Morphological Variation and Discriminating Traits of Kersting's Groundnut Accessions

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Research Article

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Morphological variation 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. Only limited information is available on the morphological traits that can discriminate accessions; without such information, appropriate breeding strategies cannot be devised. This study aimed to identify discriminating traits and assess the 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. An experiment was conducted using 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 contained 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.

1. Introduction

Orphan crops, also known as forgotten or abandoned crops, traditional or underdeveloped crops (Padulosi et al., 2013), are crop species that have received only limited attention from researchers. However, most orphan crops are highly nutritious, climate resilient (Mabhaudhi et al., 2019), and resistant to commonly occurring crop diseases (Andrew et al., 2009). Thus, orphan crops can potentially contribute toward food security and nutritional security, and should receive more research attention.

Kersting's groundnut [*Macrotyloma geocarpum* (Harms) Maréchal & Baudet)] is a multipurpose legume crop that is widely grown in West Africa and Central Africa (Adu-Gyamfi et al., 2011; Abiola and Oyetayo, 2015). It is reportedly an orphan and underutilized crop species (Adu-Gyamfi et al., 2012; Dansi et al., 2012) that thrives well in semi-arid zones with an annual rainfall of <600 mm (Achigan Dako and Vodouhè, 2006). Kersting's groundnut has high nutritional value, as it contains 21.3 g protein per 100 g of grain (Ajayi and Oyetayo, 2009). 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 (Ajayi and Oyetayo, 2009). Seeds have high vitamins contents (Leung et al., 1968). According to Adazebra (2013), 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, selling at US$ 5-7 per kilogram (Agoyi et al., 2019). Despite its nutritional and economic values, 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 (Ayenan and Ezin, 2016). It is not a priority crop for governments and researchers (Dansi et al., 2012; Adazebra, 2013; Assogba et al., 2015).

Agro-morphological characterization is a key step in assessing genetic diversity to classify germplasm of cultivated plants (Boyé et al., 2016; Radhouane, 2004). To do so, researchers use descriptors. However, descriptors for Kersting's groundnut have not been described, unlike Bambara groundnut (*Vigna subterranea* (L.) Verdc.) (IPGRI et al., 2000), peanut (*Arachis hypogaea* L.) (IBPGR and ICRISAT, 1992) and pigeonpea (*Cajanus cajan* (L.) Millsp.) (IBPGR and ICRISAT, 1981). Only a few studies have focused on studying morphological variation in Kersting's groundnut, most of which evaluated either few number of accessions or accessions from only one country. Assogba et al. (2015) and Akohoue et al. (2019) in Benin and Adu-Gyamfi et al. (2012) in Ghana and Bayorbor et al. (2010) in Nigeria reported significant variation for various traits among accessions.

The present study aimed at filling the above-mentioned gaps by i) assessing diversity among accessions in 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.

2. Materials and methods

2.1. Study area

The study was carried out at 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 generally bimodal but can also be unimodal. The soils are ferralitic, ferruginous and hydromorphic. Rainfall, temperature, sunshine, and relative humidity during the period of experimentation are presented in Table 1.

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

2.2. 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 23 August 2018. The experiment was conducted using 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.

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

2.3. Data collection and analysis

The quantitative traits evaluated were: 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. In addition, data were collected on 12 qualitative traits (Table 3).

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 (IPGRI et al. 2000) and peanut (IBPGR and ICRISAT 1992). Observations were made on 20 randomly selected plants within each plot. Standardized Shannon-Weaver index (H') was calculated for the qualitative traits (Ghimire et al., 2018; Yadav et al., 2018). 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 (Ghimire et al. 2018; Yadav et al. 2018), we 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 (Anderson et al., 2008; Sengupta and Sil, 2020). 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 to assess the level of phenotypic variation in quantitative traits, as follows:

$$\mathrm{CV}\ (\%) = \frac{\mathrm{s}}{\bar{x}} \times 100 \qquad \text{(Abdi, 2010)}$$

where s = the standard deviation and $\bar{x}$ = the mean.

