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Research Article

Morphological Variation among and Discriminating Traits of Kersting's Groundnut Accessions

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Abstract

Kersting's groundnut *[Macrotyloma geocarpum*(Harms) Maréchal & Baudet] (KG) is a nutritious subterranean grain legume in West and Central Africa. Limited information is available on the morphological traits that discriminate accessions, limiting the use of appropriate breeding strategies. This study aimed to identify discriminating traits and assess diversity among accessions of Kersting's groundnut. Eighty-one KG accessions from Benin and Burkina Faso were evaluated based on 29 qualitative and quantitative traits. Experiment was conducted as an Alpha lattice design with three replications. Standardized Shannon-Weaver index (H') and descriptive statistics were calculated for qualitative traits. Pearson correlation coefficients, stepwise discriminant analysis, principal component analysis, cluster analysis and canonical discriminant analysis were conducted. Results showed that accessions varied greatly based on growth-habit (H'= 0.68), flower-color (H' = 0.50), seed-eye shape (H' = 0.47), and stem pigmentation (H' = 0.41). Eight quantitative traits, viz.,seed width, seed thickness, number of branches per plant, petiole length, days to 50% flowering, number of seeds per pod, pod-width, and pod-length, were found to significantly discriminate the accessions. Accessions were grouped into three clusters based on quantitative traits. Cluster 1 had accessions with late flowering and good vegetative growth, cluster 2 had accessions with high germination percentage and cluster 3 had accessions with high yield performance. Seed-length varied greatly among accessions, thus indicating the potential for improving yield via seed size.

Keywords: Descriptors; Diversity; Kersting's groundnut; *Macrotyloma geocarpum*; Orphan crops

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Introduction

Orphan crops, also known as forgotten or abandoned crops, traditional or underdeveloped crops [1], are crop species that have received only limited attention from researchers. However, most orphan crops are highly nutritious, climate resilient [2], and resistant to commonly occurring crop diseases [3]. Thus, orphan crops can potentially contribute toward food security and nutritional security, and should receive greater research attention.

Kersting's groundnut [*Macrotyloma geocarpum*(Harms) Maréchal & Baudet)] is a multipurpose legume crop grown in West Africa and Central Africa [4,5], where it is endemic. It is reportedly an orphan and underutilized crop species [6,7] that thrives well in semi-arid zones with annual rainfall below 600 mm [8]. Kersting's groundnut has high nutritional value; 21.3 g of protein per 100 g of grains [9]. The seed is a rich source of crude protein with high levels of essential amino acids, such as phenylalanine (3.2/100 g), histidine (2.1/100 g), lysine and methionine [9]. Seeds have high vitamin content [10]. According to Adazebra [11], Kersting's groundnut is one of the less-known leguminous crops, but it contributes significantly toward rural nutrition, livelihoods and sustainable development. Highly appreciated in urban areas of Benin, the crop has a high market value, it is the most expensive grain legume in West Africa, costing up to US $ 5-7 per kilogram [12]. Despite its nutritional and economic importance, Kersting's groundnut cultivation continues to decline in West African countries because of constraints, such as low yield, non-availability of improved varieties, poor storage ability of the grains, and high labor requirements for production [13]. It is not a priority crop for governments and researchers [7,11,14].

Agro-morphological characterization is a key step in assessing genetic diversity to describe and classify germplasm of cultivated plants [15,16]. In assessing agro-morphological diversity of cultivated plants, researchers use descriptors. Descriptors of Kersting's groundnut have not been described, unlike Bambara groundnut (*Vigna subterranea*(L.) Verdc.) [17], peanut (*Arachis hypogaea* L.) [18] and pigeonpea (*Cajanus cajan* (L.) Millsp.) [19]. Only a few studies have focused on morphological variations in Kersting's groundnut. Assogba et al., [14] and Akohoue et al., [20] in Benin and Adu-Gyamfi et al., [6] in Ghana and Bayorbor et al., [21] in Nigeria found significant variation for various traits among accessions.

The present study aimed at filling the above-mentioned gaps by i) assessing diversity among a regional germplasm collection obtained from Benin and Burkina Faso, and ii) identifying discriminating traits that could be included in a list of descriptors to be used for morphological characterization of kersting's groundnut.

Materials and methods

Study area

The study was carried out at a research station in the Regional Center of Agricultural Research (CRA-CF) in Djidja, village of Djegbatin (7°19'04.362" N and 1°54'58.914" E). The climate of Djidja is sub-equatorial and the rainfall is bimodal, with a tendency to a unimodal pattern. The soils are ferralitic, ferruginous and hydromorphic. Rainfall, temperature, sunshine, and relative humidity during the period of experimentation are presented in table 1.

Climatic parameter	August	September	October	November	December
Rainfall (mm)	82.5	183.2	138	44.2	0
Sunshine	141.3	160.9	201.1	250.6	243.7
Relative Humidy	80	79	78	73	63
Maximum temperature (°C)	30.3	31.2	32	33.9	35.2
Minimum temperature (°C)	22.4	22.8	23.2	23.8	23.2
Average temperature (°C)	26.4	27	27.6	28.9	29.2

Table 1: Monthly average climatic data recorded on study site during experiment.

