

# Phenomic Characterization of *Crotalaria* Germplasm for Crop Improvement in Kenya

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# Abstract

**Background:** Diversification of global food systems through exploration of traditional varieties and wild edible plant species is a focal mitigation strategy for food security worldwide. The present study determined the phenomic diversity of locally available, affordable and climate-resilient cultivated and wild *Crotalaria* species for breeding purposes. Seed samples were collected from different administrative counties in Kenya spanning different climatic zones. Other seeds were provided by the Genetic Resources Research Institute of Kenya. A randomized complete block design with three replications was used for agro-morphological evaluation of the 83 accessions used in this study. Data on quantitative and qualitative traits was collected. Cluster analysis on R and R-studio was used to generate a dendrogram by the Euclidian genetic distance and dissimilarity indices while the non-metric multidimensional scaling (NMDS) method was used to determine the spatial interrelationship between the accessions. The Pearson's correlation coefficients were used to determine the relationships between qualitative and quantitative traits while the principal component analysis was used to discriminate the accessions.

**Results:** Three edible species (*C. brevidens* Benth., *C. ochroleuca* G.Don, *C. trichotoma* Bojer.) were found to be cultivated by Kenyan farmers and a significant variation ( $p < 0.0001$ ) for all parameters under study was recorded. Agglomerative hierarchical clustering grouped the accessions into 8 major clusters. The NMDS ordination formed 15 and 6 groups based on counties and regions respectively.

**Conclusions:** This study forms the basis for exploring novel breeding among cultivated species, between wild and cultivated *Crotalaria* species as well as determining molecular markers linked to phenomic traits.

## Background

The world food supply is increasingly under threat due to climate change, population explosion and crop destruction by pests and diseases. The creation, operation, and evolution of 'new' or 'alternative' food supply chains is one of the key mitigation strategies of achieving global food security (Renting et al., 2003). A critical dimension of this strategy is the conservation of genetic diversity of plant species to safeguard potential economic traits that could be vital in combating pests infestations and diseases outbreaks as well as increasing crop yields (Pandey et al., 2019). Plant breeders rely on genetic diversity of plants to improve yield, resistance to pests and diseases, and tolerance to environmental stress (Govindaraj et al., 2015). Plants respond to changing environments through phenotypic plasticity derived from both genetic and epigenetic factors. Epigenetic variation has been associated with a wide range of adaptive phenotypes in plants, but few direct means for exploiting this variation exist (Raju et al., 2018).

Morphological characterization is essential in the identification of valuable germplasm accessions as well as typification and classification of accessions into cultivar groups. Morphological characterization using conventional phenomic descriptors (morphological and agronomic descriptors) is cardinal in describing and establishing relationships among cultivar groups and accessions of genetically un-profiled crops (Plazas et al., 2014). Availability of genetic diversity within crop plants in the form of wild species, mutant lines or even breeding stocks serves as a source of important alternative gene forms and could therefore help in development of climate tolerant varieties (Fu, 2015). Since breeding goals are constantly changing, there is need to introgress different genes in both domesticated and semi-domesticated crops either as germplasm resources or otherwise (Bhandari et al., 2017).

The Genus *Crotalaria* L. consists of more than 600 species of herbs and shrubs distributed in the tropical and sub-tropical regions (Pandey et al, 2010). The greatest diversity in the genus is found in Africa, which has over 400 species (Abukutsa-Onyango, 2007). Plants in this genus have a myriad of uses including ethno-medicinal uses, nitrogen fixation, green manure, silage, fiber, cover crop for weeds control, prevention of soil erosion, control of nematodes and as ornamental plants (Ramos, et al., 2001; Rouamba et al., 2018). In Kenya, some *Crotalaria* species have been used in the

control of the parasitic weed *Striga hermonthica* and as food (Rouamba et al., 2018). Despite their potential, these species have continued to receive little attention in terms of research and improvement and as such, their diversity remains unknown (Wang et al., 2006). In Kenya, there are only two documented edible *Crotalaria* species with scanty information on their agro-morphological description (Sahou et al., 2014). A common challenge for *Crotalaria* collection holders is the lack of characterization and evaluation data, without which, the genetic material is not readily usable. Detailed plant genetic diversity that is aligned to plant phenomic profiles is essential in plant breeding and subsequently genetic improvement. This study documents the phenomic diversity of cultivated and wild *Crotalaria* species, to the information published herein is essential in enhancing understanding of the diversity within and among *Crotalaria* accessions. Such diversity is relevant for classification, management of genetic resources, selection and breeding, as well as, for research purposes.

## Methods

### Description of study sites, sample collection criteria and processing

Study samples were collected from all the three Kenyan climatic regions of agricultural importance namely; the hot and humid coastal strip, the temperate zone covering the west and south west regions with mountains and plateaus, and lastly the hot and dry north and eastern zone. These regions have considerable variations in rainfall and temperature. The arid and semi-arid zones record rainfall ranges of between 200 mm and 600 mm with temperature ranges of 23°C to 34°C. The coastal belt experiences rainfall ranging between 1000mm and 1250mm with temperature ranges of 22°C to 30°C while the temperate zone receives rainfall ranging 950mm to 3000mm with temperature ranges of 14°C to 28°C annually. Three hundred farmer-held *Crotalaria* accessions were collected from the fore-mentioned zones and 133 *Crotalaria* accessions were cordially provided by the Genetic Resources Research Institute of Kenya (GBK). All the accessions were established in field and green house conditions at the University of Embu Farm and used to establish the working germplasm stock (Figure 1). Global positioning system (GPS) coordinates for all sampled sites were recorded and used to generate a geo-spatial map (Figure 2) using the ArcGIS 10.5 software.

### Cultivation, characterization and classification

Sampled seeds were planted in the research field and the plants were continuously monitored. Individual plants in an accession were characterized morphologically using 18 plant descriptors. The descriptors were; plant height, number of leaflets, leaf length, leaf breadth, pod length, pod breadth, number of branches and number of pods per plant (quantitative variables) and stem colour, life span, growth habit, leaf/leaflet shape, stem pubescence, corolla colour, dry pod colour, seed colour and pod shape (qualitative variables) as described by Raj and Britto (2011). The data was used to cluster the accessions into 83 morphologically distinct accessions based on similarities of their morphological characteristics.

### Experimental design

Agro-morphological evaluation of the 83 accessions was done using the Randomized Complete Block Design (RCBD) with three replications. Each accession was planted in a single line in each block. Seeds were then directly sown in rows of 4m long and 45cm apart (Sadiq et al., 2019). Thinning was done after four weeks of establishment to ensure an intra row spacing of 30cm and a single plant for each sample per block randomly selected for data collection. All farm practices were adhered to except fertilizer or manure application.

### Data analysis

Questionnaire data was analysed using the Statistical Product and Service Solutions (SPSS) software version 20. Quantitative data of the study was subjected to ANOVA using the Statistical Analysis Software (SAS version 6.2.9) and mean separation done using the Student Newman Keuls (SNK) test at 95% confidence level. Quantitative and qualitative data were organized into a matrix and analysed using R and RStudio packages (Versions 3.6.2 and 1.2.5 respectively). The Euclidean genetic distance and dissimilarity indices were used to estimate similarity and generate a dendrogram using the Unweighted Pair-Group Method with Arithmetic average (UPGMA). Non-metric multidimensional scaling (NMDS) method was used to determine the spatial interrelationship between the samples based on the administrative regions (counties) from where they were collected as well as their agro-ecological regions of origin (regions). A two-dimension model with 999 permutations per run was generated using metaMDS in Vegan for NMDS (McCune & Grace, 2002). The principal component analysis (PCA) was used to discriminate accessions while the cluster analysis was used to group them based on their quantitative parameters. Pearson's correlation coefficients were used to determine the relationship between quantitative traits. A heat map was used to visually represent the contributions of the different parameters to the observed morphological diversity.