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

$$\mathrm{H'} = \frac{[\Sigma\ (\mathrm{n}\ /\ \mathrm{N}) \times \{\log 2\ (\mathrm{n}\ /\ \mathrm{N}) * (-1)\}]}{\log_2 \mathrm{k}} \quad \text{(Yadav et al.2018)}$$

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.

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):

$$Y_{ijk} = \mu + G_i + R_j + B_k + \varepsilon_{ijk} \quad \text{(Asharaf et al. 2013)}$$

where Y_{ijk} = value of the observed quantitative trait; μ = population mean; G_i = 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 relationship between 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 used to describe the clusters. All analyses were performed in R software 3.5.2. (R Core Team, 2019).

3. 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 flower color could be used as key qualitative descriptors for Kersting's groundnut.

A large majority (73%) of the accessions in the collection exhibited prostrate growth habit. This could be used to inform breeding strategies. For instance, Ndiang et al. (2012) 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). Big seed could be used as a selection criterion to improve yield.

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

Most accessions (88.88%) had greenish-white flowers (Figure 1a) and the rest of them (11.12%) had purple-tinted white flowers (Figure 1b). Similarly, 95.06% of the accessions had white pods (Figure 1c) and only 4.94% 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), while 8.64% had black seed coat (Figure 1f) and 1.24% had red-brown seeds (Figure 1g). This result could be explained by the fact that black-seeded and the brown-seeded accessions were rare and were produced by a few households on a small scale (Akohoue et al. 2018).

Similarly, three variants were recorded for seed-eye shape. A large majority of accessions (85.20%) had triangular seed eyes (Figure 1i), while 7.40% had butterfly-like seed eyes (Figure 1h), and 7.40% had irregular seed eyes (Figure 1j).

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.

Grain yield and yield components, i.e., 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 on bambara groundnut (Boyé et al. 2016), cowpea (Gbaguidi et al. 2015) and Kersting’s groundnut (Assogba et al. 2015), where high and significant coefficients of variation were observed for number of flowers per plant. The high CV values are indicative of the existence of substantial diversity among accessions, offering opportunities for improving the trait(s) studied. However, this study showed low variation for seed size (CV= 5.06 %, 4.63 %, and 5.37 % for seed length, width and thickness, respectively). Seed size is an important trait for Kersting’s groundnut, since 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 bear high chances of leaving out many pods are high. In addition, tiny seeds lead to increased loss during shelling and winnowing. The importance of seed size in Kersting's groundnut has been recognized in previous studies. For instance, Amujoyegbe et al. (2007) reported small seed size to be one of the major causes of decline of Kersting's groundnut production in Nigeria. Breeding for bigger seeds, in addition to improving yield, would relieve women from drudgery while hand-picking and shelling Kersting's groundnut pods. Consistent with Assogba et al. (2015) [100-seed weight, (range =10.70 to 14.71 g)] and Akohoue et al. (2019) [100-seed weight (range = 7.10 to 16.28 g)], the present study showed significant variation ($p < 0.001$) for 100-seed weight (range = 8.14 to 18.64 g) (Table 5). The slightly bigger seeds observed could be explained by different experimental conditions (climatic and soil conditions), as reported by Khan et al. (2010) that accumulation of reserves in seeds depends 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.

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

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 ($P<0.05$). Accessions did not vary significantly based on traits such as number of pods per plant, pod width, number of seeds per pod, number of leaves per plant and grain yield. This difference observed could be explained by genetic variation among the accessions.

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

The correlation analysis revealed strong relationships between some of the parameters assessed (Table7). 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. (2017) on Bambara groundnut. The positive correlation between some of the traits can be exploited for 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, low and negative but significant correlation (r= -0.17) was found between days to 50% flowering and number of pods per plant (Table7). These results corroborate results of Assogba et al. (2015) and Yadav et al. (2015).

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

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.

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

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 were 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 (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).

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

On their part, 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.