Plant material and experimental design

Genetic material consisted of a collection of 81 accessions, of which 70 were from Benin and 11 from Burkina Faso (Table 2). Planting was done on 23rd August, 2018. The experiment was conducted as an Alpha lattice design, with 9 plots per block × 9 blocks and three replications. Each plot consisted of three rows, each 4.5 m in length. The rows were spaced 0.75 m apart. Plant-to-plant spacing was 0.30 m, giving a plant population of 44500 plants per hectare. Distance between plots was 1 m. One seed was sown per hill at a depth of 5 cm. No fertilizer was applied and weeding was done manually 3 weeks, 7 weeks and 12 weeks after sowing.

N°	AN†		Seed color		Origin
1	BUR 9		Black seed		Burkina-Faso
2	Akl 2		Cream seed		Benin
3	Agn2		Cream seed		Benin
4	Soh		Cream seed		Benin
5	Ats		Cream seed		Benin
6	Adc		Cream seed		Benin
7	Hhg		Cream seed		Benin
8	Hou		Cream seed		Benin
9	Viv		Cream seed		Benin
10	Dkp		Cream seed		Benin
11	BUR 13		Cream seed		Burkina-Faso
12	Dgo		Cream seed		Benin
13	BUR 14		Cream seed		Burkina-Faso
14	Odm 1		Cream seed		Benin
15	Aso		Cream seed		Benin
16	Sag 1		Cream seed		Benin
17	Gbo5		Cream seed		Benin
18	Gbn1		Cream seed		Benin
19	Odm 2		Cream seed		Benin
20	BUR 18		Black seed		Burkina-Faso
21	Lal3		Cream seed		Benin
22	BUR 10		Cream seed		Burkina-Faso
23	Kah 2		Cream seed		Benin
24	Gbo3		Cream seed		Benin
25	Gbo1		Cream seed		Benin
26	Itk 2		Cream seed		Benin
27	Akl 1		Cream seed		Benin
28	Kno 1		Cream seed		Benin
29	Lal2		Black seed		Benin
30	Tow1		Cream seed		Benin
31	BUR 15		Cream seed		Burkina-Faso
32	LeAd1		Cream seed		Benin
33	Itk		Cream seed		Benin
34	Kno 2		Cream seed		Benin
35	Add		Cream seed		Benin
36	Lam 1		Cream seed		Benin
37	Gbo4		Brown seed		Benin
38	Ena2		Cream seed		Benin
39	Hay 1		Cream seed		Benin
40	Agc		Cream seed		Benin
41	Bin		Cream seed		Benin
42	Oua		Cream seed		Benin
43	Sag 2		Cream seed		Benin
44	Zku		Cream seed		Benin
45	BUR 7		Cream seed		Burkina-Faso
46	Ena1		Cream seed		Benin
47	Gbn 2		Cream seed		Benin
48	Itk 1		Cream seed		Benin
49	BUR 8		Black seed		Burkina-Faso
50	Agn1		Cream seed		Benin
51	Zhla3		Cream seed		Benin
52	Tos		Cream seed		Benin
53	Lgz		Cream seed		Benin
54	Zhla1		Cream seed		Benin
55	Gta1		Cream seed		Benin
56	Dog		Cream seed		Benin
57	BUR 12		Cream seed		Burkina-Faso
58	Gbo2		Cream seed		Benin
59	Kin		Cream seed		Benin
60	TON		Cream seed		Benin
61	Dov		Cream seed		Benin

62	Kem		Cream seed		Benin
63	Adj		Cream seed		Benin
64	Zhla2		Black seed		Benin
65	Ali 1		Cream seed		Benin
66	Ako		Cream seed		Benin
67	Hay 2		Cream seed		Benin
68	BUR 16		Black seed		Burkina-Faso
69	LeAd2		Black seed		Benin
70	Kah 1		Cream seed		Benin
71	Zke		Cream seed		Benin
72	Dra		Cream seed		Benin
73	Bod		Cream seed		Benin
74	Agb		Cream seed		Benin
75	Sem2		Cream seed		Benin
76	MLK		Cream seed		Benin
77	Sem1		Cream seed		Benin
78	Atc		Cream seed		Benin
79	Fol		Cream seed		Benin
80	BUR 3		Cream seed		Burkina-Faso
81	Gta3		Cream seed		Benin

Table 2: Name, seed color and origin of Kersting's groundnut accessions used in this study.

Data collection and analysis

Seventeen quantitative traits [germination percentage, number of leaves per plant, number of flowers per plant, number of pods per plant, yield, number of branches per plant, days to 50% flowering, 100-seed weight, seed length, seed width, seed thickness, leaf length, leaf width, petiole length, pod length, pod width, and number of seeds per pod and 12 qualitative traits were evaluated (Table 3).

Qualitatives traits		Levels of qualitative traits
Growth habit		Erect
		Semi- erect
		Prostrate
Stem pigmentation		Present
		Absent
Terminal leaflet shape		Suborbicular
		Oblong-eliptic
Leaflet color		Green-dark
		Green-intermediate
Flower color		White tinged with purple
		Greenish white
Pod shape		narrow top
		large top
Pod color		White
		White tinged with purple
Pod texture		Many grooves
		A few groove
Easy Pod detachment		Yes
		No
Seed shape		Elongated
		Spherical
Seed color		Black
		Cream
		red-brown
Seed Eye shape		Triangular
		butterfly

Table 3: Qualitative morphological traits evaluated.