## Results

### Availability and distribution of *Crotalaria* species in Kenya

The present study determined that there are 21 morphologically distinct *Crotalaria* species distributed in 15 counties in Kenya (Supplementary table 1) with three species already domesticated by two communities in the country. These species were predominantly found in Kakamega, Vihiga, Busia, Bungoma, Kisumu, Migori, Siaya and Homa Bay counties. Only 9 of 133 accessions (6.77%) provided by the Genetic Resources Research Institute of Kenya are domesticated by local communities in Kenya, and belonged to the species *C. brevidens* Benth and *C. trichotoma* Bojer.

### Edible *Crotalaria* species in Kenya

Three *Crotalaria* species were found to be cultivated by farmers in Kenya (Figure 3). These are *C. brevidens* Benth. var. *brevidens*, *C. brevidens* Benth. var. *intermedia*, *C. ochroleuca* G.Don. and *C. trichotoma* Bojer./*C. zanzibarica* Benth.. Morphologically these three species closely resemble one another but with a few differences. *C. brevidens* Benth. var. *brevidens* and *C. brevidens* Benth var. *intermedia* both have slender pods at maturity compared to *C. ochroleuca* and *C. trichotoma* which have relatively broad pods. The calyx of *C. brevidens* Benth are relatively puberulous with bright yellow petals while those of *C. ochroleuca*, and *C. trichotoma* are glabrous with pale yellow petals. The difference between the two varieties of *C. brevidens* is the colour of leaflets which are dull green in *C. brevidens* Benth. var. *brevidens* and bright green in *Crotalaria brevidens* Benth. var. *intermedia*. *C. trichotoma*, sometimes referred to as *C. zanzibarica* is an erect herb which grows taller than the other three (up to 2.70m) with a woody base. It is morphologically close to *C. ochroleuca* which is relatively shorter (up to 2.50m) but highly branching. *C. ochroleuca*, and *C. trichotoma* have a mild taste while *C. brevidens* have a bitter taste although the degree of bitterness depends on the place and time of propagation. Out of the 83 accessions used in this study, 28 (33.7%) were *C. ochroleuca*, 4 (4.8%) were *C. trichotoma* and 11 (13.3%) were *C. brevidens*.

### Cultivation aspects of *Crotalaria* vegetables.

From a sampled population of 37 farmers, 86.5% knew of two types of edible *Crotalaria* species, 10.8% knew of three types while 2.7% did not know of the different types. However, this was based on the taste of cooked vegetables, which they categorized as either mild, bitter or very bitter. Edible *Crotalaria* species in Kenya were found to be cultivated by small holder farmers, with land sizes ranging from 0.20 to 8.0 hectares. The crop was mainly cultivated in kitchen gardens with average land size under the crop observed of 0.058 Ha. Of the farmers interviewed, 89.2% planted the crop in less than 0.05 Ha (Supplementary material 3). A correlation analysis of the household size, farm size and the total

land area under *Crotalaria* vegetables revealed that there was a negative correlation between household size and farm size ( $r^2=-0.21$ ) although this was not significantly different ( $p=0.901$ ). There was a positive correlation between household size and the portion under which *Crotalaria* vegetables were grown ( $r^2=0.17$ ) which was also not significant ( $p=0.314$ ). Farm size and the portion of land under *Crotalaria* vegetables were positively correlated ( $r^2=0.665$ ,  $p=0.001$ ) (Supplementary table 4). Analysis of variance revealed that there was a significant difference in the portions of land under the crop among all the interviewed farmers ( $p=0.001$ ), with the largest being 0.8 Ha and the least being less than 0.05 Ha. Household sizes for the interviewed farmers ranged from two to eight people and this was found to have an effect on how much piece of land will be cultivated to plant *Crotalaria* vegetables, with large households cultivating more land than the small households.

It was observed that 89.2% of the farmers who cultivated *crotalaria* vegetables were aged between 31-65 years with none of the respondents aged between 0-30 years. Only 6 (16.2%) of the 37 respondent farmers who cultivated *Crotalaria* vegetables had formal education beyond the secondary level. All the farmers with tertiary education planted the vegetable for commercial and domestic purposes. However, 15 (40.5%) farmers with primary and secondary education planted the vegetables solely for domestic consumption. Majority (70.3%) of the farmers admitted to eating the vegetable weekly, while 4 farmers (1.2%) ate the vegetable daily and fortnightly. Of all the interviewed farmers, only 5 (13.5%) were male, while 32 were female, representing 86.5%.

### **Variation in Quantitative characters in Kenyan *Crotalaria* species**

Significant variations ( $p<0.0001$  for all parameters) between accessions were recorded for all the quantitative variables; height, number of leaflets, leaf length, leaf width, number of branches, pod length, pod diameter, pods per plant and leaf area (Supplementary Table 2). Mean height ranges were between 2.65 m in sample GBK-005664 (*C. trichotoma*) and 0.34 m in sample GBK-005489 (*C. greenwayi*). From the 83 samples used in this study, 76 had compound leaves with three leaflets except for *C. grahamiana* which had seven leaflets. The other seven samples had simple leaves, representing the five species; *C. juncea*, *C. spectabilis*, *C. retusa*, *C. paulina* and *C. pancira*. Leaf lengths ranged from 19.0 cm in sample GBK-005209 (*C. paulina*) to 1.13 cm in sample GBK-005664 (*C. greenwayi*). The leaf area followed the same pattern, with sample GBK-005209 (*C. paulina*) having the largest leaf area (122.51 cm<sup>2</sup>) and GBK-005664 (*C. greenwayi*) with the smallest leaf area (0.606 cm<sup>2</sup>). The most profuse branching was observed in samples GBK-005685 (*C. spectabilis*) and GBK-005201 (*C. pancira*) which had a mean of 34.33 branches while the least number of branches observed in sample Fmg 0298 (*C. pallida*) with a mean of 3.00 branches. The mean pod length ranged from 6.7 cm in sample Fvh 0119 to 0.4 cm in sample GBK-047548 (*C. cephalotes*) while mean pod diameter ranged from 5.83 cm in sample Fvh 0119 to 0.90 in sample GBK-005664 (*C. greenwayi*). Sample GBK-005189 (*C. laburnifolia*) had the highest mean number of pods while Fki 0123 (*C. ochroleuca*) had the least mean number of pods.