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

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 characterization using appropriate molecular marker systems, such as simple sequence repeats (SSRs) or single nucleotide polymorphisms (SNPs) is performed. To date, only one molecular diversity study has been reported using isozymes in this species and no diversity was observed (Pasquet et al., 2002). 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 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 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 PC2 on the negative side (Figure 4).

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

The PC2 explained vegetative growth. PC3 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 PC3 (Figure 5). The PCA showed that accessions with a high number of flowers also had long pods, long and tick 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 only three were 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.

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

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

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

The first, second and third clusters contained 68, 3 and 10 accessions, respectively. Cluster 1 (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 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 (14.7 ± 0.39) (Table 9). Overall, C1 was characterized by accessions with high vegetative growth and late flowering. 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 fewer days to 50% flowering (46.11 ± 0.11). In addition, accessions in C2 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. 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), long leaflet (69.4 ± 0.71) and wide seed width (6 ± 0.06 mm) but low number of branches per plant (6.56 ± 0.37). Overall, 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 C3 showed high performance 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 C3 to obtain improved pure cream varieties with high yield performance.

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

4. 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, 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 discriminated the accessions. This could be a starting point for the establishment of a list of descriptors to be measured while studying the crop.

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3. 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.

4. Authors contributions

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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Figures

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 1 f) Black coat seed, Fig 1g) brown coat seed, Fig 1h) butterfly shape eyes, Fig 1i) triangular eyes, Fig 1j) irregular eyes.