The traits measured were adapted from the lists of descriptors of closely related and similar subterranean legume species, such as Bambara groundnut [*Vigna subterranea* (L.) Verdc] and peanut (*Arachis hypogaea* L.), described in IPGRI et al., [17] and IBPGR and ICRISAT [18], respectively. Observations were made on 20 randomly selected plants within each plot. Standardized Shannon-Weaver index (H') was calculated for the qualitative traits [22,23]. For the quantitative traits, descriptive statistics (mean, standard deviation, minimum, maximum, coefficient of variation) were calculated.

Although studies have used the standardized Shannon-Weaver diversity index (H') for both quantitative and qualitative traits [22,23], this study used coefficient of variation for quantitative traits. In fact, the calculation of H' requires recording continuous data into a set of discrete categories (i.e., binning). However, evidence shows that binning often results in loss of information because of reduction in data points [24,25]. Besides, the choice of the cut-off point and the amplitude of the defined phenotypic classes are totally arbitrary and left to the discretion of the researcher, leading to difficulties in comparing results across studies.

Coefficient of Variation (CV) was computed as the ratio between standard deviation and the mean, multiplied by 100, which was used to assess the level of genetic variation in quantitative traits.

$$CV = \frac{s}{x} \times 100 \quad [26]$$

where *CV* = the Coefficient of Variation, *S* = the standard deviation and = the mean.

H' for qualitative traits was calculated in Microsoft Excel based on phenotypic frequencies of each trait to evaluate the variability among accessions using the following formula

$$H' = \left|\sum \left(\frac{n}{N}\right) \times \{\log 2\}\left(\frac{n}{N}\right) * (-1)\}\right| \Big/ \log_2 k \quad [23]$$

where *H'* is the standardized Shannon-Weaver diversity index, *k* is the number of phenotypic classes for a given qualitative trait, *n* is the frequency of the phenotypic class for each trait and *N* is the total number of observations.

Qualitative traits	Levels of variables	Accessions								
		Benin			Burkina			Collection		
		Frequency	Proportion	H'	Frequency	Proportion	H'	Frequency	Proportions	H'
Growth habit	erect	5	7.14	0.67	1	9.09	0.68	6	7.4	0.67
	prostrate	49	70		10	90.9		59	72.83	
	semi- erect	16	22.85		0	0		16	19.75	
Stem pigmentation	Present	5	7.14	0.40	7	63.63	0.41	12	14.81	0.40
	Absent	65	92.85		4	36.36		69	85.18	
Terminal leaflet form	Suborbicular	69	98.57	0.22	9	81.81	0.23	78	96.29	0.22
	Oblong-eliptic	1	1.42		2	18.18		3	3.70	
Leaflet color	Green-dark	68	97.14	0.22	10	90.9	0.23	78	96.29	0.22
	Green-intermediate	2	2.85		1	9.09		3	3.70	
Flower color	White tinted with purple	6	8.57	0.50	3	27.27	0.50	9	11.11	0.50
	Greenish white	64	91.42		8	72.72		72	88.88	
Pod shape	narrow top	68	97.14	0.22	10	90.90	0.23	78	96.29	0.22
	large top	2	2.85		1	9.09		3	3.70	
Pod color	White	66	94.28	0.28	11	100	0.28	77	95.06	0.28
	White tinted withe purple	4	5.71		0	0		4	4.93	
Pod texture	Many grooves	69	98.57	0.16	10	90.90	0.16	79	97.53	0.16
	A few grooves	1	1.42		1	9.09		2	2.46	
Easy Pod detachment	Yes	31	44.28	0.47	6	54.54	0.46	45	55.55	0.47
	No	39	55.71		5	45.45		36	44.44	
Seed shape	Elongated	67	95.71	0.22	11	100	0.23	78	96.29	0.22
	Spherical	3	4.28		0	0		3	3.7	
Seed coat color	Black	3	4.28	0.32	4	36,36	0.33	7	8.64	0.32
	Cream	66	94.28		7	63.63		73	90.12	
	Brown	1	1.42		0	0		1	1.23	
Seed Eye shape	Irregular	0	0	0.47	6	54.54	0.47	6	7.4	0.47
	Triangular	2	2.85		4	36.36		6	7.4	
	butterfly	68	97.14		1	9.09		69	85.18	

Table 4: Phenotypic variability observed in accessions based on the calculation of the Standardized Shannon-Weaver index (H').

Analysis of Variance (ANOVA) using a linear mixed model for alpha lattice designs was used to test for differences among accessions for quantitative traits. The linear mixed model used was as follows (genotypes were considered fixed effects and replications and blocks random effects): $Y_{ijk} = \mu + G_i + R_J + B_k + \varepsilon_{ijk}$ [27]

where Y_{ijk} = value of the observed quantitative trait; μ = population mean; = fixed effect of the i^{th} accession; R_J = effect of the j^{th} replicate (superblock); B_k = effect of the k^{th} incomplete block within the j^{th} replicate; and ε_{ijk} = experimental error.

Pearson's correlation was used to examine the relationship between yield and other quantitative traits. Further, quantitative trait data were subjected to stepwise discriminant analysis to determine the traits that best discriminated the accessions. Canonical discriminant analysis was performed to describe seed and flower color based on discriminating traits. Principal Component Analysis (PCA) was performed to determine the patterns of agro-morphological variation. Hierarchical classification was done to group the accessions. Thereafter, descriptive statistics and Analysis of Variance (ANOVA) were

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used to describe the clusters. All analyses were performed in R software 3.5.2 [28].

