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Research Article

Agro-Morphological Characterization of Kenyan Slender Leaf (*Crotalaria brevidens* and *C. ochroleuca*) Accessions

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Slender leaf (*Crotalaria spp*) is among the indigenous and underutilized vegetables in Kenya whose production is limited to the Western and Coastal regions of the country. For a long time, this crop has been neglected in terms of research and genetic improvement. There is therefore scanty information on its morphological diversity and agronomic performance, hence the need for this study. Field experiments were carried out for two seasons in October to December 2018 and March to May 2019. The experiments were laid out in Randomized Complete Block Design with 29 accessions and replicated three times. Both qualitative and quantitative data were recorded from the accessions based on the *Crotalaria* descriptors. Quantitative data were subjected to analysis of variance using XLSTAT Version 2019, and accession means were separated using Student's Newman Keuls test at 95% level of confidence. Both qualitative and quantitative data were subjected to multivariate cluster analysis, and a dendrogram was constructed using the unweighted pair-group method with arithmetic average. The principal component analysis was conducted to obtain information on the importance of the characters. Significant variation in agro-morphological traits was found within and between the two species. Cluster analysis grouped the accessions into seven major classes with a between-classes diversity of 75.13% and a within-classes diversity of 24.87%. This study sets the basis for genetic improvement of slender leaf in Kenya since the observed diversity can be exploited in selection for intraspecific and interspecific hybridization.

1. Introduction

Leafy vegetables contribute to additional taste and flavour as well as a source of protein, fibre, mineral, and vitamins, to human diet [1]. Slender leaf (*Crotalaria spp*) is one of the African indigenous leafy vegetables available in Kenya. The vegetable belongs to Fabaceae/Leguminosae family which comprise of annuals and short-lived perennials of agronomic importance [2]. Their largest centre of diversity is in Africa with approximately 500 species [3]. India is a secondary centre of origin, from where several species may have been dispersed to Australia and the East of the New World [4]. There are two edible species of slender leaf, *Crotolaria brevidens* and *C. orchroleuca*, grown and consumed in Kenya, Tanzania, and Uganda among the East African countries [5]. The local name depends on the location. It is known as "marejea" in Tanzania, "mitoo" in Kenya, and "alaju" in Uganda [6]. Slender leaf grows prolifically in the wild as weeds in many parts of Kenya but its domestication and cultivation as a food crop are limited only to the western and coastal regions of the country.

Most of the African indigenous leafy vegetables have been proven to have the potential to contribute to the dietary improvement and food security for small households and less privileged families [7]. Slender leaf is not exceptional. The consumed portions of the slender leaf include young leaves and shoots and contribute 100% of the daily dietary requirement for vitamin A, vitamin C, iron, calcium, and proteins [8]. It provides 29.6 mg/g of zinc, 0.2 mg/g of calcium, 9.9 mg/g of vitamins, 11.1 mg/g of iron, and 1.9 mg/g of proteins [9]. Slender leaf also has medicinal applications where it has been implicated in treating stomach-related ailments and malaria [10]. Furthermore, the crop has additional agronomic advantages like the ability to fix atmospheric nitrogen. It is also an important green manure legume due to high biomass production and with antagonistic effect on parasitic nematodes [11]. The slender leaf is, therefore, among the promising indigenous vegetables in Kenya whose potential is yet to be fully utilized.

Despite the high economic value of slender leaf, it has been for a long time neglected by researchers, farmers, and other stakeholders, who have concentrated on exotic and already improved popular vegetables [1]. To realize the agronomic potential and commercial viability of this high-value vegetable, there is a need to develop its agronomic package, improve its leaf yields, and promote the production of quality seeds. This can only be achieved through intensive domestication, breeding, and selection. However, the success of every breeding programme relies on the level of genetic variability of the available germplasm [12, 13] which forms the basis for selection. For the slender leaf, there is scanty information available on its genetic and agronomic attributes. This knowledge needs to be harnessed to enable genetic and agronomic improvement of the crop. Therefore, this study assessed the agro-morphological diversity of the two consumed species of slender leaf available in Kenya.

2. Materials and Methods

2.1. Description of Study Site. Field experiments were set up at the University of Embu research farm for two seasons in October to December 2018 and March to May 2019. The University of Embu lies at 0° 35′ 25″ S and 37° 25′ 31″ E with an elevation of 1494 m above sea level. It lies within the upper midland 2 agro-ecological zone. The area receives an annual rainfall of up to 1230 mm. The rainfall pattern is bimodal with long rains falling between March and June and short rains between October and December [14]. The mean annual temperature is 19.5°C with a maximum temperature of 25°C and a minimum of 14.1°C [14]. The major soils are Humic Nitisols which are derived from basic volcanic rocks. These soils are deep, highly weathered with friable clay texture and moderate-to-high inherent fertility [15].

2.2. Collection of the Test Accessions. The seeds of thirteen accessions of *C. brevidens* and sixteen *C. ochroleuca* were collected from farmer's seed stock from nine Kenyan Counties, namely, Kakamega, Bungoma, Vihiga, Busia, Siaya, Homabay, Kisumu, Migori, and Kisii (Figure 1). Geographical coordinates of the various collection points are presented in Table 1.