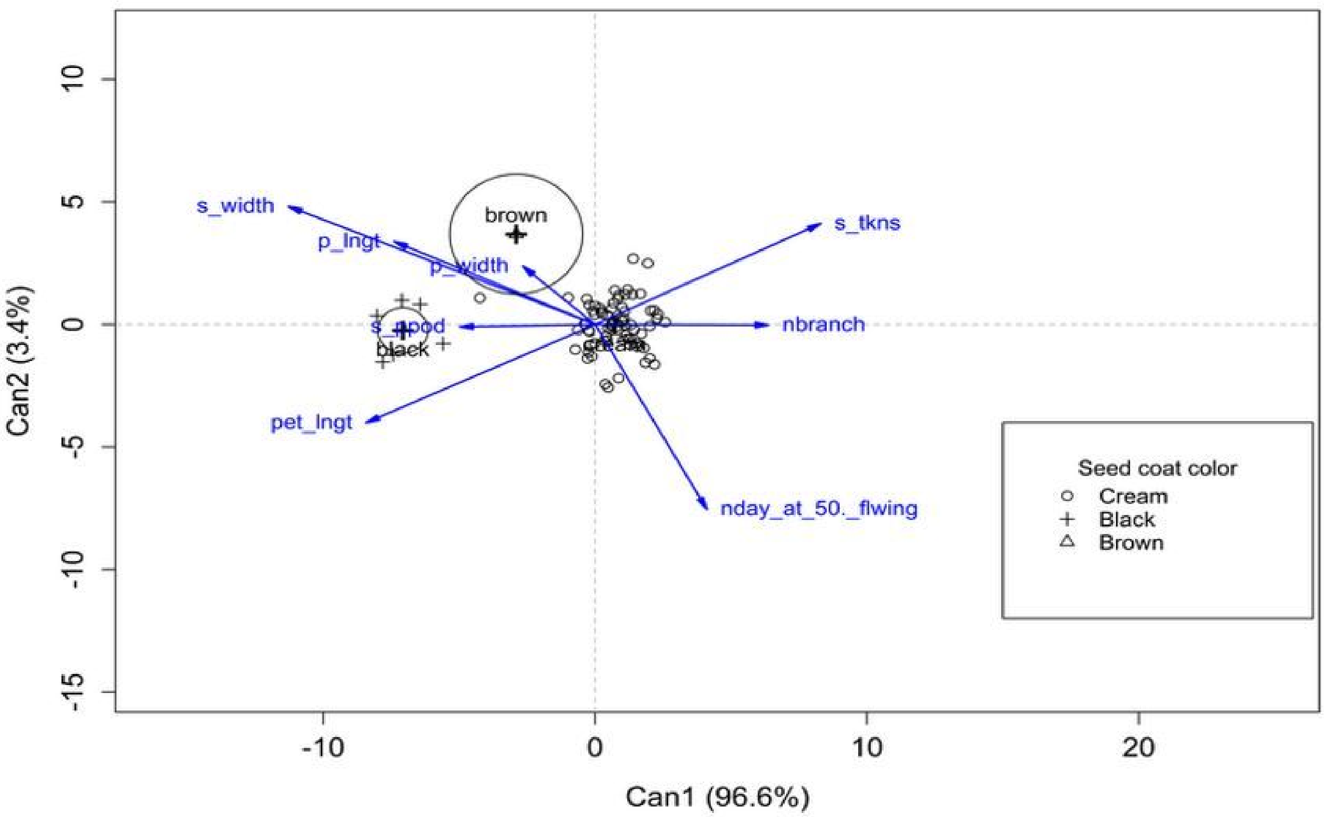

Figure 2

projection of discriminating traits with seed coat color onto the canonical axes 1 and 2.