Results and Discussion

The standardized Shannon Weaver diversity index (H′) values ranged from 0.16 to 0.68 (Table 4). There was a high level of phenotypic variation among accessions for plant growth habit (H'=0.68) and flower color (H'=0.50). Moderate variation was observed for seed eye shape, easy pod detachment, stem pigmentation and seed coat color. Pod color, terminal leaflet shape, terminal leaflet color, pod shape and pod texture exhibited a relatively low level of variation (Table 4). Growth habit and flowers color could be used as key qualitative descriptors for Kersting's groundnut.

The majority (73%) of the accessions in the collection exhibited prostrate growth habit. This was insightful and could inform breeding strategies. For instance, Ndiang et al., [29] reported prostrate growth habit as a good yield predictor in Bambara groundnut, a subterranean legume crop similar to Kersting's groundnut. In addition, all accessions from Burkina Faso had elongated seed (Table 4), which could be used for yield improvement and selection for big seed.

Most accessions (88.88%) had greenish-white flowers (Figure 1a) and 11.11% had purple-tinted white flowers (Figure 1b). Similarly, 95.06% of the accessions had white pods (Figure 1c) and only 4.93% had white pods with purple tint (Figure 1d). Three colors of seed coat were observed among the germplasm collection. Most accessions (90.12%) had cream seed coat (Figure 1e), whereas 8.64% had black seed coat (Figure 1f) and 1.23% brown-red seeds (Figure 1g). This result could be explained by the fact that black-seeded and the brown-seeded accessions were rare and produced by a few households at a small scale [30].

Similarly, three variants were observed regarding seed eye shapes. Most accessions (85.18%) had triangular seed eyes (Figure 1i), whereas 7.40% had butterfly-like seed eyes (Figure 1h), and 7.40% had irregular seed eyes (Figure 1j).

Grain yield and yield components, such as number of pods, number of flowers and number of branches per plant, showed relatively high coefficients of variation (34.78% - 50.19%) (Table 5). This is consistent with findings from previous studies, which reported high and significant coefficients of variation for number of flower per plant of bambara groundnut [15] cowpea [31], and Kersting's groundnut [14]. These relatively high CV values are indicative of the existence of substantial diversity among accessions, offering opportunities for yield improvement in the crop. Similarly, the traits, such as number of pods per plant (CV = 50.19%), number of flowers per plant (CV = 49.51%), germination percentage (CV = 37.42%) and grain yield (CV = 57.84%), exhibited relatively high coefficients of variation, suggesting a wide dispersion around the mean for these traits. This is consistent with findings of previous studies on various leguminous crops, where high and significant coefficients of variation were reported for number of flowers per plant in Bambara groundnut [15], in cowpea [30] and Kersting's groundnut [14]. However, the study showed low variation for seed size (CV= 5.06 %, 4.63 %, and 5.37 % for seed length, width and thickness, respectively). This observation contrasts with the findings of Assogba et al., [14], who assessed diversity among 31 local Kersting's groundnut accessions and reported higher CV (14.97% and 15.83%) for seed length and width, respectively. Seed size is an important trait for Kersting's groundnut, since

Figure 1: 1a) greenish-white flowers, Fig 1b) purple-tinted white flowers, Fig 1c) white pod Fig 1d) purple tinted-white, Fig 1e) Cream coat seed, Fig 1f) Black coat seed, Fig 1g) brown coat seed, Fig 1h) butterfly shape eyes, Fig 1i) triangular eyes, Fig 1j) irregular eyes.

the tiny seeds make harvesting difficult and cause significant yield loss. In fact, harvesting of Kersting's groundnut is done by hand-picking pods and shelling consists of thrashing dry pods. Hand-picking of pods with tiny seeds is difficult, and the chances of leaving out many pods are high. In addition, tiny seeds lead to increased loss during shelling. The importance of seed size in Kersting's groundnut has been recognized in previous studies. For instance, Amujoyegbe et al., [32] reported small seed size to be one of the major causes of a decline in Kersting's groundnut production in Nigeria. Breeding for bigger seeds, in addition to improving yield, would remove women's drudgery when hand-picking and shelling Kersting's groundnut pods. Consistent with Assogba et al., [14] [100-seed weight, (10.70 to 14.71 g)] and Akohoue et al., [20] [100-seed weight (7.10 to 16.28 g)], the present study showed significant variation ($p < 0.001$) for 100-seed weight (8.14 to 18.64 g) (Table 5).

This could be explained by different experimental conditions (climatic and soil conditions), as reported by Khan et al., [33] that accumulation of reserves in seeds depended on the type of genotypes but also climatic factors. In fact, the present study was conducted on a well-watered fallow in the top Kersting's groundnut-producing area, known as the food basket of southern Benin. Nevertheless, investigations need to be pursued further, with multi-location trials to fully understand the determinants of yield variation in the crop.

