

## **Genetic diversity in some Nigerian kenaf (*Hibiscus cannabinus* L.) genotypes as assessed by agro-morphological traits and seed protein markers**

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### **Abstract**

**Identifying and assessing genetic diversity in kenaf (*Hibiscus cannabinus* L.) using agro-morphological markers is problematic, thus, necessitating the use of biochemical markers. Thirty five kenaf genotypes were evaluated for genetic diversity using 13 agro-morphological characters and seed proteins. The field experiment was laid out in a Randomized Complete Block Design with three replications; the data were subjected to Principal Component Analysis (PCA) and Cluster Analysis (CA) to group the genotypes. The observed genetic diversity among the genotypes based on Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of seed proteins was presented in the form of a dendrogram generated by the UPGMA cluster analysis. The relative effectiveness of the two types of markers in assessing the diversity among the genotypes was investigated. The CA grouped the 35 genotypes into eight clusters; the PCA revealed that the first three principal components accounted for 66.60% of the total variation among the genotypes. Plant height, stalk height, internode distance, number of seeds/pod, basal stem diameter, number of pods/plant, pod length and seed yield/plant were identified as traits that best described the genotypes. The seed protein dendrogram, in addition to grouping the 35 genotypes into four major clusters, was able to identify duplicates within the population.**

Keywords: Biochemical markers, principal components analysis, dendrogram, duplicates.

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### **Introduction**

Kenaf (*Hibiscus cannabinus* L.) an herbaceous annual crop of the Malvaceae family grows in tropical and temperate climates and thrives with abundant solar radiation and high rainfall. Under good conditions kenaf grows to a height of 5 – 6 meters in 6 – 8 months and produces up to 30 tonnes per hectare of dry stem material (Wood, 2003). The kenaf plant is composed of multiple useful components (stalks, leaves, and seeds) and all these components have industrial importance; it is thus, a plant of high economic potential to exploit. In the past, the importance of the crop was mainly focused on

paper production, while the last two decades, kenaf has been characterized as a multipurpose crop owing to its high number of industrial applications (fibres and fibre strands, protein, oils, and allelopathic chemicals), medicines and oil absorbents. (Alexopoulou and Monti, 2013; Falasca et al., 2014).

The total production of kenaf in Africa was just 6 % of the world production in the year 2015 (FAO, 2015). Although kenaf is not new to Nigeria, the need to identify and develop commercial varieties necessitates the evaluation of available accessions in the germplasm. Identification of kenaf varieties is problematic, which significantly

hinder their effective utilization and conservation. Characterization and understanding the plant characteristics will provide information about genetic diversity, identification of diverse parental combinations to develop segregating progenies with maximum genetic variability for further selection (Ojo et al., 2012). Agronomic, morphological and physiological traits are generally used to characterize the varieties. However, such data usually vary with environments and evaluation of traits is often laborious and time consuming (Ayo-Vaughan et al., 2010). In view of these difficulties, bio-chemical and molecular markers received more attention in recent years from the crop geneticists and breeders for the assessment of genetic variability (Rabbani et al., 2001; Ayo-Vaughan et al., 2010). Among bio-chemical techniques, Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is one of the most widely used due to its validity and simplicity in estimation of genetic diversity of crop germplasm in comparison with other classes of molecular markers. Because it is simple to analyze the technique, it is inexpensive, affordable and commonly used in practical plant breeding (Abd El-Hady et al., 2010). It is based on the separation of seed proteins according to their electrophoretic mobility which is largely independent of environmental fluctuation. This

study was conducted to investigate the genetic diversity in thirty five kenaf genotypes on the basis of agro-morphological traits and seed protein, and to ascertain their genetic relatedness.

### Materials and Methods

Thirty five kenaf genotypes used in the study were obtained from the germplasm of the Institute of Agricultural Research and Training (IAR&T) Ibadan, Oyo State, Nigeria (Table 1). The experiment was conducted at the Teaching and Research Farm, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria during the late rainy season (September-December, 2013). The experiment was laid out in a Randomized Complete Block Design with three replications. Three seeds each of the genotypes were sown to a two-row plot of 3m long each. Each row was spaced 0.50m apart, within row spacing was 0.25m. The plants were later thinned to two plants per hole at two weeks after planting to give a total of 52 plants per plot, per replicate. Immediately after thinning, NPK 12:12:17 fertilizer was applied to the plants at the rate of 7 g per plant. Field pests were controlled using insecticide (Cypermethrin 10% EC) at the rate of 13 mls in 20 litres of water and weeding was done manually when necessary.