### **Variation in qualitative characters**

The qualitative characters that were studied include; leaf shape, pod shape, leaf type, flower colour, stem colour, hairiness of the stem, dry pod colour, seed colour and growth habit (Figure 4). There were 6 leaf shapes observed within the genus, of which three were observed in 74 accessions, accounting for 89.1% of the shape diversity observed. Oblanceolate leaves/leaflets were the most common and were observed in 27 (32.5%) samples, followed by the elliptical 25 (30.1%) and linear 22 (26.5%). The obovate, orbicular and ovate leaves/leaflets constituted 5 (6%), 2 (2.4%) and 2 (2.4%) accessions respectively. The long broad smooth pod was the most frequent and was observed in 49 (59%) accessions, followed by the long slender smooth, observed in 22 (26.5%) accessions. The long broad hairy, short broad hairy and short slender smooth pods were the least frequent, with each constituting 1.2%. The short broad smooth and long slender hairy pods constituted 7.2% and 3.6% respectively. The compound leaf type was observed in 76 (91.6%) accessions while 7 (8.4%) accessions had simple leaves. The pale yellow corolla with purple stripes were observed in 29 (34.9%)

samples while the deep yellow with purple stripes were observed in 15 (18.1%) samples. The lemon yellow corolla with purple stripes and the chartreuse yellow were the least, representing only 1.2% of the samples. The brown-green stems were the most abundant in the population (51.8%), while green and pale green were 14.5% and 13.3% respectively. Purple-green and shiny-green stems each represented 6% of the samples. Dark-green and yellow-green stems were the least abundant in the samples representing 4.8% and 3.6% respectively. The stems were either smooth (75.9%) or pubescent (24.1%). Black dry pods constituted 63.9% while brown dry pods represented 33.7%. Brown with black striped pods and green with brown striped pods were 1.2% each. Pale-yellow seeds were the majority (32.5%), while the mixture of pale-yellow and red-orange, and the purely red-orange seeds constituted 16.9%. Other seed colours observed were black (6%), brown (7.2%), grey (6%), pale grey (2.4%), black and grey mixture (2.4%), grey and pale-yellow mixture (1.2%) striped grey (3.6%), maroon (2.4%) and purple (2.4%). Most of the cultivated accessions had mixed seed colours in a pod whereas the wild species had distinct uniform seed colours.

### **Correlation between quantitative variables**

A weak positive correlation was observed between height and the following traits: number of leaflets, number of branches, pod length, pod diameter and the number of pods per plant (Table 1). Height and leaf length had no correlation. However, height only correlated significantly with the number of leaflets, ( $r^2=0.158$ ,  $p=0.0128$ ), number of branches ( $r^2=0.160$ ,  $p=0.0111$ ), pod length ( $r^2=0.191$ ,  $p=0.0024$ ) and pod diameter ( $r^2=0.125$ ,  $p=0.0481$ ). Both leaf width ( $r^2=-0.170$ ,  $p=0.007$ ) and leaf area ( $r^2=-0.149$ ,  $p=0.018$ ) had a significant inverse correlation with height. All parameters correlated negatively with the number of leaflets except pod length ( $r^2=0.192$ ,  $p=0.002$ ) and pods per plant ( $r^2=0.074$ ,  $p=0.240$ ). Among the negatively correlating parameters, a moderate negative correlation was observed only between the number of leaflets and leaf area ( $r^2=-0.561$ ), which was significantly different ( $p<0.0001$ ). Although leaf length and leaf width were moderately negatively correlating with number of leaflets, this correlation was significantly different with  $p<0.0001$  for both parameters. Leaf length correlated positively with all the other parameters. However, only leaf area ( $r^2=0.74$ ;  $p<0.0001$ ) and had strong positive correlations with leaf length, with leaf width ( $r^2=0.531$ ;  $p<0.0001$ ), number of branches ( $r^2=0.297$ ,  $p<0.0001$ ), pod length ( $r^2=0.241$ ,  $p=0.0001$ ), pod diameter ( $r^2=0.227$ ,  $p=0.0003$ ) and pods per plant ( $r^2=0.201$ ,  $p=0.0015$ ) all showing moderate to weak positive correlations. Leaf width had no correlation with pod length ( $r^2=-0.052$ ,  $p=0.4057$ ), but had positive correlation with all the other parameters. However, only leaf area strongly correlated with leaf width, ( $r^2=0.886$ ,  $p<0.0001$ ). The number of branches had a positive weak correlation which was significantly different from zero with the following parameters: pod length ( $r^2=0.157$ ,  $p=0.013$ ), pod diameter ( $r^2=0.214$ ,  $p=0.0007$ ), pods per plant ( $r^2=0.220$ ,  $p=0.0005$ ) and leaf area ( $r^2=0.153$ ,  $p=0.015$ ). Pod length had a significantly moderate positive correlation with pod diameter ( $r^2=0.654$ ,  $p<0.0001$ ) but had no correlation with pods per plant and leaf area. Pod diameter and pods per plant were weakly negatively correlated, which was significantly different from zero ( $r^2=-0.257$ ,  $p<0.0001$ ). A significant weak positive correlation was observed between pods per plant and leaf area ( $r^2=0.197$ ,  $p=0.0018$ ).

### **Agglomerative hierarchical clustering of qualitative and quantitative traits**

Agglomerative hierarchical clustering of both qualitative and quantitative traits of the 83 *Crotalaria* accessions assembled them into eight major clusters at 7.5 Euclidean distance (Figure 5). The first cluster (A) had only one wild accession, *C. spectabilis*. The distinct feature of this accession was the simple leaf type with the highest leaf area index. The second cluster (B) consisted of four wild accessions of different species with two sub-clusters. The first sub-group consisted of the species *C. incana* and *C. endecaphylla* while the second consisted of *C. cephalotes* and *C. greenwayi* species. All these species have trifoliolate compound leaves and other similarities in qualitative traits such as brown dry pods and hairy stems. The third cluster (C) comprised three wild accessions representing two species; *C. juncea* and *C. greenwayi*. These species are herbaceous with brown dry pods. Cluster four (D) consisted of four wild accessions with

four different species; *C. spectabilis*, *C. retusa*, *C. pancira* and *C. paulina*. These accessions are perennial shrubs with simple leaves, their dry pods are brown with black or grey seeds and have shiny green stems. Cluster five (E) was made up of 14 accessions representing 10 species. All the accessions in this cluster were wild perennial shrubs with compound leaves. The first sub-group consisted of five accessions of the species *C. pallida*, *C. laburnifolia*, *C. intermedia* and *C. anagyroides*, all with three leaflets and brown dry pods. The other sub-group in this cluster consisted of a single accession of the species *C. grahamiana* which has seven leaflets. The other sub-cluster in the group consisted of two accessions namely; *C. scassellatii* and accession Fkj 0294 (collected from Kajiado county). The last sub-cluster in this group consisted of six accessions, representing only two species; *C. recta*, and *C. endecaphylla*, characterised by compound trifoliolate leaves and black dry pods. The sixth cluster (F) consisted of 36 cultivated accessions. A common feature among these accessions is that their dry pods are black, and their flowers have purple stripes. The cluster consisted of the two species, *C. brevidens* and *C. ochroleuca*. This cluster had three sub-clusters, two entirely made of *C. ochroleuca* samples while the third consisted of a mixture of *C. brevidens* and *C. ochroleuca* accessions. The Seventh cluster (G) consisted of the wild type perennial herb *C. intermedia*. The last cluster (H) was made up of 20 accessions, comprising of both cultivated and wild species. This cluster contained three sub-clusters, representing six species; *C. brevidens*, *C. intermedia*, *C. incana*, *C. deserticola*, *C. lanceolata*, and *C. trichotoma*.

