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Research Article
Digital Imaging Characterization of Kenaf (*Hibiscus cannabinus* L.) Seeds

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Abstract
Background and Objectives: Digital imaging which had been identified as a fast and reliable method for cultivar discrimination. Therefore, the objective of this study was to characterize 15 kenaf accessions using digital imaging. **Materials and Methods:** Twenty five seeds each of the kenaf accessions were subjected to digital imaging analysis using the WinSEEDLE™ software to distinguish the seed morphometric traits of available kenaf accession and the possibility of using the morphometric data to determine variations between the accessions. Data on seed imaging were subjected to one-way analysis of variance, principal component analysis, Pearson's correlation analysis and the single linkage cluster analysis. **Results:** Seed area (0.343), straight width (0.327), curved width (0.305), volume circle (0.337), surface area circle (0.340) and shape factor (0.310) contributed highly to the variation among the seeds suggesting their potential usage for cultivar discrimination by digital imaging of kenaf seeds. **Conclusion:** Variations existed among the seeds, breeders interested in seed improvement of kenaf may consider these seed traits when selecting for seed quality.

Key words: Morphometry, principal component analysis, digital imaging, dendrogram, seed quality

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Kenaf (*Hibiscus cannabinus* L.) is a fibre plant native to East-Central Africa where it has been grown for several thousand years for food and fibre[1]. It is an annual spring crop cultivated for long (4000 BC) and its origin is from Africa[2]. Kenaf grows in tropical and temperate climates and thrives with abundant solar radiation and high rainfall. Under good conditions, kenaf will grow to a height of 5-6 m in 6-8 months and produce up to 30 t ha^{-1} of dry stem material[3]. Kenaf yields approximately 3-5 times as much fibre as southern pine[1]. Essentially, kenaf is a traditional, 3rd world crop that is poised to be introduced as a new, annually renewable source of industrial fibre in the so called developed economies[4].

The kenaf plant is composed of multiple useful components (e.g., stalks, leaves and seeds) and within each of these plant components there are various usable portions (e.g., fibres and fibre strands, proteins, oils and allelopathic chemicals). The combined attributes of these components provide ample potential product diversity to continue the use and development of this crop[5].

Kenaf is one of the most important fibre crops in the world. It has been cultivated and used as cordage crop to produce twine, rope, gunny-bag and sackcloth for over six millennia[2,6].

Seed analysis is becoming increasingly important, both for quality control in seed production and for harvest classification. Fast and easy to achieve image-based measurements can thus provide data correlating with genetic properties of germination and growth performance[7].

Various methods (e.g., use of charge-coupled device camera, flat bed scanner, X-ray scanning or nuclear magnetic resonance imaging) can be used to obtain seed images showing external or internal features of certain quality factors, such as size, shape, colours and defects[8].

Bio-morphological seed features may be analysed by computer-aided image analysis systems and data quickly processed and stored in the hard disk, plotted or statistically elaborated[9]. These data include relationships among seed size and shape and growth time-course and understanding of growth patterns that produce curvature and inflection points. One of the practical applications of image analysis is to assist researchers and seed analysts in monitoring seed swelling and viability and, so, to overcome some operative limitations of the standard germination test, as approved by ISTA[10] and AOSA[11]. In addition, the assessment of RGB index of each individual seed within a large seed sample may allow the development of non-destructive methods in sorting seed sub-samples with different germination capability[12]. These implications suggest computer-aided image analysis to be a promising technique that should be employed in setting the first approach to seed morphology investigation.

Identification of kenaf varieties is problematic and the understanding of the characteristics and relationships between kenaf germplasm is limited, which significantly hinders their effective utilization and conservation. To date, the identification of a particular kenaf variety remains complex. Digital image analysis offers an objective and quantitative method for estimation of morphological parameters. With the evolution of imaging and computing hardware, several imaging systems have been developed for characterization and classification of many varieties of crops. This study therefore employed this tool in characterizing the kenaf accessions. The aim of this experiment was to characterize the accessions through digital image analysis and subsequently use statistical means to identify and distinguish them from one another.

MATERIALS AND METHODS

Data were collected on 25 seeds from each accession by using an EPSON scanner which was connected to a computer device to acquire image and the Regent Instrument (Regent Instrument Inc, Canada) was used for the image analysis by running the custom written software WinSEEDLE™ (Pro Version). For every replication, 25 seeds was placed on lighting hood in such a way that embryo axis of seed faces image analysis system and longitudinal axis running parallel to the surface of the scanner. Seeds were automatically analyzed by the scanner and the image of the seed recorded by the 'WinSEEDLE™'. The procedure was carried out 3 times in the Laboratory of the Department of Plant Breeding and Seed Technology, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria in 2015.