Analysis of variance (ANOVA) performed on quantitative traits showed highly significant differences ($p < 0.001$) among accessions for seed thickness, percentage of germination, number of flowers per plant, number of days to 50% flowering, seed weight, petiole length and pod length (Table 6). Accessions differed significantly for number of branches per plant, leaflet length ($p < 0.01$), and leaflet width ($P<0.05$); other traits, *viz.*, number of pods per plant, pod width,

Descriptors†	Min	Max	Mean ± SE	CV
PG	6.98	90.7	46.37±1.11	37.42
nLe	21.29	132.9	65.75±1.27	30.05
nFlw	3	53.8	16.47±0.52	49.51
nPd	12	264.86	90.32±2.91	50.19
nBrh	2.3	20.8	9.51±0.21	34.78
nDFlw	46	56	48.83±0.12	3.93
SeWt(g)	8.14	18.64	12.92±0.1	11.56
SeL(mm)	6.91	9.69	8.21±0.03	5.06
SeW(mm)	4.74	6.42	5.68±0.02	4.63
Sth (mm)	3.33	4.92	4.26±0.01	5.37
LeL(mm)	52.71	77.96	65.75±0.26	6.19
LeW (mm)	38.03	57	48.66±0.23	7.37
PeL(mm)	81.04	208.83	143.38±1.32	14.41
PoL (mm)	6.45	17.45	12.32±0.11	13.96
PdW(mm)	3.62	9.16	7.72±0.04	8.08
nSP	1	1.73	1.3±0.01	13.67
yield (kg/ha)	239.47	9243.9	2466.89±91.54	57.84

Table 5: Minimum, maximum, mean and variation in traits of Kersting's groundnut accessions from Benin and Burkina Faso.

number of seeds per pod, number of leaves per plant and grain yield, did not show significant differences. This difference observed could be explained by genetic variation among the accessions, especially since all the accessions in this study did not have a single origin.

Mean squares of the 17 quantitative traits of Macrotyloma geocarpum				
	Sources of variation			
Variables†	Replication	Block	Accession	Residual
	Df=2	Df=8	Df=80	Df=152
nPd	3796.67 ns	2859.94 ns	3009.66 ns	3674.19
Sth	0.72 ***	0.01 ns	0.07 ***	0.03
PdW	0.85 ns	0.42 ns	0.45 ns	0.35
nSP	0.64 ***	0.02 ns	0.03 ns	0.02
nLe	11701.59 ***	892.41 ***	271.92 ns	224.76
LeW	342.11 ***	9.12 ns	12.67 *	8.22
PG	590.31 **	456.66 ***	615.9 ***	121.36
nDFlw	1546.21 ***	44.24 ns	84.04 ***	38.82
nBrh	85.67 ***	37.52 ***	11.22 **	6.83
nFlw	20.77 ***	5.38 **	6.03 ***	2.03
SeWt	10.94 ***	1.93 ns	3.86 ***	1.2
SeL	0.57 ***	0.11 **	0.34 ***	0.08
SeW	0.28 **	0.02 ns	0.12 ***	0.04
LeL	251.01 ***	17.98 ns	19.30 **	11.92
PeL	10870.68 ***	1145.13 ***	519.38 ***	220.83
PoL	19.70 ***	2.22 ns	4.06 ***	2.11
yield	122789.82 ns	165756.29 *	174349.26 ns	145845.5

Table 6: ANOVA of the 17 quantitative traits of kersting's groundnut.

The correlation analysis revealed strong relationships between some of the parameters assessed (Table 7). A positive correlation was observed between 100-seed weight and seed length (r = 0.68), leaflet length (r=0.38) and petiole length (r=0.42). This result corroborates the observations made by Gbaguidi et al., [34] on Bambara groundnut. The positive correlation between some of the traits can be exploited in indirect selection. For instance, the positive correlation between number of pods and yield (r=0.59), is an indication that elite plants can be selected based on visual assessment of pod number. On the other hand, moderate negative and significant correlation (r= -0.17) was found between 50% flowering and number of pods per plant (Table 7). These results corroborate results of Assogba et al., and Yadav et al., [14,35].

Stepwise Discriminant Analysis (SDA) performed on quantitative traits revealed 8 traits, *viz*., seed width, seed thickness, number of branches per plant, petiole length, days to 50% flowering, number of seeds per pod, pod width, and pod length, which discriminated the accessions (Table 8). These discriminating traits could be used as descriptors for describing Kersting's groundnut accessions. In fact, being under-researched, Kersting's groundnut does not have a list of described descriptors to be used for characterizations, unlike many well-studied crops, whose lists of descriptors for morphological traits have been developed and made available by IPGRI, Bioversity International, USDA, ICRISAT or other well-known or international agricultural research institutes. The eight discriminating traits that were identified constitute a starting point for the establishment of a list of descriptors for the crop.

Canonical Discriminant Analysis (CDA), performed to describe seed color of accessions based on discriminating traits, showed two axes that explained 100% of the variation, with the axis 1 capturing 96.6% of the variation (Figure 2). Seed thickness, number of branches per plant and days to 50% flowering were correlated with the first axis on the positive side, whereas seed width, petiole length, number of seeds per pod was on the negative side. Thus, axis 1 can be considered indicative of vegetative growth. Seed width, seed thickness, pod length and pod width were correlated with the second axis on the positive side, whereas number of branches per plant and petiole length were correlated with the second axis on the negative side. Most of these traits were related to yield. Thus, axis 2 can be considered a yield-components axis (Figure 2). Overall, black-seeded accessions had wide seed, long pods, a high number of seeds per pod and long petioles; brown seeds had high pod width, whereas cream-colored seed took more days to reach 50% flowering, and had higher number of branches and thicker seeds (Figure 2).