Table 1: Correlation of nine quantitative traits of 83 *Crotalaria* accessions from Kenya

Variable	Height (ft)	No of leaflets	Leaf length (cm)	Leaf width (cm)	Number of Branches	Pod length (cm)	Pod Diameter (cm)	Pods per Plant	Leaf area (cm <sup>2</sup> )
Height (ft)	1.00000								
No of leaflets	<b>0.15752</b>	1.00000							
Leaf length (cm)	0.04413	<b>-0.42131</b>	1.00000						
Leaf width (cm)	<b>-0.17014</b>	<b>-0.43052</b>	<b>0.5311</b>	1.00000					
Number of Branches	<b>0.16074</b>	-0.09821	<b>0.29659</b>	0.00957	1.00000				
Pod length (cm)	<b>0.19154</b>	<b>0.19164</b>	<b>0.24146</b>	-0.05292	<b>0.15774</b>	1.00000			
Pod Diameter (cm)	<b>0.12537</b>	-0.01905	<b>0.22668</b>	0.07796	<b>0.21449</b>	<b>0.65484</b>	1.00000		
Pods per Plant	0.11343	0.07467	<b>0.2005</b>	<b>0.179</b>	<b>0.22013</b>	-0.00918	<b>-0.25797</b>	1.00000	
Leaf area (cm <sup>2</sup> )	<b>-0.14985</b>	<b>-0.56131</b>	<b>0.74386</b>	<b>0.88564</b>	<b>0.15342</b>	-0.00792	0.10351	<b>0.1971</b>	1.0000

Values in bold are different from 0 at a significance level of p<0.05.

Table 2: The contribution of variables to the first five Principal Components of 83 *Crotalaria* accessions in Kenya.

Traits	PC 1	PC 2	PC 3	PC 4	PC 5
Eigenvalue	2.961	1.730	1.398	0.838	0.764
Variance %	0.329	0.192	0.155	0.093	0.085
Cumulative %	0.329	0.521	0.676	0.770	0.855
<b>Factor loading</b>					
Height (ft)	0.092	<b>-0.346</b>	<b>0.394</b>	<b>-0.467</b>	<b>0.6803</b>
No of leaflets	<b>0.379</b>	-0.212	0.232	<b>0.458</b>	-0.1205
Leaf length	<b>-0.524</b>	-0.117	0.034	-0.050	-0.0489
Leaf width	<b>-0.421</b>	0.158	-0.101	<b>0.342</b>	<b>0.4087</b>
Number of branches	-0.234	<b>-0.399</b>	0.294	<b>-0.344</b>	<b>-0.5601</b>
Pod length	-0.001	<b>-0.616</b>	-0.149	<b>0.346</b>	0.1838
Pod diameter	-0.045	<b>-0.507</b>	<b>-0.488</b>	0.072	-0.0548
Pods per plant	-0.167	-0.026	<b>0.657</b>	<b>0.459</b>	-0.0482
Leaf area	<b>-0.558</b>	0.006	-0.013	0.023	0.0214

### variable-reduction by Principal Components

The principal component analysis (PCA) grouped the nine quantitative traits into nine principal components, which accounted for all the genetic variation observed in the 83 *Crotalaria* accessions. The first five Principal Components (PCs) explain 85.5% of the observed variation in the accessions, out of which 52.1% of the variability was accounted for by the first two PCs (Table 2). The traits that contributed to the most variability among accessions both in the negative and positive loadings in the first PC1 were leaf area, leaf length, leaf width and number of leaflets. With an Eigen value of 1.73, the second PC accounted for 19.2% of the phenomic variation. The phenotypic attributes that mainly contributed to PC2 were pod length, pod diameter number of branches and plant height. Pods per plant, pod diameter, and height were the traits that contributed to the variation accounted for by PC3, whose Eigen value was 1.39.

The correlation between variables and PCs revealed that the variables height, leaf length, and leaf area are well explained by the first two PCs, since they appear at the periphery of the correlation circle. These two PCs however cannot properly explain the variables pod length and pod diameter as they appear at the center of the correlation circle (Figure 6a). Based on this bi-plot, PCs 1 and 2 have almost no information on pods per plant but provide adequate information on diversity aspects based on leaf area and leaf length. A bi-plot on the correlation between accessions and PCs revealed a similar grouping as that of the cluster dendrogram, with the furthest accession in the bottom left quartile clustering as a distant relative of the other accessions (Figure 6b). The bi-plots also revealed that the variables leaf area and pod length and also leaf length and height are orthogonal.

Most of the observed variation was contributed by the quantitative traits, with the qualitative traits contributing little to the clustering. The magnitude of contribution of quantitative and qualitative traits to variation as depicted by the heat

maps generated based on the counties of collection (Figure 7) and agro-ecological regions (supplementary figure 1) resonates with the PCA output. The most important quantitative traits were the number of pods per plant, the leaf area, number of branches, leaf length, and pod length. Seed colour followed by pod shape were the most contributing qualitative traits to diversity.

### NMDS clustering

According to the Non-metric multidimensional scaling (NMDS) analysis, the close affinities among the accessions in each sample type are supported by their proximity to one another though there is still a little morphological overlap with other samples. The NMDS ordination with a stress value of 0.165 indicated an acceptable ordination of the data. Characteristic of the sampling counties, fifteen NMDS groups were formed (Figure 8). Similarly, six groups were formed representing the six agro-ecological sampling regions (Figures 9). The two most significant variables that generated the clusters in the two-dimension model at  $P < 0.05$  were number of pods per plant and leaf area index (with a score of 12% and 0.8% respectively). Based on the administrative clusters (Figure 8), accessions collected from Kakamega county and the neighbouring counties exhibited similar morphological traits with exception of Nairobi county. A unique overlap between accessions collected in Migori and Kajiado counties was observed. Samples from Makueni and Embu counties as well as GBK accessions exhibited peculiar characteristics and thus showed no significant relatedness to the other accessions. Based on the agro-ecological regions, accessions collected from Western region and the neighbouring Nyanza and Rift valley regions exhibited similar morphological traits. Accessions collected from Nyanza region shared a noticeable proportion of similarities with accessions collected from the Rift valley region. Accessions collected from the Eastern region and gene bank exhibited peculiar characteristics thus showed no significant relatedness to the other study accessions. The ANOSIM value of ( $R=0.4807$ ,  $P=0.001$ ) supported the even distribution of high and low ranks within and between groups of *Crotalaria* species in the different counties and agro-ecological regions. Although distantly separated, the accessions from Nairobi clustered together with those from Kakamega and exhibited similar morphological appearance.

## Discussion

This is the first study documenting the phenomic diversity of cultivated and wild *Crotalaria* species from the different agro-ecological regions in Kenya. In the present study, three *Crotalaria* species are reported as edible and distributed among Kenyan farmers, compared to 15 species which are reported as edible in the genus worldwide (Barrett, 1990). In earlier studies, in Kenya, only two species, *C. brevidens* and *C. ochroleuca*, were reported as edible (Abukutsa-Onyango, 2007). The distinction between the two varieties of *C. brevidens* and the availability of *C. trichotoma* among the Kenyan farmers are hereby reported for the first time. The edible *Crotalaria* species reported before was only *C. brevidens* Benth. Var *brevidens* although *C. brevidens* Benth. Var *intermedia* is more common among the Kenyan farmers. The two varieties of *C. brevidens* Benth are found in several African countries including Nigeria, Ethiopia, Kenya, Uganda and Tanzania as well as the Americas. *C. ochroleuca* is wide spread throughout all African countries except the north and south most parts and outside Africa in Australia, Brazil, China, New Guinea and the United States. *C. trichotoma* is found predominantly in East Africa, Taiwan and Vietnam (Chauvet, 2004). In Kenya, the edible *Crotalaria* species are only common among the Luo and Luhya communities and are therefore predominantly found cultivated in the western part of the country. The preference of each of these varieties of *C. brevidens* Benth. among Kenyan farmers could be investigated further and awareness on the different species created among the farmers, consumers and breeders.