Data collection: Data was viewed from the review and mean data for each parameter and summed up for average value in the WinSEEDLE™ itself. The parameters that were recorded included:

- Projected Area (PA): Calculated as product of values of the seed length and seed width. (Area = length × width). It is expressed in mm^2
- Straight Length (SL): This is the straight distance between 2 points stretching from the base of the embryo axis to the tip of the endosperm of the kenaf seed. It is expressed in mm

- Curved Length (CL): This is the curved distance between 2 points stretching from the base of the embryo axis to the tip of the endosperm of the kenaf seed. This is expressed in mm
- Straight Width (SW): This is the maximum width measured perpendicular to straight length. It is expressed in mm
- Curved Width (CW): This is the maximum width measured perpendicular to curved length. It is expressed in mm
- Curvature (mm)
- Volume Circle (VC): This is the volume from circular cross-section method. It is expressed in mm^2
- Surface Area Circle (SAC): This is the surface area from circular cross-section method. It is expressed in mm^2
- Eccentricity Index (EI): Estimated as the ratio of seed length to seed width
- Projected Perimeter: This is the perimeter of a circle drawn around the seed touching all edges. It is expressed in mm
- Shape Factor (SF): Seed shape descriptor calculated using the formula $4\pi A/P^2$ where A is the seed area and P represents the perimeter

Analysis of morphometric data: The data collected were subjected to Statistical Analysis System (SAS)[13] procedures for one-way Analysis of Variance (ANOVA) at 5% probability level, Principal Component Analysis (PCA), Pearson correlation analysis and the Single linkage Cluster Analysis (SLCA).

RESULTS

Analysis of variance of seed morphometric parameters of the kenaf accessions is presented in Table 1. Results showed that there were very highly significant differences ($p \leq 0.01$) between the accessions in respect to all the seed morphometric parameters measured except curvature.

Table 2 shows the simple descriptive statistics which include means, standard deviation and standard error. Seed size based on projected area, straight and curved width, curvature, volume circle, eccentricity index and shape factor were lowest in HIB 16. HIB 20 recorded the lowest values of projected perimeter and straight and curved length. The highest projected area, straight and curved width, volume circle and eccentricity index was recorded in HIB 14 while HIB 36 gave the highest straight and curved

Table 1: Result of analysis of variance for seed morphometric parameters of the kenaf accessions

Source of variation	Accessions (df = 14)	Error (df = 30)
Projected area (mm^2)	38.50**	0.85
Straight length (mm)	0.68**	0.09
Curved length (mm)	0.65**	0.08
Straight width(mm)	0.45**	0.01
Curved width(mm)	0.46**	0.00
Curvature (mm)	1.05	1.09
Volume circle (mm^2)	665.36**	0.93
Surface area circle (mm^2)	422.78**	4.68
Eccentricity index	0.006**	0.00
Projected perimeter (mm)	74.09**	0.96
Shape factor	0.098**	0.00

**Significant at $p \leq 0.01$

Table 2: Simple descriptive statistics of seed morphometric traits of the kenaf accessions

Seed morphometric parameters	Mean	Minimum	Maximum	Standard error	Standard deviation
Projected area (mm^2)	21.47	12.20 (HIB 16)	26.40(HIB 14)	0.93	3.58
Straight length (mm)	7.68	6.84 (HIB 20)	8.71(HIB 36)	0.12	0.48
Curved length (mm)	8.29	7.54 (HIB 20)	9.15(HIB 36)	0.12	0.47
Straight width (mm)	3.62	2.53(HIB 16)	4.18(HIB 14)	0.10	0.39
Curved width (mm)	3.89	2.87(HIB 16)	4.65(HIB 14)	0.10	0.39
Curvature	0.31	0.11(HIB 16)	2.44(HIB 36)	0.15	0.59
Volume circle (mm^2)	54.73	20.58(HIB 16)	77.21(HIB 14)	3.85	14.89
Surface area circle (mm^2)	68.43	38.45(HIB 16)	87.24(HIB 14)	3.07	11.87
Eccentricity index	0.64	0.53(HIB 16)	0.73(HIB 14)	0.01	0.05
Projected perimeter (mm)	24.49	20.21(HIB 20)	37.83(HIB 36)	1.28	4.97
Shape factor	0.63	0.20(HIB 16)	0.80(HIB 20)	0.05	0.18