Overall, accessions with white flowers had thick seeds, whereas accessions with purple flowers had thin seed, and low seed length, leaflet width and petiole length (Figure 3). Thus, white-flower accessions exhibited higher performance for yield components and could be used as donor parents in breeding programs.

In total, significant morphological variation, beyond seed and flower colors, existed among accessions of Kersting's groundnut. However, the genetic nature of such variation can only be understood if molecular characterization using appropriate marker systems, such as Simple Sequence Repeat (SSR) or Single Nucleotide Polymorphism (SNP) is performed. To date, only one molecular diversity study has been reported using isozymes in this species and no diversity was observed [36]. This state of knowledge needs to be improved and the use of the Next Generation Sequencing Technology may help broaden our knowledge of the genetic diversity in the species.

Principal Component Analysis (PCA) revealed that the first three components had eigenvalues of >1.00 and accounted for 56.4 % of the total variability. The first Principal Component (PC1), which

Quantitative traits†	nPd	Sth	PdW	nSP	nLe	LeW	PG	nFlw	nBrh	nDFlw	SeWt	SeL	SeW	LeL	PeL	PoL
nPd																
Sth	0.10ns															
PdW	0.05ns	0.08ns														
nSP	0.02ns	-0.06ns	0.01ns													
nLe	0.08ns	0.02ns	0.10ns	0.11ns												
LeW	0.06ns	0.02ns	0.00ns	0.03ns	0.46***											
PG	0.05ns	0.24***	0.10ns	0.08ns	0.07ns	0.12ns										
nFlw	0.12ns	0.06ns	-0.04ns	0.31***	0.25***	0.21**	0.23***									
nBrh	0.00ns	0.08ns	-0.06ns	-0.04ns	0.75***	0.26***	-0.01ns	-0.07ns								
nDFlw	-0.17**	0-.18**	-0.08ns	-0.04ns	0.04ns	0.10ns	0.30***	0.27***	0.08ns							
SeWt	0.15*	0.41***	0.14*	0.08ns	-0.06ns	0.02ns	0.29***	0.34***	-0.14*	-0.42***						
SeL	0.12ns	0.22***	0.16*	0.14*	0.00ns	0.13*	0.31***	0.36***	-0.14*	-0.37***	0/.00.68***					
SeW	0.17**	0.39***	0.21**	0.07ns	0.08ns	0.28***	0.35***	0.28***	-0.04ns	-0.27***	0.66***	0.80***				
LeL	0.12ns	0.18**	-0.02ns	0.08ns	0.23***	0.76***	0.34***	0.43***	0.03ns	-0.26***	0.38***	0.43***	0.46***			
PeL	0.10ns	0.25***	0.00ns	0.06ns	0.30***	0.58***	0.50***	0.50***	0.13*	-0.41***	0.42***	0.47***	0.51***	0.77***		
PoL	0.08ns	0.03ns	0.59***	0.76***	-0.02ns	-0.02ns	0.18**	0.29***	-0.12ns	-0.19**	0.29***	0.34***	0.27***	0.12ns	0.12ns	
yield	0.59***	0.12ns	0.05ns	0.35***	0.17**	0.17**	0.07ns	0.30***	0.01ns	-0.17**	0.33***	0.23***	0.32***	0.31***	0.22***	0.38***

Table 7: Correlations between agronomic traits for 81 accessions of Kersting's groundnut.

Step	Traits†	Wilks. lambda	F.statistics. overall	p.value. overall	F.statistics. diff	p.value.diff	Can1	Can2
1	PG	0.319	4.31	0	4.31	0	0.31	-0.64
2	SeL	0.11	4	2.39E-26	3.72	0	0.69	-0.08
3	Sth	0.04	3.56	1.22E-32	2.81	0	-0.33	-0.43
4	SeW	0.01	3.47	4.15E-41	3.23	0	-0.27	0.29
5	nDFlw	0	3.11	1.36E-42	1.92	0	-0.26	0.6
6	SeWt	0	2.84	6.08E-43	1.69	0	0.45	-0.49
7	nBrh	0	2.6	7.80E-42	1.4	0.03	0.69	-0.34

Table 8: Summary of the stepwise discriminant analysis identifying quantitatives traits that differentiated of kersting's groundnut accessions and correlation between discriminating traits and the canonical axes.

explained 33.3% of the total variation, was positively associated with seed width, petiole length, leaflet length, germination percentage, pod length, seed length, number of flowers per plant and 100-seed weight; whereas days to 50% flowering was correlated with PC1 on the negative side. The first principal component (PC1) explained yield traits. The PC2 explained 13.8 % of the total variation and was positively correlated with number of branches per plant, number of leaves per plant and leaflet width. Seed thickness was correlated with the second PC on the negative side (Figure 4).