The present study reports 21 *Crotalaria* species present in 15 counties of Kenya, a relatively high diversity considering the high species rich mountainous regions were not sampled. However, based on the Flora of Tropical East Africa (FTEA) data, over 93 species of *Crotalaria* are thought to be present in Kenya (Zhou et al., 2017). This difference could be attributed to sampling time and species extinction factors. FTEA is a data base which receives contributions from many

botanists and has been collecting samples since the 1940s compared to the current study which collected samples over a period of five months. In the current study only 16.2 % of *Crotalaria* farmers had tertiary education and the crop is cultivated in small parcels of land. This indicates that the crop has been neglected and most farmers with economic resources are not planting it despite its benefits. Variability was observed in all the qualitative and quantitative traits investigated. Contrary to a previous study by Mwakha et al. (2020), who reported lack of diversity in stem colour, dry pod colour, and hairiness of the stem. The study by Mwakha et al. (2020), investigated two domesticated species hence the diversity could have been lost over time due to selection pressure. In agreement with the findings of Raj & Britto (2011b), the present study established that most of the traits observed were not exclusive to a particular *Crotalaria* species with the leaf shape trait being among the most diverse qualitative trait. Therefore, agro-morphological characterization alone is not adequate for diversity assessment of *Crotalaria* and should be supported by other methods such as DNA based techniques. However, Phenomic characterization of plants can be ideal in identification of suitable morphological markers for crop improvement by breeders and researchers.

Diverse leaf/leaflet shapes including oblanceolate, elliptical, linear, obovate, orbicular and ovate were observed in current study. However, the distinctiveness of this trait in most of the cultivated species was lacking, with observations of different leaflet shapes in different plants of the same species. Overlap in leaf types in the species *C. brevidens* and *C. ochroleuca* which are common cultivated species in Kenya has been previously reported (Mwakha et al. 2020). Therefore, this trait may not a good qualitative parameter for inheritance studies. In all the wild accessions examined in this study, only one leaf/leaflet shape was observed per accession. This is in agreement with the findings of Soyewo & Omiyale (2015) who observed distinct leaf shapes per species in different *Crotalaria* species in Nigeria. The other trait which was also found to be inconsistent in cultivated species but consistent in wild species was seed colour. In the cultivated species *C. brevidens*, *C. ochroleuca*, and *C. trichotoma*, there were mixtures of different seed colours in the same pod. Most of these species had mixtures of pale yellow and red orange seeds, with rare cases of a uniform seed colour being observed. In the wild species, mixed seed colour was not observed in one pod. Other studies on the seed morphology of *Crotalaria*, have reported mixed seed colour phenomenon in different *Crotalaria* species (Robert H. Miller, 1967; Subramaniam et al. 2015). Except for inter-species crosses involving wild types and cultivated species, seed colour might also not be a good qualitative trait for species improvement.

This study reported a strong positive correlation between, leaf length and leaf area and between leaf width and leaf area. Leaf width and length being factors of leaf area would be expected to positively correlate with each other and with leaf area. Correlation among quantitative variables means that it is possible to improve one of them by targeting the other. Other studies also allude that strongly correlating traits are possibly under the control of same genes and therefore when breeding, selecting for one of the traits could result in the improvement of the other trait (Neyhart et al., 2019). In the present study, other significant positive correlation was observed between leaf length and number of branches, pod length, pod diameter as well as pods per plant. This therefore could imply that some of the genes controlling the leaf parameters and those controlling pod parameters could be having a pleiotropic effect on the opposite parameters. Significant correlation was also observed between the number of branches and the pod parameters; pod length, pod diameter as well as pods per plant, suggesting a possible pleiotropic relationship between the genes controlling branching and these pod parameters.

Cluster analysis grouped the 83 *Crotalaria* accessions into eight clusters. Most of the cultivated accessions clustered together while most of the wild species also clustered together. The assembly profile of most cultivated accessions could be attributed to selection pressure which is known to reduce variability in plants. The high homogeneity observed in cluster F which consists of the cultivated species *C. brevidens* and *C. ochroleuca* could be largely due to unique selection pressure that aimed at improving these species as vegetables. The phenomenon of selection pressure shaping grouping in cluster analysis has been previously reported in jute mallow by Ngomuo et al. (2017). *C. spectabilis* in cluster A and *C. intermedia* in cluster G appeared as singletons after clustering. This means that these accessions could be having

unique morphological traits which were not found in the other accessions. Similarly, singletons in cluster a dendrogram were observed by Dube et al. (2019) in different *Corchorus* accessions and suggested that such samples should be given more attention during selection since they are more diverse. The cluster H had both cultivated and wild species, suggesting that the domesticated species are closely related to these wild species. This could explain why *C. deserticola*, and *C. lanceolata* closely resemble *C. brevidens*. Morphologically similar species could also be close genetically hence such clusters can be useful in improving the cultivated varieties using the wild accessions.

The overlap of NMDS groups in some regions could be an indication of germplasm exchange, similar domestication practices and reduced epigenetic effects asserted by characteristics of the different agro-ecological zones of the study area. The domestication of some *Crotalaria* species by certain communities in Western and Nyanza regions of Kenya coupled with their migratory patterns to other different regions including Nairobi could have greatly contributed to the narrow diversity and similarity in morphological characteristics among accessions collected in these regions. Overlaps in NMDS groups are common observations in genetic and community structure analysis of plant populations. Closely located NMDS groups are an indication of close geographical or genetic distance between accessions as indicated by Wang et al., (2009).

In the present study, the first two principal components contributed to 52.1% of the observed variation among the accessions. This was lower than a cumulative variance of 84.3% observed by Mwakha et al. (2020) who worked with two *Crotalaria* species. Leaf length, leaf width, leaf area, number of leaflets, pod length, pod diameter, number of branches and plant height were the highest contributing factors to the observed variability. These parameters therefore constitute the most important attributes for any breeder seeking to improve these species.

## Conclusions

This study provides a detailed phenomic description of 21 *Crotalaria* species in Kenya. Whereas *C. brevidens* has been previously documented as an edible species in Kenya, the present study for the first time differentiates the *C. brevidens* in Kenya into distinct varieties; *C. brevidens* Benth. var. *brevdens*, *C. brevidens* Benth. var. *intermedia*. The study also documents *C. trichotoma* for the first time as an edible cultivated species in Kenya. Results of this study further highlight important target agronomic traits as revealed by morphological diversity analysis and hence serve as a reference point for genetic improvement of the cultivated *Crotalaria* species. The phenomic relationship profile between cultivated and wild *Crotalaria* species provides a baseline for novel *Crotalaria* breeding programs since wild species form a rich reservoir of adaptive genes. This study therefore is a critical foundation for genomic characterization of this genus.