The accessions that recorded the values are indicated in parenthesis

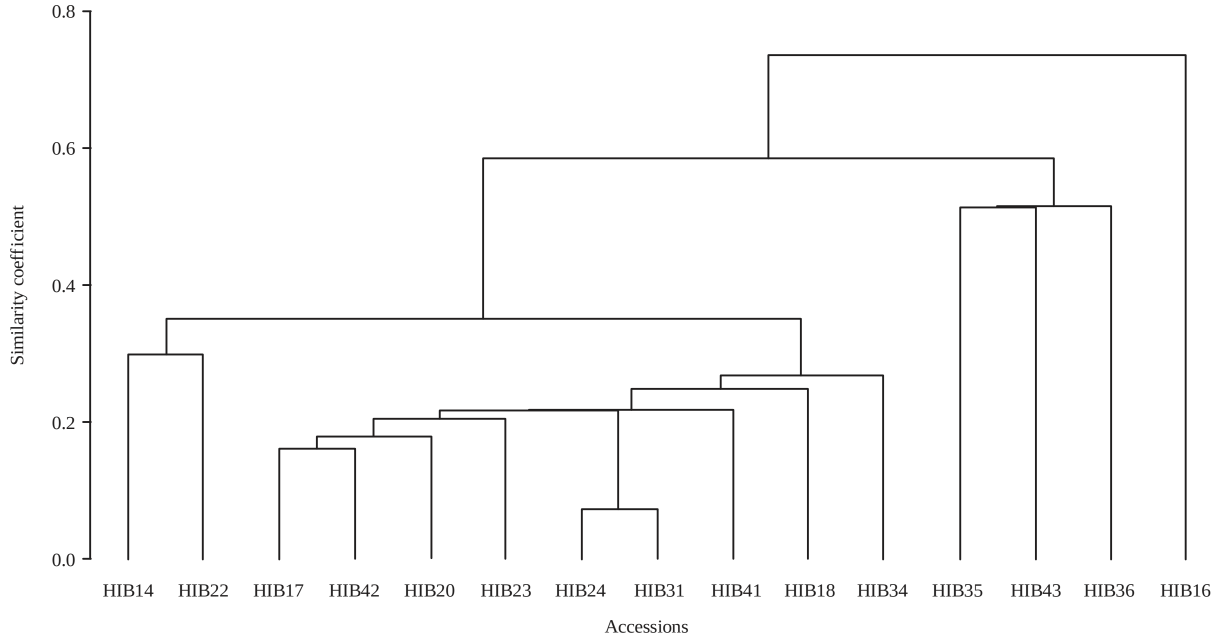

Fig. 1: Dendrogram from euclidean paired group cluster analysis of seed samples of 15 kenaf accessions

Table 3: Correlation coefficients among seed morphometric parameters of the kenaf accessions

	SL	CL	SW	CW	C	VC	SAC	EI	PP	SF
PA	-0.08	0.17	0.87**	0.83**	-0.28	0.93**	0.94**	0.66**	-0.53	0.80**
SL		0.77**	-0.04	-0.08	0.39	-0.17	-0.11	-0.19	0.64**	-0.44
CL			0.24	0.35*	0.11	0.07	0.17	0.05	0.54	-0.22
SW				0.91**	-0.17	0.82**	0.87**	0.83**	-0.51	0.80**
CW					-0.05	0.79**	0.86**	0.82**	-0.36	0.64**
C						-0.27	-0.24	-0.07	-0.39	-0.37
VC							0.95**	0.64**	-0.55	0.76**
SAC								0.72**	-0.51	0.76**
EI									-0.50	0.66**
PP										-0.88**

*Significant at $p \leq 0.05$, **Significant at $p \leq 0.01$, PA: Projected area, SL: Straight length, CL: Curved length, SW: Straight width, CW: Curved width, C: Curvature, VC: Volume circle, SAC: Surface area circle, EI: Eccentricity index, PP: Projected perimeter, SF: Shape factor

length, curvature and projected perimeter. The highest shape factor was also recorded in HIB 20.

The Pearson's correlation coefficients of the studied morphometric traits are presented in Table 3. Correlation coefficient among the 15 kenaf accessions showed that highly significant ($p \leq 0.01$) positive correlation exists between projected area and most other seed morphometric parameters except straight length, curved length, curvature and projected perimeter. Also, straight length was significant ($p \leq 0.05$) and positively correlated with curved length and projected perimeter. Curved length also had significant ($p \leq 0.01$) positive association with curved width. Other measured traits, like curved width, curvature, volume circle, surface area circle, eccentricity index had highly significant and positive correlation with each other while projected perimeter recorded highly significant ($p \leq 0.01$) but negative correlation with shape factor.

Table 4 presents the result of the principal component analysis showing the Eigen values and percentage contributions. The result showed that only two of the principal axes had Eigen values higher than one and they both accounted for 85.14% of the total variance. PC1 accounted for 61.780% and PC2, 23.360%. Principal component 1 was largely loaded with projected area, straight width, curved width, volume circle, surface area circle and shape factor. PC2 was largely loaded with curved length followed by straight length, projected perimeter and curvature. The relative discriminating power of the PCA as revealed by the eigen values was high in PC1 and lower in PC2.