2.3. Experiment Layout and Design. Field experiments were laid out in Randomized Complete Block Design (RCBD) with the 29 accessions as the treatments and replicated three times. Each treatment/accession was allocated a plot measuring 2 m × 2 m with a 0.5 m alley between plots. The seeds were directly sown through drilling in rows spaced 30 cm apart and then covered lightly with soil. Thinning was done four weeks after sowing to attain a spacing of 30 cm × 15 cm [16]. The experiments were purely rain-fed, and no fertilizer was applied. Manual weeding was carried out when necessary to maintain weed-free plots.

2.4. Data Collection. Twenty quantitative and qualitative morphological traits were used to estimate the level of variation among the accessions based on *Crotalaria* descriptors adopted from Raj and Britto [17] with some slight modifications. The qualitative traits that were recorded include leaf type, leaf colour, stem colour, hairiness of stem, flower colour, dry pod colour, and seed colour. The quantitative data were collected on growth and agronomic traits including plant height, number of branches, petiole length, leaf length, leaf width, days to 50% flowering, days to first mature pod, pod length, pod width, number of pods per plant, number of seeds per pod, and weight of 1000 seeds. The leaf area was estimated at maximum leaf expansion using the formula $A_L = 0.73\ (L_L \times W_L)$ adopted from Musyimi et al. [18], where A_L is the leaf area, L_L is the leaf width, and W_L is the maximum the leaf width.

2.5. Data Analysis. Qualitative and quantitative data were organized into a matrix and subjected to cluster analysis using XLSTAT Version 2019. Estimates of similarity among the morphotypes were calculated using Euclidean genetic distance, and dissimilarity indices were used to generate a dendrogram using the unweighted pair-group method with arithmetic average (UPGMA). Principal component analysis (PCA) was conducted to get information on the importance of the characters. A two-way analysis of variance (ANOVA) was used to analyze the quantitative data using XLSTAT Version 2019 to obtain the accessional variations, seasonal variations, and interactions between accessions and seasons. The means were then separated using Student's Newman Keuls (SNK) test at 95% level of confidence. Pearson's correlation coefficients were performed to compare the degree of association between quantitative traits.

3. Results

3.1. Variation in Qualitative Characters. A summary of the qualitative characters of slender leaf accessions that were studied is shown in Table 2. Out of the seven qualitative traits that were studied, only three were diverse among the accessions. The three variable qualitative traits were leaf type, flower colour, and seed colour. Accessions were found to be uniform for the rest of the traits, namely, stem colour, stem hairiness, leaf colour, and dry pod colour All the 29 accessions were glabrous (not hairy) with green-coloured stem and leaf. All the accessions also developed black dry pods at

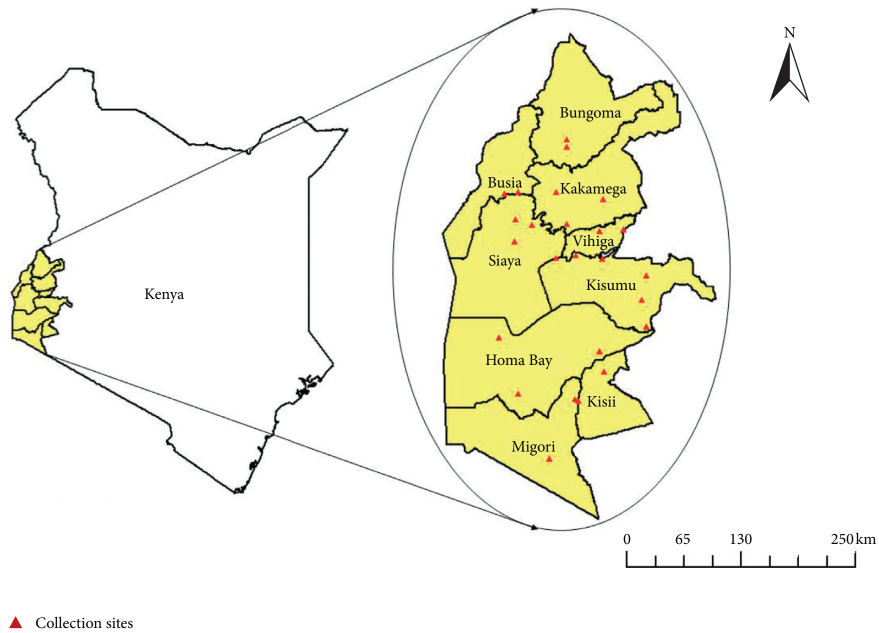

Figure 1: Map of Kenya showing the sampled counties and the collection points.

Table 1: Twenty-nine slender leaf accessions collected from nine counties in Kenya.