## Abbreviations

ANOVA: Analysis of Variance; GBK: Genetic Resources Research Institute of Kenya; NMDS: Non- Metric Multidimensional Scaling; PCA: Principal Component Analysis; PCs: Principal Components; RCBD: Randomized Complete Block Design; UPGMA: Unweighted Pair-Group Method with Arithmetic average

## Declarations

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### Authors' contributions

JKM, JN, and NLMB designed the experiments. JKM and JN Analysed the data and wrote the manuscript. JKM Performed the experiments. NLMB Guided the research. NLMB, JN, PK and EO Conceptualized the idea, secured funds and supervised the work. All authors read and approved the final manuscript.

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## Availability of data and materials

All the data generated or analyzed from this study are included in this published article and its additional files.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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## Figures



Figure 1

Established *Crotalaria* accessions at the research fields and green house conditions. *Crotalaria* accessions established in the field (a) and green house (b) before establishment of germplasm stock.

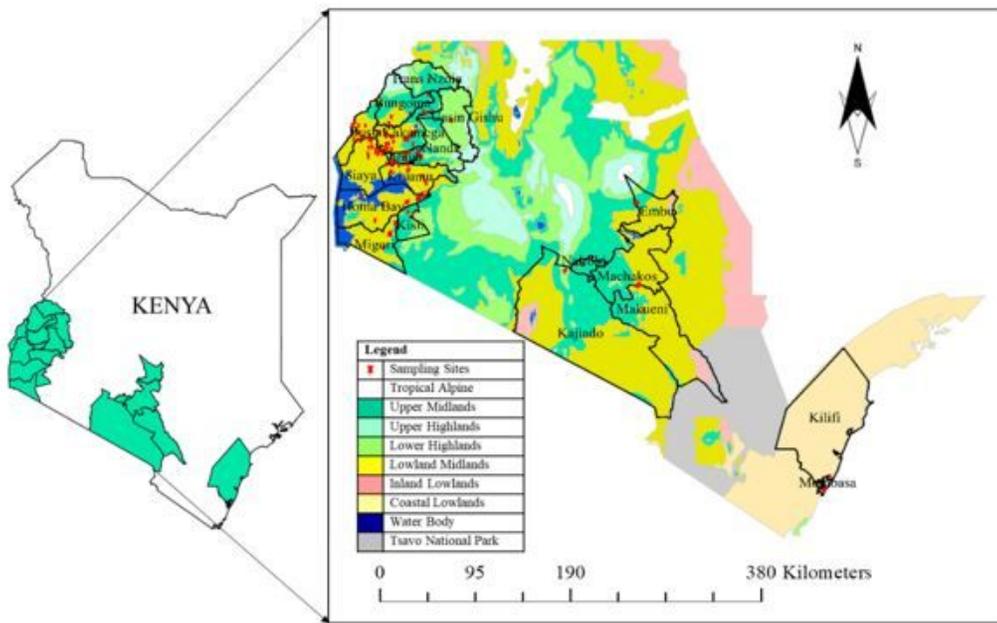


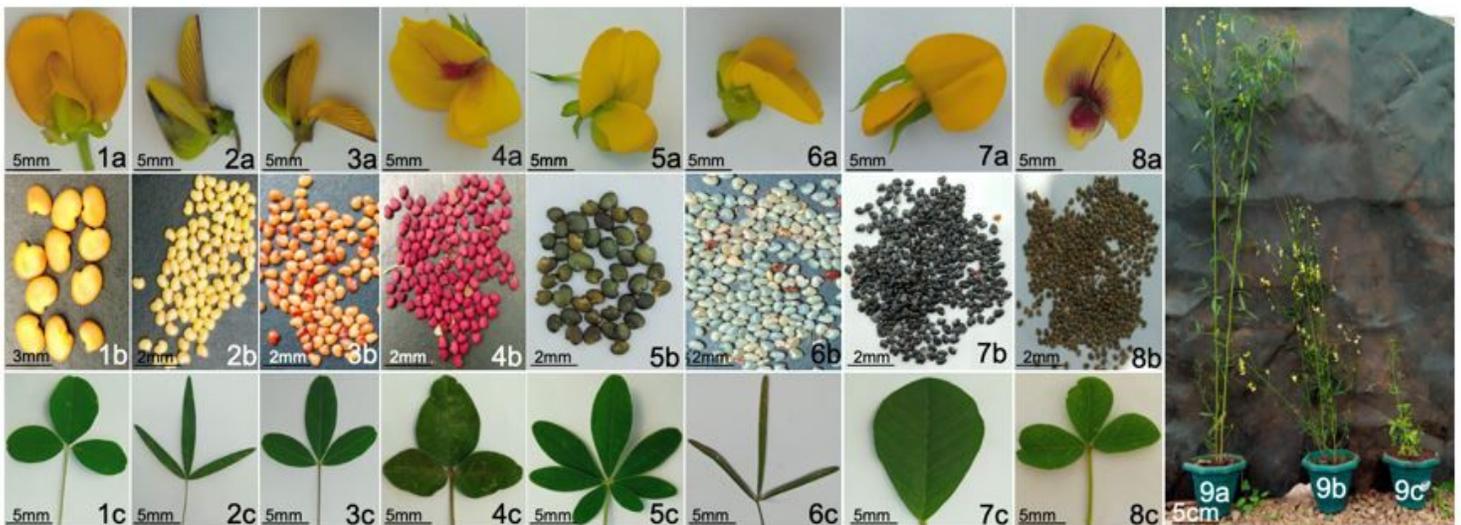
Figure 2

Kenyan Agro-ecological zones and the *Crotalaria* sampling sites



**Figure 3**

Edible *Crotalaria* species in Kenya. A). *C. brevidens* Benth. var. *intermedia*. With bright green leaves and slender pods B). *C. brevidens* Benth. var. *brevidens*. With dull green leaves and slender pods C). *C. ochroleuca* G. Don with many branches and broad podded D). *C. trichotoma* Bojer which is taller, less branching and with broad pods.



**Figure 4**

Qualitative and Quantitative characters of different *Crotalaria* species in Kenya Qualitative (petal colours(a), Seed colours(b) and leaf shapes(c)). Quantitative (plant height (9a. 1.95m, 9b. 1.10m, 9c. 0.9m)).

