables about their predictive values and relative importance in distinguishing all genotypes. Generally, we assume that the choice of variables measured may have a strong impact on the success of a diversity study regardless of the statistical technique used. The best discriminating variables will differ among species and the identification of these may be challenging. Determining the variables that are closely associated with attributes of interest in colocynth will be beneficial therefore since only those desired traits are to be investigated.

CONCLUSIONS

The study presented here permits an easy understanding of the status of *Citrullus colocynthis* in Benin and West Africa. Morpho-agronomic characterizations allow to conclude that each genotype of *C. colocynthis* is heterogenous and the diversity is very high. The statistical analyses performed here were useful to identify a great variability among evaluated traits and to distinguish the most divergent variables from colocynth. This work provided an important contribution to morphological and agronomic characterization of polymorphic traits from different organs of colocynth, which can help breeders in the future in the direction of identifying elite genotypes to attempt breeding programs. The study allowed a better knowledge of the cultivated colocynth germplasm collection. Multivariate techniques demonstrated better significant intra- and intergenotypic differences for morpho-agronomic characterization Moreover, the report provides an important contribution to the characterization of polymorphic traits from different organs of the colocynth species. In conclusion, there is a need to fulfill the collection, evaluation and selection of genotypes, which can help breeders enhance and promote the crop regarding its potential.

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