S/no.	Accession code	County	Species	Altitude (m.a.s.l.)	GPS coordinates
1	BHM198	Homabay	*C. brevidens*	1080	−0.4367°S, 34.2060°E
2	BHM213	Homabay	*C. brevidens*	1198	−0.5108°S, 34.7351°E
3	BKK091	Kakamega	*C. brevidens*	1103	0.5999°S, 34.5627°E
4	BKK129	Kakamega	*C. brevidens*	1483	0.1561°S, 34.5627°E
5	BKS220	Kisii	*C. brevidens*	1525	−0.7709°S, 34.6242°E
6	BKM197	Kisumu	*C. brevidens*	1641	−0.3819°S, 34.9791°E
7	BKM203	Kisumu	*C. brevidens*	1170	−0.1097°S, 34.9792°E
8	BMG234	Migori	*C. brevidens*	1366	−1.0693°S, 34.4694°E
9	BMG239	Migori	*C. brevidens*	1481	−0.7573°S, 34.6027°E
10	BSY111	Siaya	*C. brevidens*	1310	0.0650°S, 34.2884°E
11	BSY113	Siaya	*C. brevidens*	1321	0.1552°S, 34.3799°E
12	BVG001	Vihiga	*C. brevidens*	1790	0.1282°S, 34.8610°E
13	BVG004	Vihiga	*C. brevidens*	1608	0.1179°S, 34.7331°E
14	MBG086	Bungoma	*C. ochroleuca*	1427	0.5611°S, 34.5605°E
15	MBS064	Busia	*C. ochroleuca*	1302	0.3157°S, 34.2346°E
16	MBS065	Busia	*C. ochroleuca*	1290	0.3263°S, 34.3063°E
17	MHM207	Homabay	*C. ochroleuca*	1364	−0.7296°S, 34.3074°E
18	MHM215	Homabay	*C. ochroleuca*	1421	−0.5108°S, 34.7351°E
19	MKK011	Kakamega	*C. ochroleuca*	1338	0.3259°S, 34.5036°E
20	MKS221	Kisii	*C. ochroleuca*	1526	−0.7695°S, 34.6239°E
21	MKS226	Kisii	*C. ochroleuca*	1556	−0.6137°S, 34.7568°E
22	MKM204	Kisumu	*C. ochroleuca*	1152	−0.2414°S, 34.9580°E
23	MKM218	Kisumu	*C. ochroleuca*	1560	−0.0045°S, 34.6115°E
24	MMG029	Migori	*C. ochroleuca*	1561	0.2864°S, 34.7547°E
25	MMG234	Migori	*C. ochroleuca*	1366	−1.0693°S, 34.4694°E
26	MSY110	Siaya	*C. ochroleuca*	1251	0.1805°S, 34.2952°E
27	MSY216	Siaya	*C. ochroleuca*	1373	−0.0210°S, 34.5031°E
28	MVG003	Vihiga	*C. ochroleuca*	1608	0.1179°S, 34.7331°E
29	MVG125	Vihiga	*C. ochroleuca*	1528	−0.0245°S, 34.7474°E

maturity but with variable seed colour ranging from pale yellow (45%), reddish (21%), and black (3%). The rest of the accessions had a mixture of two or three seed colours with 21% having a mixture of a pale yellow and reddish colour and 10% having a mixture of reddish, black, and pale yellow colours. Three leaf types were identified among the

Table 2: Variation in qualitative characters among the 29 slender leaf accessions.

S/no.	Accession code	Stem hairiness	Stem colour	Leaf type	Leaf colour	Flower colour	Dry pod colour	Seed colour
1	BHM198	Absent	Green	Ovate	Green	Cream-yellow	Black	Mixture 1
2	BHM213	Absent	Green	Lanceolate	Green	Yellow	Black	Pale yellow
3	BKK091	Absent	Green	Lanceolate	Green	Yellow	Black	Pale yellow
4	BKK129	Absent	Green	Lanceolate	Green	Yellow	Black	Mixture 1
5	BKS220	Absent	Green	Ovate	Green	Cream-yellow	Black	Pale yellow
6	BKM197	Absent	Green	Lanceolate	Green	Yellow	Black	Mixture 2
7	BKM203	Absent	Green	Lanceolate	Green	Yellow	Black	Pale yellow
8	BMG234	Absent	Green	Lanceolate	Green	Yellow	Black	Pale yellow
9	BMG239	Absent	Green	Lanceolate	Green	Yellow	Black	Mixture 1
10	BSY111	Absent	Green	Ovate	Green	Yellow	Black	Pale yellow
11	BSY113	Absent	Green	Linear	Green	Yellow	Black	Mixture 1
12	BVG001	Absent	Green	Lanceolate	Green	Yellow	Black	Pale yellow
13	BVG004	Absent	Green	Linear	Green	Yellow	Black	Black
14	MBG086	Absent	Green	Linear	Green	Yellow	Black	Pale yellow
15	MBS064	Absent	Green	Lanceolate	Green	Yellow	Black	Pale yellow
16	MBS065	Absent	Green	Lanceolate	Green	Cream-yellow	Black	Mixture 1
17	MHM207	Absent	Green	Ovate	Green	Yellow	Black	Reddish
18	MHM215	Absent	Green	Linear	Green	Cream-yellow	Black	Mixture 2
19	MKK011	Absent	Green	Linear	Green	Cream-yellow	Black	Reddish
20	MKS221	Absent	Green	Lanceolate	Green	Cream-yellow	Black	Mixture 1
21	MKS226	Absent	Green	Lanceolate	Green	Cream-yellow	Black	Reddish
22	MKM204	Absent	Green	Linear	Green	Cream-yellow	Black	Pale yellow
23	MKM218	Absent	Green	Ovate	Green	Yellow	Black	Reddish
24	MMG029	Absent	Green	Ovate	Green	Cream-yellow	Black	Pale yellow
25	MMG234	Absent	Green	Ovate	Green	Cream-yellow	Black	Mixture 2
26	MSY110	Absent	Green	Ovate	Green	Cream-yellow	Black	Pale yellow
27	MSY216	Absent	Green	Lanceolate	Green	Yellow	Black	Pale yellow
28	MVG003	Absent	Green	Ovate	Green	Yellow	Black	Reddish
29	MVG125	Absent	Green	Ovate	Green	Cream-yellow	Black	Reddish