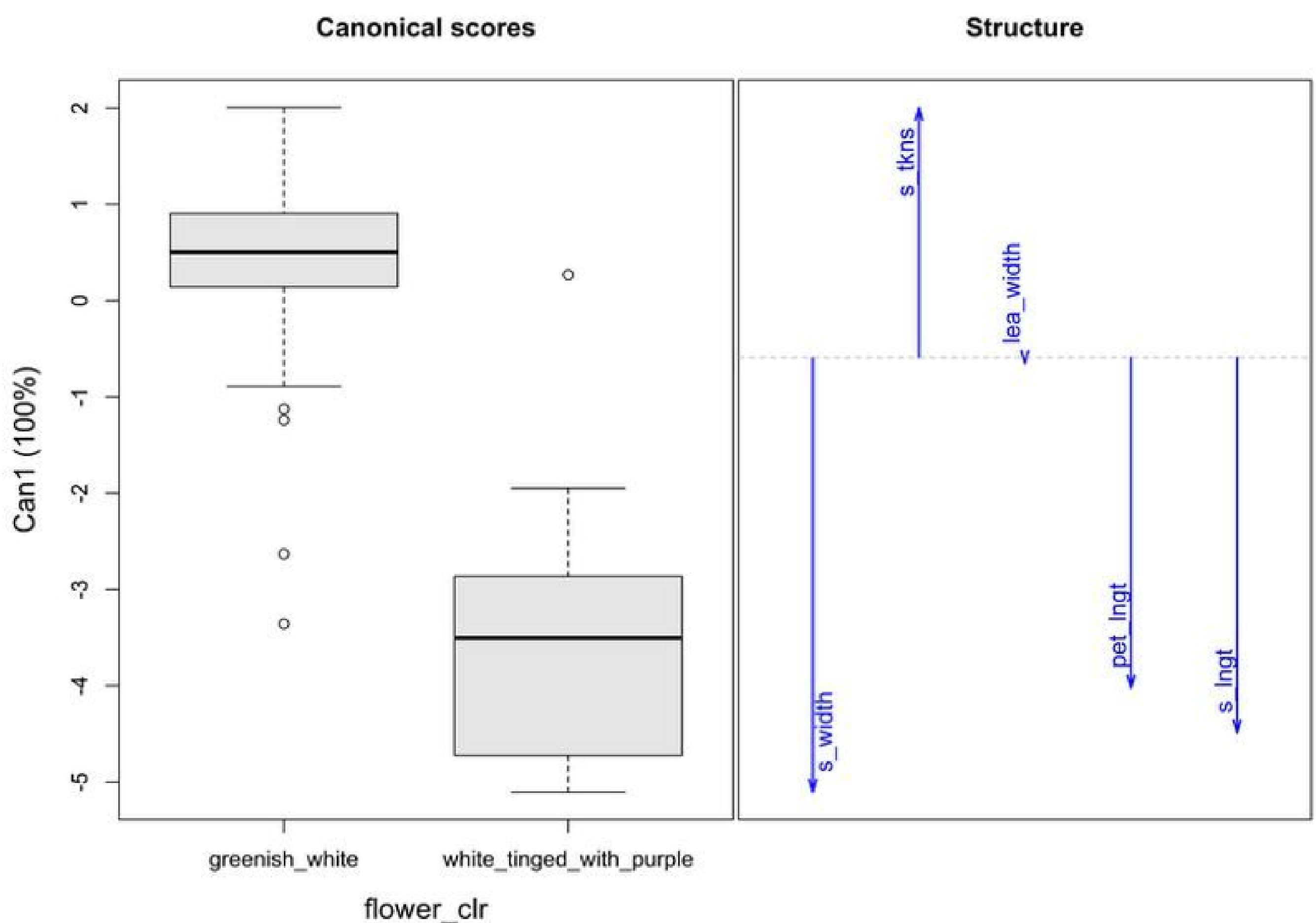

Figure 3

Boxplots showing relationship between flower color and quantitative traits.