The dendrogram drawn from mean of seed morphometric parameters using Single Linkage Cluster Analysis (SLCA) to show the relationship between the seed of the kenaf accessions is shown in Fig. 1. From the result, the accessions were different from each other at a minimum

Table 4: Eigen values and percentage contributions of each seed's parameters as revealed by principal component analysis

Seed parameters	PC1	PC2
PA	0.343*	0.107
SL	-0.096	0.514*
CL	0.013	0.557*
SW	0.327*	0.128
CW	0.305*	0.222
C	-0.189	0.365*
VC	0.337*	0.043
SAC	0.340*	0.097
EI	0.281	0.074
PP	0.237	0.377*
SF	0.310*	-0.201
Eigen value	8.031	3.036
Variance (%)	61.780	23.360
Cumulative variance (%)	61.780	85.140

*Significant contribution, PA: Projected area, SL: Straight length, CL: Curved length, SW: Straight width, CW: Curved width, C: Curvature, VC: Volume circle, SAC: Surface area circle, EI: Eccentricity index , PP: Projected perimeter, SF: Shape factor, PC: Principal component

distance of 1.00. At 0.63 distance, 3 main clusters were identified with HIB 14 and 22 forming a cluster while seeds of HIB 17, 42, 20, 23, 24 31, 41, 18 and 34 clustered together as a group and HIB 35, 43, 36 and 16 formed the last cluster. At a distance of 0.42, two main clusters were identified. Seed of HIB16 was clearly different from others at a distance of 0.25.

DISCUSSION

Varietal identification or discrimination of cultivars is essential for quality seed production. The measurement of single seed is possible with image analysis technique which is an attractive system easily employed in many environments, non destructive, give a real time analysis and inexpensive. Analysis of seed morphometric is an aspect of image analysis technique (machine vision system) that offers the prospect of studying seed surface features more closely, thereby increasing the available character set. It also has potential use in a wide range of tasks such as determining the cultivar identity of seed lots and testing the distinctness of new cultivars for the award of breeders' right and cultivar registration[14]. The result of the seed morphometric analysis from this study revealed that the kenaf accessions were different from each other as there were differences among the parameters measured at 5% probability level. The kenaf seeds differ significantly ($p \leq 0.05$) from each other in all the morphometric characters measured except curvature. This supports earlier findings by Daniel *et al.*[15] and Adetumbi[16]. From the result, it is clear that variation exist among the seeds of 15 kenaf accessions with respect to some of the characters evaluated. This indicated that descriptors of seed differences can be effectively exploited in physiological studies as stated by Dell'Aquila[9] and in cultivar and genotype description.

Correlation coefficients of most of the morphometric traits in this study revealed significant positive correlation ($r \geq 0.60$) between projected area and most other seed morphometric parameters except straight length, curved length, curvature and projected perimeter.

Also, seed straight length positively and significantly correlated (r = 0.77) with curved length and projected perimeter (r = 0.64).

Seed straight width also had significant and positive correlation ($r \geq 0.80$) with all the parameters measured except curvature and projected perimeter. The positive and significant correlation that exists between these seed metric characters indicates that improving on any of the parameter will improve other characters. Also, since they are significantly correlated, each can be used as substitute in any evaluation and classification studies and as such these characters possessed greater practical value for seed discrimination in kenaf. Wyllie-Echeverria *et al.*[17] did show that seed size and seed metrics were important discriminators of *Zostera marina*. Kaushik *et al.*[18] also reported similar result in Jathropa.

The result of the principal component analysis shows that different characters contributed differently to the total variation as indicated by the eigen values as well as loading on different principal axis. Between the two principal axes that had eigen values higher than one, PC1 that accounted for 61.78% of the variance recorded among the seeds was loaded largely with projected area, seed straight width, curved width, volume circle, surface area circle and projected form coefficient. These results suggested that these traits are the main seed metric variables to select for effective discrimination among kenaf seed. This support earlier findings by Daniel *et al.*[19] who reported that seed area, seed length, perimeter and flatness index contributed largely to the variability in the first two principal component axes of tropical inbred maize genotypes. Adetumbi *et al.*[20] also reported that seed area contributed largely to the variation that exists in PC 1 of 15 kenaf accessions because the seed area is the function of other two variables (seed length and seed width). The dendrogram resulting from the cluster analysis of this study showed that 3 main clusters were identified among the kenaf accessions which further confirm the variation that existed between them.

CONCLUSION

It was concluded that seed area, seed straight width, curved width, volume circle, surface area circle and shape factor contributed significantly to the variation that existed among the seeds. Therefore, breeders interested in seed improvement of kenaf can consider the seed traits when selecting for seed quality.

SIGNIFICANCE STATEMENTS

This study has been able to use software and scanner technology to rapidly and accurately analyse kenaf seeds and to determine its physical characteristics without damaging the seed sample. This is an advancement over the manual way measurement which lacks precision and prone to human errors.

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