Mixture 1 = pale yellow and reddish; Mixture 2 = pale yellow, reddish, and black; the accessions whose code starts with letter B belongs to *C. brevidens* while those whose code starts with letter M belongs to *C. ochroleuca*.

accessions with most accessions (45%) having the lanceolate type, 34% having the ovate type, and 20% linear leaf type. A majority (62%) of the *C. brevidens* accessions had lanceolate leaf type while *C. ochroleuca* had more variable leaf type comprising of 44% ovate, 31% lanceolate, and 25% linear. Most of the accessions (59%) had bright yellow flower corolla out of which 38% belonged to *C. brevidens* while 21% belonged to *C. ochroleuca*. The accessions with creamish yellow corolla comprised of 41% out of which 34% belonged to *C. ochroleuca* while 7% belonged to *C. brevidens*.

3.2. Variation in Quantitative and Agronomic Variables. Significant variations ($p < 0.05$) between accessions were observed for leaf area index ($p < 0.05$), pod width ($p < 0.01$), and weight of 1000 seeds ($p < 0.05$). The rest of the quantitative variables were not significantly different ($p > 0.05$) (Table 3). Leaf area index ranged from 12.54 in accession MKS221 from Kisii to 7.17 in accession BSY0113 from Siaya. Accession MMGR234 from Migori recorded the biggest pod width of 2.2 cm while BVG001 from Vihiga had the smallest pod width of 0.04 cm. The accessions were also found to vary significantly in weight of 1000 seeds with BKM203 from Kisumu recording the highest weight of 7.842 g while BKM197 from 5.447 Kisumu had the lowest seed weight. There were relatively higher variations in agronomic variables as compared to the quantitative genetic variables. The accessions recorded highly significant ($p < 0.001$) differences in days to 50% flowering which ranged from 64 to 74 days. There were also highly significant ($p < 0.001$) variations between accessions for days to maturity which ranged from 95 to 117 days.

3.3. Pearson Correlations between Quantitative Variables. There were significant ($p < 0.05$) and positive correlations between several quantitative variables (Table 4). Plant height was found to be significantly correlated to leaf width ($p < 0.040$), pod width ($p < 0.002$), pod length ($p < 0.0001$), days to 50% flowering ($p < 0.002$), and days to maturity ($p < 0.004$). The leaf characteristics were found to have significant correlations among them with petiole length correlating highly with leaf length ($p < 0.0001$) which in turn correlated significantly with leaf width ($p < 0.002$) and leaf area ($p < 0.0001$). The leaf length and leaf area were also significantly correlated to days to 50% flowering ($p < 0.03$). Significant correlations were also observed between pod width and pod length ($p < 0.0001$), the number of seeds per pod ($p < 0.002$), days to flowering ($p < 0.002$), and days to maturity ($p < 0.0001$). The pod length was also positively correlated to the number of seeds per pod ($p < 0.006$), days to 50% flowering ($p < 0.0001$), and days to maturity ($p < 0.003$). The number of seeds per pod was also found to be significantly correlated with days to 50% flowering

Table 3: Means of quantitative growth and yield variables of selected slender leaf accessions.