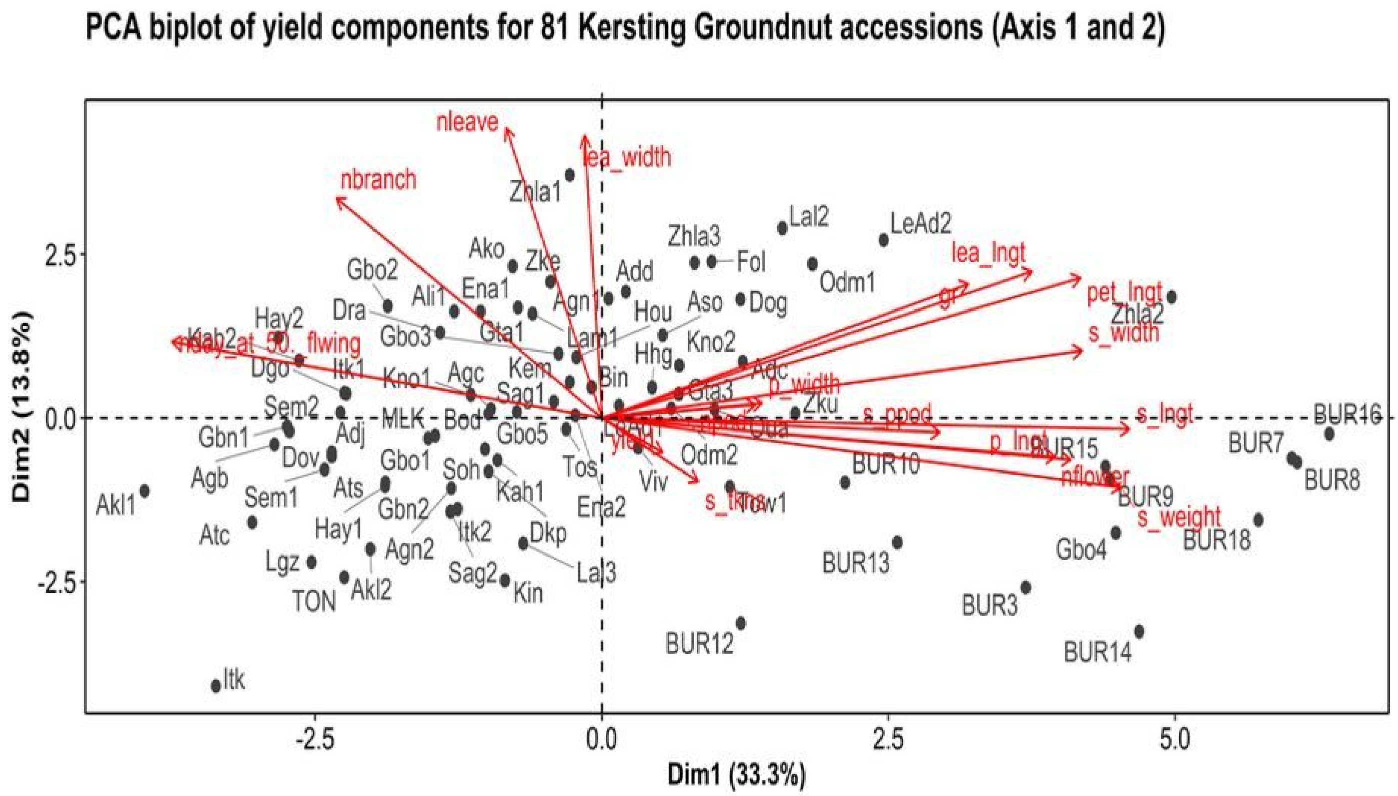

Figure 4

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

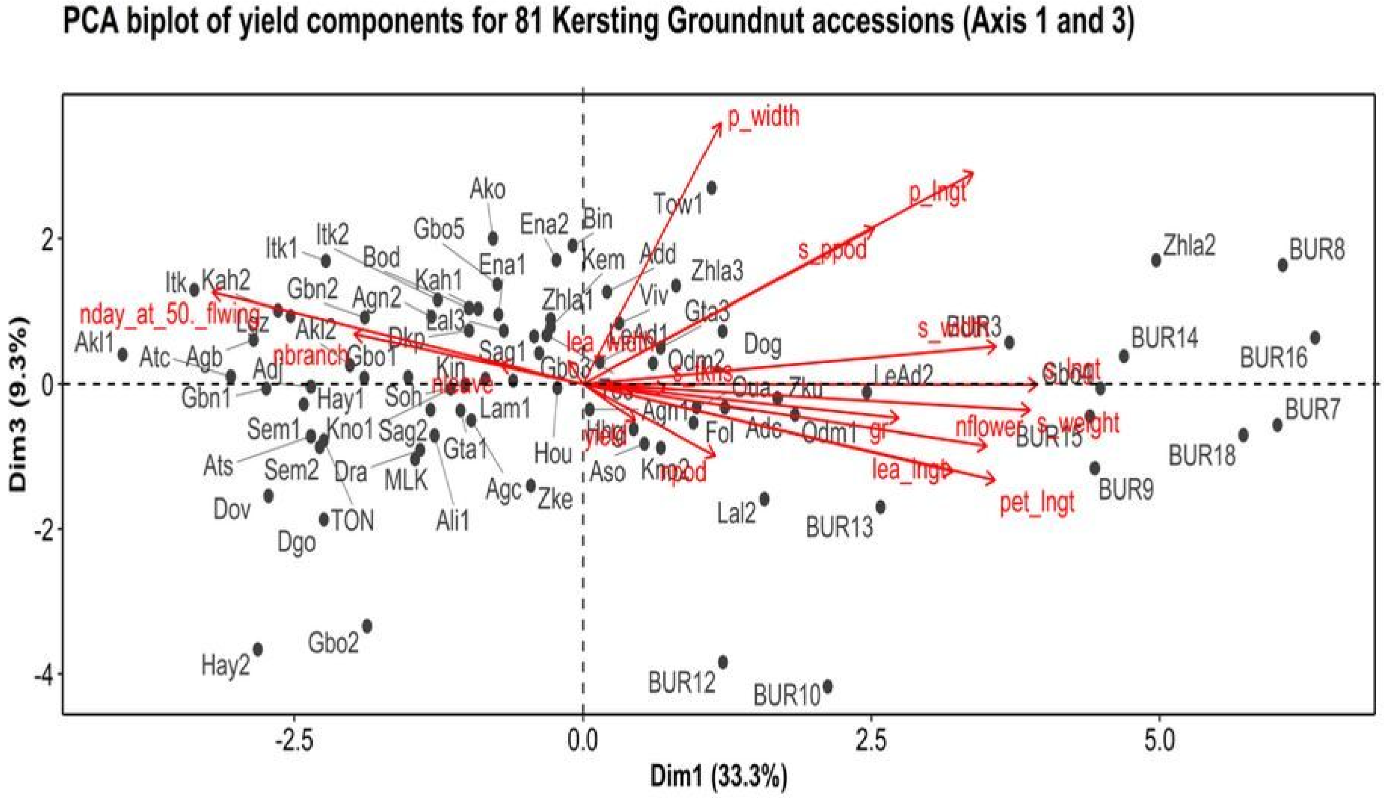

Figure 5