The second Principal Component (PC2) explained vegetative growth. The third PC accounted for 9.3 % of the total variation and was positively correlated with pod width and leaflet width; whereas yield and number of pods per plant were negatively correlated with the third principal component (Figure 5). The PCA showed that accessions with a high number of flowers also had long pods, long and heavy seeds, long leaflets and high 100-seed weight. Most of the accessions in that group were from Burkina Faso (BUR3, BUR7, BUR8, BUR9, BUR13, BUR14, BUR15, BUR16 and BUR18) and a few from Benin (Gbo4, LeAd2 and Zhla2) (Figure 4). Moreover, the accessions that had a high leaflet length also had high number of leaves and branches. Accessions falling into this category were from Benin (Zhla1, Ako and Zke) (Figure 5). In addition, accessions that had high seed thickness also had high leaflet width and high number of pods per plant (Figure 5). Such accessions were Odm2, Agn1, Aso, Kno2, Fol, and Tos, all from Benin.

The UPGMA (un weighted pair group method with arithmetic mean) dendrogram based on discriminating quantitative traits classified the accessions into three clusters (Figure 6).

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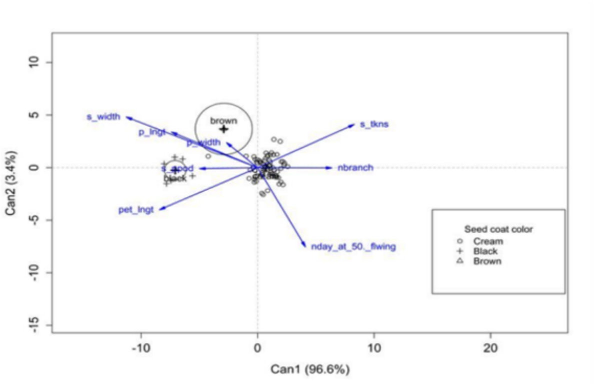

Figure 2: Projection of discriminating traits with seed coat color onto the canonical axes 1 and 2.

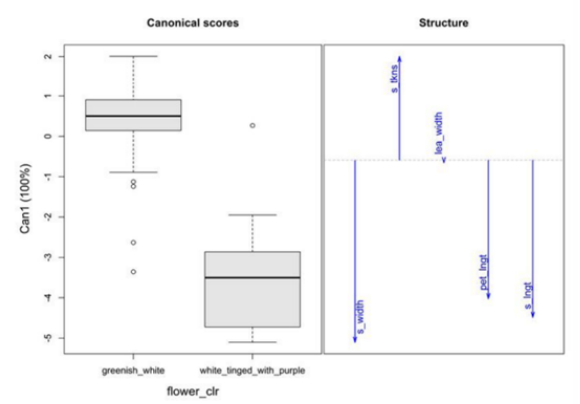

Figure 3: Boxplots showing relationship between flower color and quantitative traits.

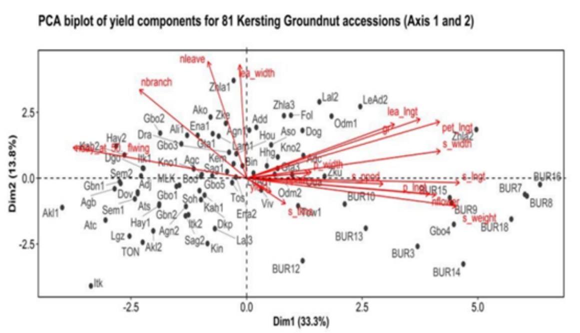

Figure 4: PCA biplot of quantitative trait for 81 Kersting's groundnut accessions (Axis 1 and 2).

The first, second and third clusters contained 68, 3 and 10 accessions, respectively. The first cluster (C1) was composed of high number of branches per plant (10.03 ± 0.23), high number of leaves per plant (67±1.3), high number of days to 50% flowering (49.26 ± 0.13) and wide leaflet width (49.03±0.21 mm). Accessions belonging to cluster C1 also had low 100-seed weight (12.54 ± 0.09 g), small seed length (8.11 ± 0.03 mm) and low number of flowers per plant

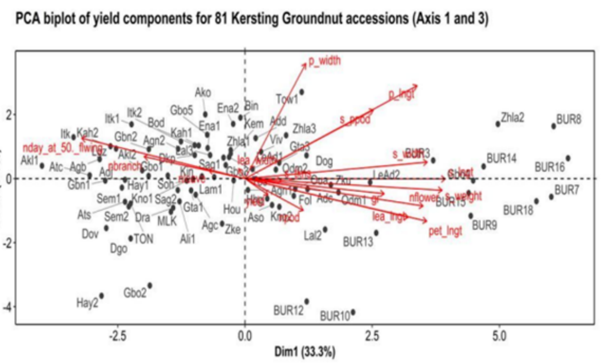

Figure 5: PCA biplot of quantitative trait for 81 Kersting's groundnut accessions (Axis 1 and 3).

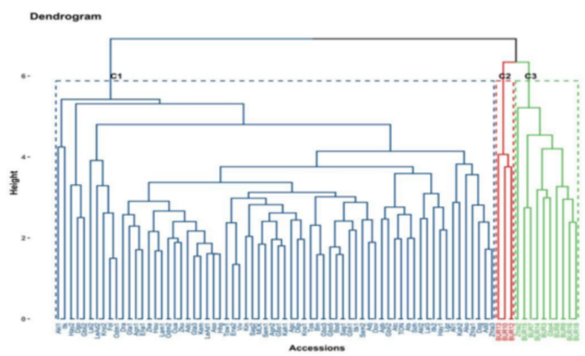

Figure 6: Hierarchical clustering of Kersting's groundnut accessions based on quantitative traits.