S/no.	Accession code	Plant height (cm)	Branch number	Leaf area index	Petiole length (cm)	Pod width (cm)	Pod length (cm)	Pods/plant	Seeds/pod	1000 seed wgt (g)	Days to 50% flowering	Days to maturity
1	BHM198	85.73	9.08	9.58^{ab}	4.11	1.76^{ab}	5.63	13.69	88.05	6.87bc	73.66^{a}	113.66^{a}
2	BHM213	73.91	6.95	11.10^{ab}	4.06	1.66^{ab}	5.42	14.97	99.55	6.60bc	68.33^{ab}	113.00^{a}
3	BKK091	73.07	7.56	9.24^{ab}	3.78	1.83^{ab}	5.33	14.50	88.38	5.70bc	72.33^{a}	113.33^{a}
4	BKK129	75.42	6.56	9.46^{ab}	3.58	1.66^{ab}	5.48	12.70	92.77	5.61bc	74.33^{a}	115.00^{a}
5	BKS220	69.55	7.33	9.30^{ab}	3.81	1.25^{b}	5.06	17.70	78.44	6.00bc	64.00^{b}	105.60^{ab}
6	BKM197	77.41	9.92	12.02^{ab}	3.90	1.41^{ab}	5.16	14.49	66.11	6.35bc	71.00^{ab}	104.83^{ab}
7	BKM203	70.88	8.08	10.49^{ab}	4.35	1.69^{ab}	5.50	15.25	93.22	7.50b	74.33^{a}	115.33^{a}
8	BMG234	83.09	9.63	11.93^{ab}	4.15	1.70^{ab}	5.47	11.87	88.11	7.28b	71.33^{ab}	111.66^{a}
9	BMG239	72.52	8.06	9.08^{ab}	3.85	1.30^{ab}	5.19	12.79	73.38	6.18bc	68.83^{ab}	111.16^{a}
10	BSY111	90.87	7.01	11.16^{ab}	4.20	1.85^{ab}	5.58	14.02	96.27	7.43b	73.33^{a}	117.00^{a}
11	BSY113	72.02	8.65	7.17^{b}	3.40	1.48^{ab}	5.18	17.80	77.72	5.44c	70.00^{ab}	111.33^{a}
12	BVG001	70.23	7.25	10.35^{ab}	3.80	1.04^{b}	5.30	14.16	79.61	5.84bc	68.00^{ab}	101.00^{ab}
13	BVG004	76.37	8.93	9.74^{ab}	4.05	1.43^{ab}	5.44	17.56	80.72	6.54bc	70.67^{ab}	103.66^{a}
14	MBG086	76.44	7.56	11.35^{ab}	3.88	1.71^{ab}	5.62	14.53	71.05	6.23bc	72.00^{ab}	107.60^{ab}
15	MBS064	73.97	9.53	10.11^{ab}	3.87	1.70^{ab}	5.55	15.31	91.05	6.19bc	72.33^{a}	110.00^{a}
16	MBS065	73.28	7.57	9.11^{ab}	4.07	1.60^{ab}	5.44	9.71	92.94	6.82bc	71.00^{ab}	111.00^{a}
17	MHM207	84.11	9.87	10.90^{ab}	3.99	1.85^{ab}	5.87	15.39	90.44	6.37bc	74.33^{a}	116.33^{a}
18	MHM215	80.13	7.85	11.07^{ab}	4.06	1.78^{ab}	5.47	15.29	81.99	6.56bc	70.67^{ab}	113.50^{a}
19	MKK011	86.27	6.46	12.19^{ab}	4.16	1.74^{ab}	5.73	15.96	91.44	7.36b	73.66^{a}	112.60^{a}
20	MKS221	62.18	8.22	12.54^{a}	4.59	1.53^{ab}	5.25	17.66	87.66	7.84a	71.67^{ab}	110.33^{a}
21	MKS226	82.25	9.33	10.65^{ab}	4.07	1.78^{ab}	5.62	13.10	83.66	6.61bc	74.00^{a}	114.00^{a}
22	MKM204	76.55	8.60	10.17^{ab}	3.84	1.71^{ab}	5.55	15.07	88.83	6.06bc	67.67^{ab}	112.00^{a}
23	MKM218	78.47	8.76	10.55^{ab}	3.71	1.67^{ab}	5.63	15.31	91.77	5.68bc	74.33^{a}	106.00^{ab}
24	MMG029	65.41	7.65	8.82^{ab}	3.81	1.25^{b}	5.13	15.38	82.05	5.98bc	67.00^{ab}	95.00^{b}
25	MMG234	75.29	8.00	10.38^{ab}	4.15	2.20^{a}	5.48	10.97	93.16	7.03bc	70.67^{ab}	112.30^{a}
26	MSY110	70.49	8.20	9.63^{ab}	4.10	1.69^{ab}	5.43	12.91	82.33	6.86bc	68.33^{ab}	111.33^{a}
27	MSY216	72.03	8.78	10.65^{ab}	3.95	1.42^{ab}	5.16	15.76	76.61	6.37bc	71.66^{ab}	106.00^{ab}
28	MVG003	81.02	8.16	12.9^{a}	3.89	1.83^{ab}	5.55	12.87	85.50	6.30bc	72.00^{ab}	114.33^{a}
29	MVG125	75.19	8.98	10.57^{ab}	3.84	1.83^{ab}	5.20	18.00	88.83	6.18bc	68.83^{ab}	112.33^{a}
	P value	0.079^{NS}	0.092^{NS}	**0.034***	0.151^{NS}	**0.004****	0.168^{NS}	0.911^{NS}	0.128^{NS}	**0.012***	**<0.0001*****	**0.002****
	Standard error	5.233	0.800	0.970	0.202	0.161	0.175	2.550	6.734	0.449	1.591	3.318

Means with the same letter within the column are not significantly different. *Significant at 5%; **significant at 1%; ***significant at 0.1%; NS: not significant; The accessions whose code starts with letter B belongs to *C. brevidens* while those whose code starts with letter M belongs to *C. ochroleuca*.

Table 4: Pearson correlation between selected quantitative variables.

Variables	Plant height	Petiole length	Leaf length	Leaf width	Leaf area	Pod width	Pod length	No. of seeds/pod	Days to flowering
Petiole length	0.097								
Leaf length	0.198	**0.705**							
Leaf width	**0.384**	0.306	**0.544**						
Leaf area	0.349	**0.559**	**0.886**	**0.862**					
Pod width	**0.543**	0.287	0.313	0.176	0.297				
Pod length	**0.700**	0.257	0.322	0.242	0.344	**0.631**			
No. of seeds per pod	0.251	0.313	0.273	−0.121	0.100	**0.558**	**0.500**		
Days to flowering	**0.552**	0.310	**0.412**	0.278	**0.403**	**0.551**	**0.706**	**0.405**	
Days to maturity	**0.517**	0.268	0.295	0.002	0.199	**0.750**	**0.539**	**0.522**	**0.537**

Values in bold are different from 0 with a significance level of $p = 0.05$.

($p < 0.029$) and days to maturity ($p < 0.004$), both of which were also significantly correlated ($p < 0.003$).