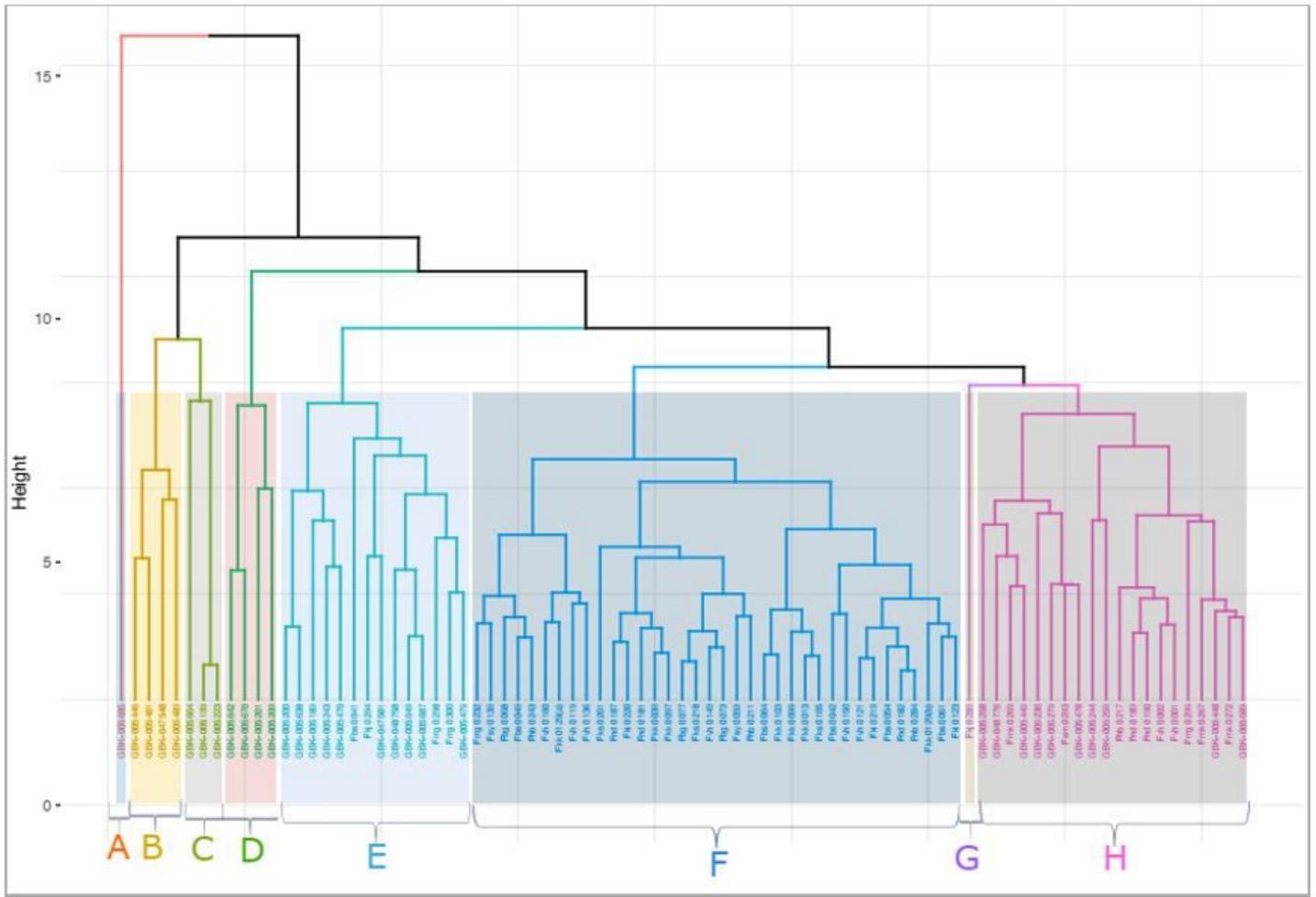


Figure 5

Morphological diversity of 83 accessions of *Crotalaria* spp. from Kenya

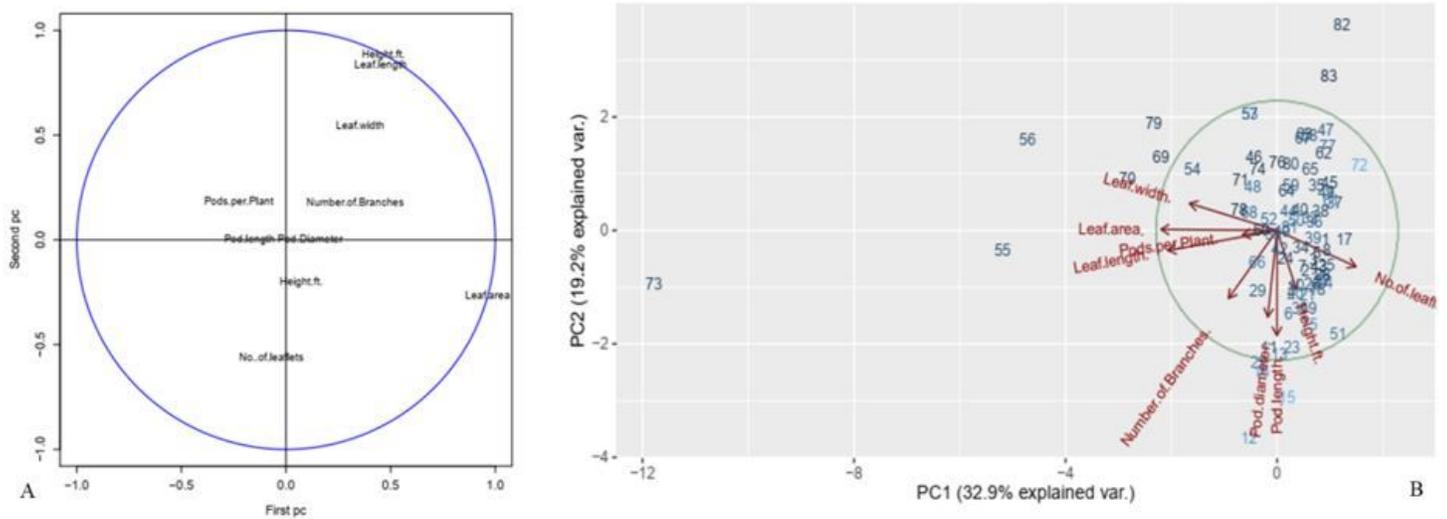
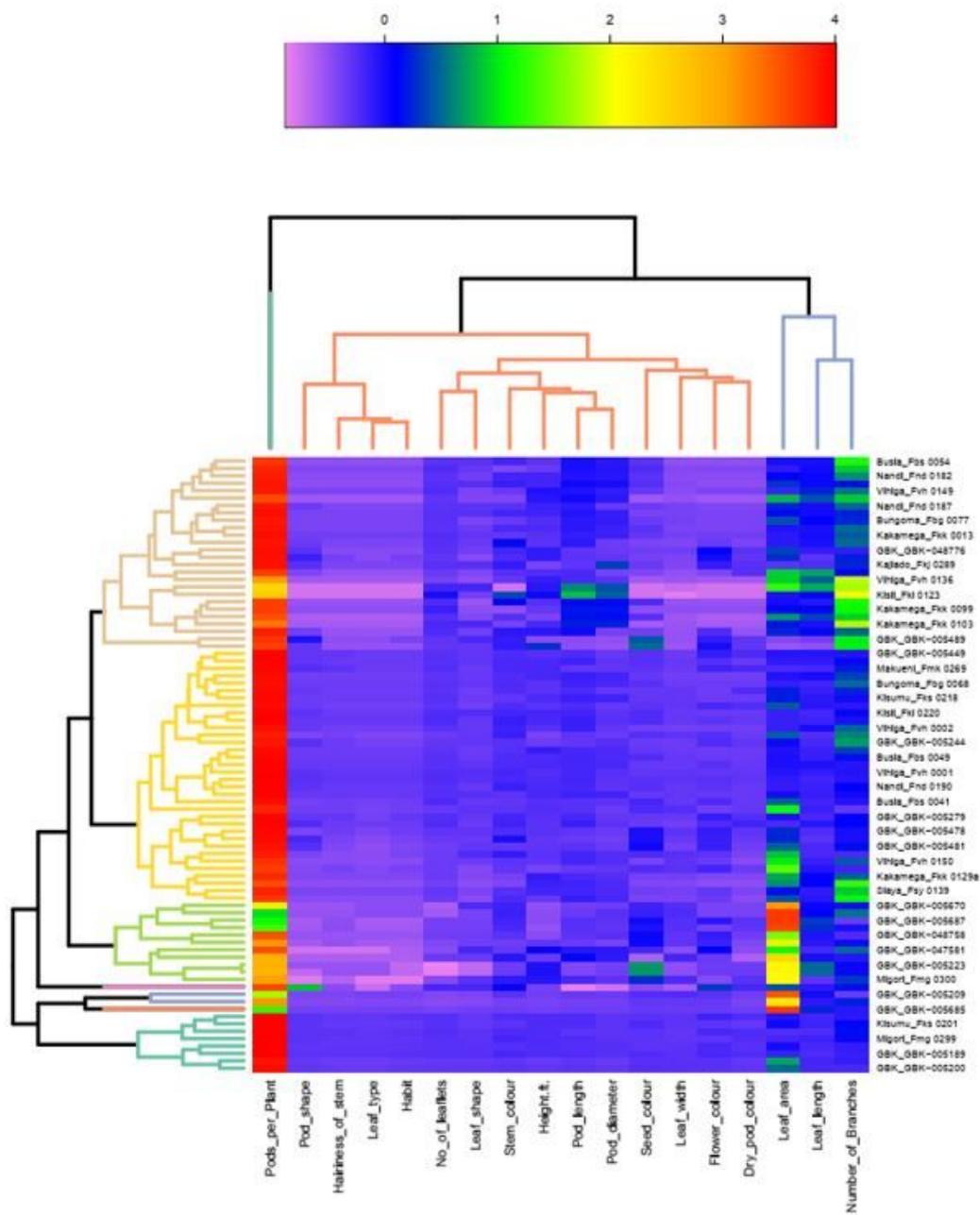