(14.7 ± 0.39) (Table 9). Overall, cluster C1 was characterized by accessions with high vegetative growth and late flowering. Cluster C2 was characterized by a significant ($p<0.001$) germination percentage (60.47 ± 4.72), high number of flowers per plant (25.8±0.6) and wide petiole length (165.82 ± 2.53 mm), and lower number of days to 50% flowering (46.11 ± 0.11). In addition, accessions in cluster 2 had medium number of branches per plant (7.57 ± 1.17) and medium seed length (8.30 ± 0.12 mm) (Table 9) compared to accessions in clusters 1 and 3. Cluster C3 was characterized by high 100-seed weight (15.18 ± 0.29g), high seed length (8.88 ± 0.12 mm), high pod width (7.94± 0.06), wide leaflet length (69.4 ± 0.71) and wide seed width (6 ± 0.06 mm) but low number of branches per plant (6.56 ± 0.37). Overall, cluster C3 was characterized by accessions with high performance for yield components (Table 9). In Benin, accessions with cream seed coat and eye color (pure cream) are preferred the most. Accessions in cluster 3 showed high performances for yield-related traits, such as seed weight, seed length and seed width. These accessions were Zhla 2, Gbo 4, BUR 3, BUR 7, BUR 8, BUR 9, BUR 14, BUR 16 and BUR 18, all having black or brown seed coat or black-eyed seeds; none of these accessions had pure cream color. Breeding efforts could therefore perform backcross between pure cream accessions and accessions from cluster 3 to obtain improved pure cream varieties with high yield performance.

Variables†	Cluster 1(n=68)				Cluster 2(n=3)				Cluster 3(n=10)				F-test
	Mean ±SE	CV(%)	Min	Max	Mean ±SE	CV(%)	Min	Max	Mean ±SE	CV(%)	Min	Max	
PdW	7.73±0.04a	4.52	6.27	8.28	6.84±0.36b	9.06	6.22	7.46	7.94±0.06a	2.39	7.72	8.39	***
nLe	67.53±1.30a	15.92	38.11	94.3	55.73±3.19b	9.92	49.37	59.33	56.61±2.92b	16.31	40	72.1	**
LeW	49.03±0.21a	3.53	44.88	53.25	43.76±0.88b	3.48	42.34	45.37	47.57±0.88a	5.87	43.08	53.06	***
PG	44.61±1.71a	31.66	20.93	79.84	60.47±4.72a	13.51	51.94	68.22	54.11±4.25a	24.82	37.21	78.29	*
nFlw	14.72±0.39b	22.12	9.03	23.25	25.8±2.06a	13.84	21.7	28.23	25.59±1.54a	19.03	17.63	33.67	***
nBrh	10.03±0.23a	18.58	6.31	15.2	7.57±1.70ab	38.95	4.17	9.4	6.56±0.37b	17.66	5.2	8.46	***
nDFlw	49.26±0.13a	2.21	47.33	53	46.11±0.11b	0.42	46	46.33	46.7±0.27b	1.79	46	48.33	***
SeWt	12.54±0.09b	5.71	11.06	13.87	14.03±0.19a	2.34	13.83	14.41	15.18±0.29a	6	13.63	16.73	***
SeL	8.11±0.03b	2.6	7.61	8.67	8.3±0.12ab	2.58	8.08	8.5	8.88 ± 0.12a	4.12	8.36	9.29	***
SeW	5.64±0.02b	2.83	5.31	6.16	5.58±0.06b	1.91	5.51	5.7	6 ±0.06a	3.07	5.72	6.29	***
LeL	65.21±0.26b	3.32	59.88	70.44	65.8±0.94b	2.46	64.56	67.64	69.4±0.71a	3.23	66.1	72.82	***
PeL	140.29±1.30b	7.65	110.42	165	165.82±2.53a	2.64	160.78	168.56	157.6±3.3a	6.62	140.41	175.8	***
PoL	12.05±0.11b	7.49	9.36	14.35	11.55±0.56b	8.36	10.73	12.61	14.41±0.25a	5.54	13.07	15.75	***

Table 9: Mean values and standard errors of discriminating traits in Kersting's groundnut accessions.

Conclusion

The evaluation of 81 KG accessions based on the 29 traits revealed high diversity, both for qualitative and quantitative traits. Three diversity groups were identified based on the quantitative traits. Three clusters were characterized by late flowering, good vegetative growth, high germination percentage and high yield performance. Besides, the study identified seed width, seed thickness, number of branches per plant, petiole length, days to 50% flowering, number of seeds per pod, pod width, and pod length as the quantitative traits that best discriminate the accessions. This could be a starting point for the establishment of a list of descriptors to be measured while studying the crop. Additional accessions should be investigated and molecular characterization studies be conducted to fully understand diversity patterns.

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Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author's Contribution

AEE, SFAK, HS and VR designed the study. CYG, AEE and HS conducted the experiments. CYG and SA actively participated in data collection. KK and SA analysed the data with support from AEE. HAT provided study site and technical guidance. CYG, AEE, KK, HS and SFAK developed the manuscript. VR, AS, AA, CJF, AAE and BS provided guidance throughout experiment, data collection and management, and manuscript development. All authors reviewed, improved the manuscript and agree to be accountable for the final manuscript.

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