3.4. Cluster Analysis. Agglomerative hierarchical cluster analysis of both qualitative and quantitative traits grouped the 29 accessions into seven major classes (Figure 2). The diversity within classes was 24.87% while diversity between classes was 75.13%. Class 1 had only one accession of *C. brevidens* from Siaya county that was distinct with highest plant height, highest number of days to flowering (late flowering), largest pod length, and highest number of days to

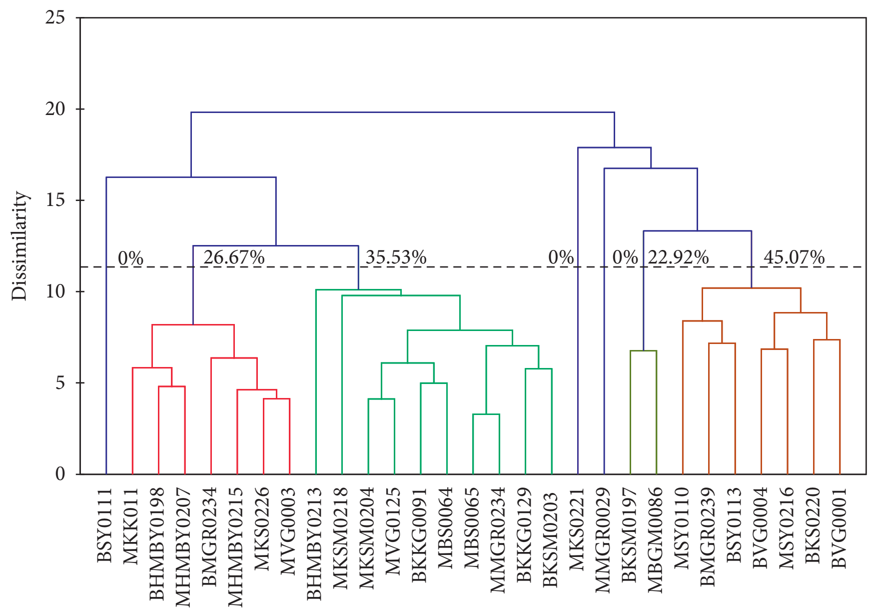

Figure 2: Dendrogram showing morphological diversity of 29 accessions of *Crotalaria spp*. The figures at the truncation line show the percent diversity within different supported clusters.

maturity (late maturity). Cluster 2 contained seven accessions with two *C. brevidens* accessions and five *C. ochroleuca* accessions from five different counties, three of which were from Homabay County. The seven accessions had 26.67 diversity between each other. Cluster 3 had ten accessions comprising of six *C. ochroleuca* and four *C. brevidens* accessions. The ten accessions had 35.53% diversity between each other. Clusters 4 and 5 had one accession each from Kisii and Migori counties, respectively, and both of them belonged to *C. ochroleuca* species. Cluster 6 contained only two accessions including one *C. brevidens* and one *C. ochroleuca* from Kisumu and Bungoma counties, respectively. The two accessions differed from each other with 22.92%. The highest within class diversity of 45.07% was observed in class 7 which contained seven accessions comprising of five *C. brevidens* accessions and two *C. ochroleuca* accessions from four different counties.

3.5. Principal Component Analysis. The first three diversity factors accounted for 92.36% of the total agro-morphological variability among the accessions, out of which 84.30% variation was explained by the first two factors (Table 5). The number of seeds per pod, plant height, days to maturity, and days to 50% flowering were the principal components that made the highest contribution to factor 1 ($F1$) which explained 58.28% of the total variability. Factor 2 ($F2$) explained 26.03% of the total agro-morphological variation with plant height and number of seeds per pod making the highest contribution to this variability factor. Days to maturity, plant height, and number of seeds per pod were the principal components in F3 which explained to 8.06% of the total variation.

Correlations between variables and factors and between accessions and factors are illustrated in Figure 3. The accessions separated in a similar way as in hierarchical cluster dendrogram (Figure 2). The eight accessions in Clusters 1 and 2 (Figure 2) were plotted relatively closer in the upper right quartile of the two-dimension plot (Figure 3(b)). These accessions were characterized by high plant height, late flowering, long pods, and late maturity. The 10 accessions in Cluster 3 (Figure 2) were also plotted together in the lower right quartile of the two-dimension plot (Figure 3(b)). These accessions combined a high number of seeds per pod with long petioles and high seed weight. The accessions in the most diverse Cluster 7 were plotted in the upper left quartile of the two-dimension plot and were characterized by high leaf area and average branch number.

Table 5: Contribution of the variables to the first three diversity factors.

Variables	$F1$	$F2$	$F3$
Plant height	**19.237**	**61.620**	**15.582**
Branch number	0.019	0.254	0.010
Petiole length	0.007	0.001	0.004
Leaf length	0.038	0.001	0.014
Leaf width	0.000	0.009	0.006
Leaf area	0.115	0.324	0.040
Days to 50% flowering	**3.189**	1.580	0.333
Pod width	0.037	0.005	0.028
Pod length	0.025	0.012	0.004
Days to maturity	**17.337**	**2.949**	**76.902**
Weight of 1000 seeds	0.002	0.070	0.008
No. of seeds per pod	**59.565**	**32.897**	**6.982**
No. of pods per plant	0.411	0.178	0.088
Leaf type	0.005	0.008	0.001
Seed colour	0.013	0.092	0.001
Flower colour	0.000	0.000	0.000
Eigenvalue	79.504	35.505	10.989
Variability (%)	58.278	26.026	8.055
Cumulative (%)	58.278	84.304	92.360