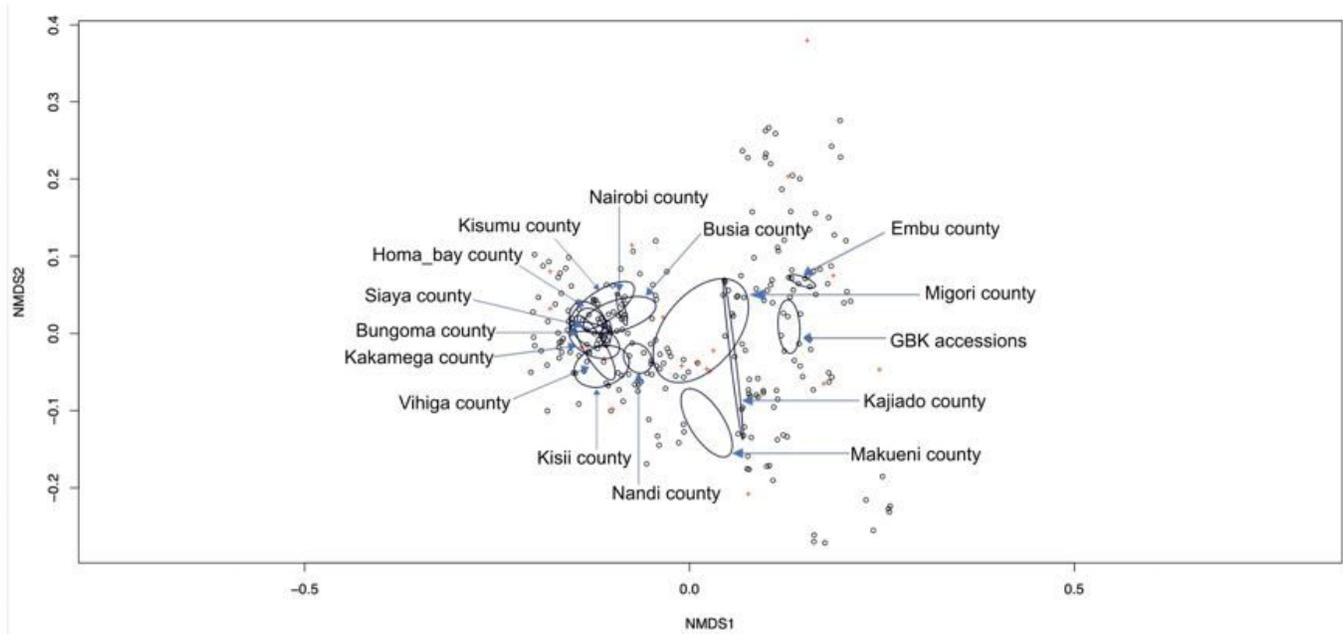
Figure 6

A) Correlation Between Variables and Principal Components, B) Correlation between 83 *Crotalaria* Accessions from Kenya and Principal Components.



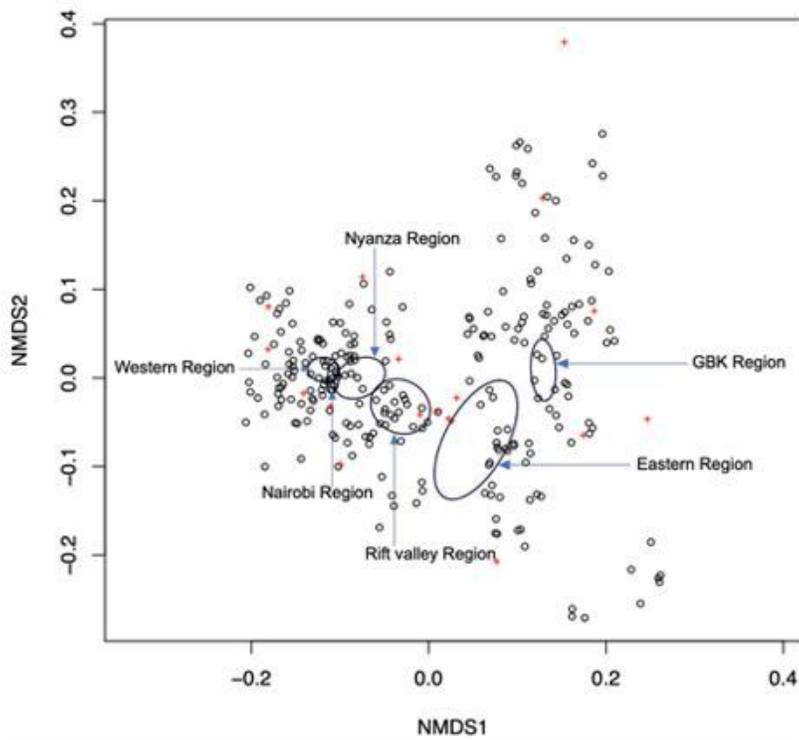
**Figure 7**

Sample clustering of 83 *Crotalaria* accessions from Kenya based on their counties of origin using both quantitative and qualitative characters.



**Figure 8**

Non-metric multidimensional scaling (NMDS) plot of 18 morpho-descriptors for 83 *Crotalaria* accessions sampled from 15 counties in Kenya.



**Figure 9**

Non-metric multidimensional scaling (NMDS) plot of 18 morpho-descriptors for 83 Kenyan *Crotalaria* accessions sampled in 6 regions.

## Supplementary Files

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