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

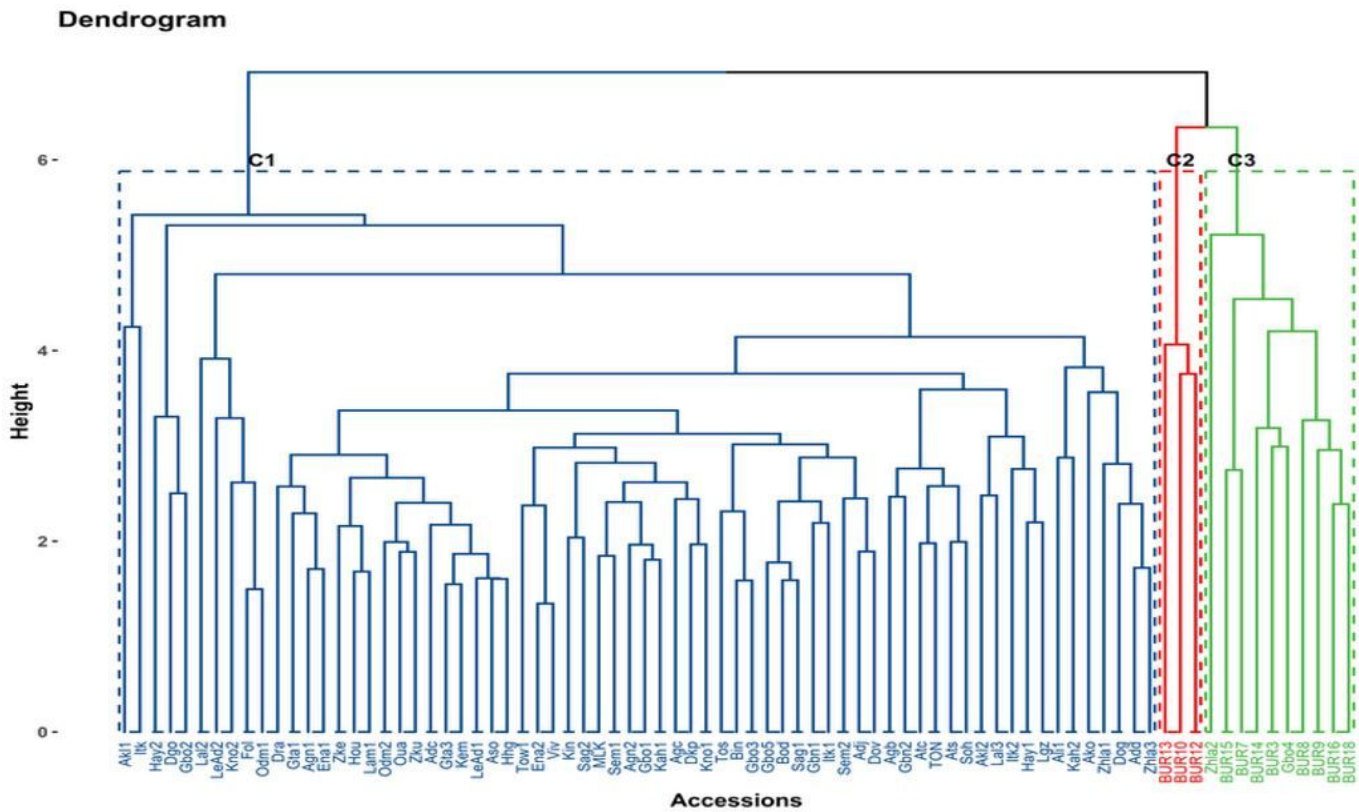

Figure 6

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