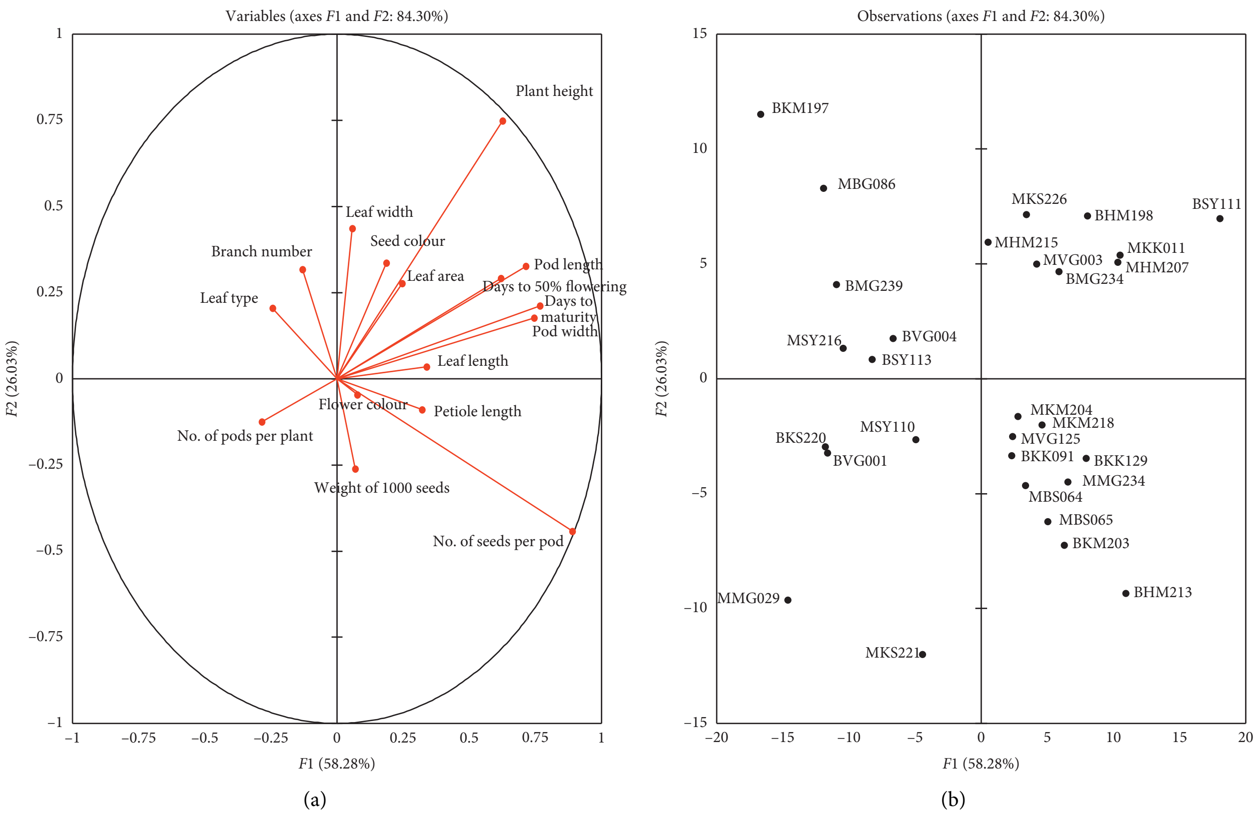

Figure 3: Pictorial illustration of correlations between variables and factors (a) and biplot between accessions and factors (b).

4. Discussion

The success of crop breeding programmes depends on the availability and identification of genetic diversity among the breeding materials [19]. Understanding the genetic diversity and agronomic performance of *Crotalaria spp* is the basis for enhanced adoption and genetic improvement of this high-value vegetable crop. Insight on direct selection on leaf and seed yield can be provided by analysis of agronomic and related traits. In this study, agro-morphological diversity was observed among the different accessions of *Crotalaria brevidens* and *C. ochroleuca* in both qualitative and quantitative traits. However, this study did not identify any agro-morphological characters that could be uniquely linked to any of the two species as the characters were either similar in all the studied accessions or variables within and among the two species. Out of the seven qualitative characters that were studied, four of them, namely, leaf colour, stem colour, dry pod colour, and hairiness of the stem, were found to be similar among species. This observation concurred with Raj and Britto [17] and Schippers [20] who documented that *C. brevidens* and *ochroleuca* are closely related and their phenotypic information cannot be attributed to either of them.

Variation within and among species was observed in only three out of the seven qualitative characters. The three variable traits were leaf type, seed colour, and flower colour. The flower colour was observed to vary from pale yellow to creamish yellow with the former being more dominant in *Crotalaria brevidens* while the latter was more dominant in *C. ochroleuca*. This slightly differs from Schippers [20] who stated that *C. ochroleuca* can best be distinguished by the pale yellow or creamish colour of the flower, unlike *C. brevidens* whose flower is usually bright yellow. The leaf shape comprises of lanceolate, ovate, and linear types, with *C. ochroleuca* having more leaf type variations compared to *C. brevidens* in which the lanceolate type dominated. Soyewo and Omiyale [21] characterized the leaf morphology of the genus *Crotalaria* in Nigeria and reported that leaf features overlap within the genus with leaf shape varying among species from lanceolate to oblanceolate to ovate. The seed colour was found to be the most variable trait among and within the two species ranging from pale yellow to reddish to black and a mixture of either pale yellow and reddish colour or pale yellow, reddish, and black colours. A similar variation in seed colour was reported by Subramaniam et al. [22] among 38 *Crotalaria spp* in India including *C. ochroleuca* where the seed colour ranged from black, brown, steel grey, pale yellow, and orange red. They concluded that seed colour is of little taxonomic significance in species characterization because there exists intraspecific variability in seed colour.

Similar to qualitative variables, most of the quantitative variables did not differ significantly among and within accessions. This could have been limited by the relatively fewer number of each species that were characterized in this study. Significant variation was demonstrated in only five out of the eleven quantitative variables studied. These included three morphological variables, namely, leaf area index (LAI), pod width, and weight of 1000 seeds and two agronomic variables, namely, days to 50% flowering and days to maturity. According to Roux and Wyk [23], growth habit, leaf type, and quantitative morphometrics like petiole length, pod length, and width are phylogenetically important in the morphological characterization of *Crotalaria*. Variation in LAI was attributed to differences in leaf types. Leaf size reportedly contributes to the biomass yield and leaf yield and is, therefore, an important aspect in selecting candidates for leaf production [24]. The weight of 1000 seeds is an indicator of good quality seed and is an essential trait to consider during the selection of accessions to be used as parental stock in seed production during propagation or breeding projects. Variation in days to 50% flowering and days to maturity indicated that there were early and late flowering accessions and this can be attributed to the agro-ecological diversity of their areas of origin. However, early and prolific flowering is a limiting factor to leaf production and yield [25] and this should be considered during selection.

Significant and positive correlations were observed between several quantitative variables. This was an indication that some variables can be indirectly targeted in a selection criterion by using other related variables especially if the target trait does not provide better grounds for discriminative selection [26]. For example, although there was no significant variation between accessions for the variable plant height, the variable was found to be significantly correlated with leaf width, pod width, pod length, days to 50% flowering, and days to maturity. Plant height is considered one of the important traits that should be put into consideration in the agronomic improvement of many vegetables. A similar positive observation was made in soybean mutant accessions by Malek et al. [27]. There was also a strong positive correlation between the leaf area and leaf width and leaf length. This was expected because the leaf area is a factor of leaf width and length. Similar findings were reported among amaranth genotypes by Gerrano et al. [28]. A significant positive correlation was also observed between days to 50% flowering and days to maturity. Besides, these two agronomic variables were also found to be positively correlated with the seed yield traits, namely, pod width, pod length, and the number of seeds per pod. Similar findings were reported in *Corchorus* accessions by Dube et al. [7]. These traits have high levels of heritability and hence can be transferred to accessions of interest through breeding [29].

A cluster dendrogram is a good measure of diversity among and within species as it groups similar entries under one cluster [27]. Cluster analysis grouped the 29 slender leaf accessions into seven supported clusters based on the level of variation among the accessions. A similar strategy was applied by Zhang et al. [30] and Mekonnen et al. [31] on the agro-morphological characterization of *Cucumis melo* and lentils accessions, respectively. Diversity within supported clusters ranged from 0% (for singletons) to 45.07% as observed in Cluster 7. The high morphological diversity indicates a high potential of phenotypic selection which is a desirable aspect to plant breeders. Some accessions were placed separately in their clusters. These were one Siaya accession of *C. brevidens* (Cluster 1), one Kisii accession of *C. ochroleuca* (Cluster 3), and one Migori accession of *C. ochroleuca* (Cluster 4). Such accessions are referred to as singletons and are given much attention in selection as they are suggested to be superior over other accessions [32]. Singletons were also observed in other genetic characterization studies [7, 28, 32].

Principal component analysis highlights and measures how each component contributes to total variation [33]. The factor loading through the eigenvectors indicates the level of contribution of each trait [7] and indicates which trait is the best target in genetic improvement of a certain crop. In this study, the first two eigenfactors accounted for 84.30% of the total phenotypic variability among the accessions. This variation was higher than what was reported by Akin-Idowu et al. [34] in grain amaranth and Ngomuo et al. [24] in jute mallow where the first two Eigen factors recorded a cumulative variance of 42% and 46%, respectively. Days to maturity, plant height, and number of seeds per pod made the most contribution to the morphological variation observed among and within the 29 slender leaf accessions that were studied. This indicates that these three traits can be utilized as target traits during hybridization. Comparing the correlations between variables, accessions, and factors offered a simple method of identifying the principal components in particular accessions and thus can be effective in aiding selection and hybridization.

5. Conclusions

This study revealed a moderate level of morphological diversity between and within *Crotalaria brevidens* and *C. ochroleuca*. However, there is potential of selection for some particular traits of agronomic importance including leaf area index, pod width, days to 50% flowering, and days to maturity. These traits can be targeted for subsequent hybridization between and within the two *Crotalaria* species for crop improvement. The study did not identify any phenotypic characters that can be uniquely associated with any of the two species. Uniformity in phenotypic traits such as stem hairiness, stem colour, leaf colour, and dry pod colour is of little taxonomic significance in the characterization of the two species since the said traits cannot be effectively used to discriminate between the two species. Similarly, seed colour cannot be effectively used in discriminating between the two *Crotalaria* species since it varies between species. This study sets the basis for genetic improvement of slender leaf in Kenya since the observed diversity can be exploited in selection and intraspecific and interspecific hybridization. The authors recommend further analysis of genetic diversity between and within the two species using molecular markers to provide deeper insights into the existing variability. Future studies should also consider a relatively higher number of accessions per species in order to increase the chances of identifying unique traits that can be used to clearly distinguish between the two species.

Data Availability

Some of the data used to support the findings of this study are included in the article. Additional data are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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