**Table 1:** Kenaf genotypes used in the study

S/No	Genotype	S/No	Genotype
1	Ex-Giwa34 <sup>1</sup>	19	AU 2452 1
2	Purple Flower	20	AU 2452 3
3	2QQ17 <sup>2</sup>	21	A-60-282-15 <sup>7</sup>
4	B1 100 10 <sup>1</sup>	22	Tainung 221 <sup>4</sup>
5	Fanek 39	23	Tainung 221 <sup>2</sup>
6	S 108 14 47 <sup>3</sup>	24	Cuba-Ovate 5 <sup>1</sup>
7	HC 583 31 <sup>2</sup>	25	Cuba 19 <sup>3</sup>
8	25 Krad 3 <sup>1</sup>	26	Cuba 19 <sup>2</sup>
9	G45 2 <sup>3</sup>	27	AC-313 30 <sup>2</sup>
10	AU 245 2 <sup>4</sup>	28	AC 313 24 <sup>4</sup>
11	Local Line 35	29	AU-245 2 <sup>5</sup>
12	AU 72 4 <sup>8</sup>	30	Ex- Shika 24 <sup>2</sup>
13	A-60-282 15 <sup>3</sup>	31	2QQ1 <sup>3</sup>
14	HC-583 31 <sup>1</sup>	32	A-60 282 5 <sup>1</sup>
15	AU-71 9 <sup>3</sup>	33	G422
16	AU-75 41 <sup>4</sup>	34	AU 2452
17	AU-7541 <sup>3</sup>	35	2QQ17 <sup>3</sup>
18	AU 71 9 <sup>2</sup>		

Agro-morphological data were collected on 10 randomly selected plants per genotype in each replicate for number of branches, plant height (cm), stalk height (cm), basal stem diameter (cm), internode distance (cm), days to flowering, number of pods per plant, pod length (cm), pod weight (g), number of seeds per pod, stalk weight per plant (g), 100 seed weight (g) and seed yield per plant (g). The data collected were subjected to principal component and cluster analyses using procedure of the SAS/PC version 9. Principal component grouping of the characters was employed to examine the percentage contribution of each character to the total genetic variation while the cluster analysis was performed to summarize relationship among the genotypes into a dendrogram using the un-weighted pair group method with arithmetic mean (UPGMA). The genotypes were also subjected to a classificatory programme, FASTCLUS technique of SAS, which grouped the genotypes into clusters.

#### *SDS-PAGE protocol:*

This experiment was carried out at the Central Biotechnology Laboratory of the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

Freshly harvested seeds of each kenaf genotypes were ground into powder using a sterilised laboratory plastic mortar and pestle. 0.03g sieved flour of each genotype was transferred into eppendorf tubes. 800 $\mu$ L extraction buffer (0.1  $\mu$ L Tris, 30  $\mu$ L distilled water which were mixed and their pH adjusted to 7.6 and then the final volume was adjusted to 100 $\mu$ L) with 100  $\mu$ L 2- $\beta$  mercaptoethanol and vortexed. The eppendorf tubes were then kept in the freezer for 15 minutes, then at room temperature for another 15 minutes. Vortexing and freezing were repeated three times. Finally, the samples were centrifuged at 1000 rpm for 2 minutes, and the supernatant were transferred to new eppendorf tubes and stored at 4°C.

#### *Acrylamide gel electrophoresis of the soluble proteins:*

Acrylamide gel was prepared, rinsing the butanol from the top of the gel with water, the water was then drained by inverting the gel. 0.2  $\mu$ L of 10% ammonium persulfate was added, it was TEMED for every 20  $\mu$ L of stacking gel solution and filled to the top of the cassette with

the mixture. Comb was inserted until the teeth were 5mm from the resolving gel, the comb which rested on the top of the well, also divided and leveled with the top of the short plate. Oxygen was excluded while ensuring the dividers fully separated the wells, the gel was then allowed to polymerise for about 15 – 20 minutes.

After preparing the electrophoresis system, protein staining solution (0.75g Tris, 8g sucrose, 2g sodium dodecylsulfate, 0.06g bromophenol blue, 30 $\mu$ L distilled water which after dissolving and adjusting its pH at 6.8, its final volume was reduced to 10 $\mu$ L) and extracted proteins were mixed at the ratio of 1:3 and a sample with the weight of 30 $\mu$ L was loaded into each of the wells of the gel with the aid of a micro-pipette (Firoozi et al., 2010). A standard molecular weight sizing of protein marker (Proteoladder 150) was loaded as a check in the first well. The gel was run at 200V (until bromophenol blue dye was off) for 50 minutes. This was done at room temperature. At the end of electrophoresis, the gel was separated from the system and carefully put in staining solution (the mixture of Coomasi blue, methanol, acetic acid and twice-distilled water with the quantities of 144mg, 60, 24 and 60 $\mu$ L, respectively) for 45 minutes. Destaining of the gels was carried out for 24hrs using a mixture of 60mL methanol, 20mL acetic acid and 120mL twice-distilled water to uncover the bands. The protein bands in the gel were observed under Ultraviolet (UV) lamp and photographed using a digital camera.

#### *Gel band scoring analysis*

The emerged bands on the gels were scored on the basis of presence (1) and absence (0) in each kenaf genotype. Electrophoretic data were documented using a gel documentation system (Bio-Rad, USA) and analysed by using Quantity I – D analysis software and also the dendrogram was constructed with 4.0% tolerance in Un-weighted Pair-Group Arithmetic Mean (UPGMA).

## **Results**

The first three axes accounted for 66.60% of the total variation among the 13 characters that described the 35 genotypes of kenaf (Table 2). Characters with high factor score ( $\geq 0.30$ ) under first principal components (PC1) include plant height, stalk height, internode distance and number of seeds per pod, PC2 was

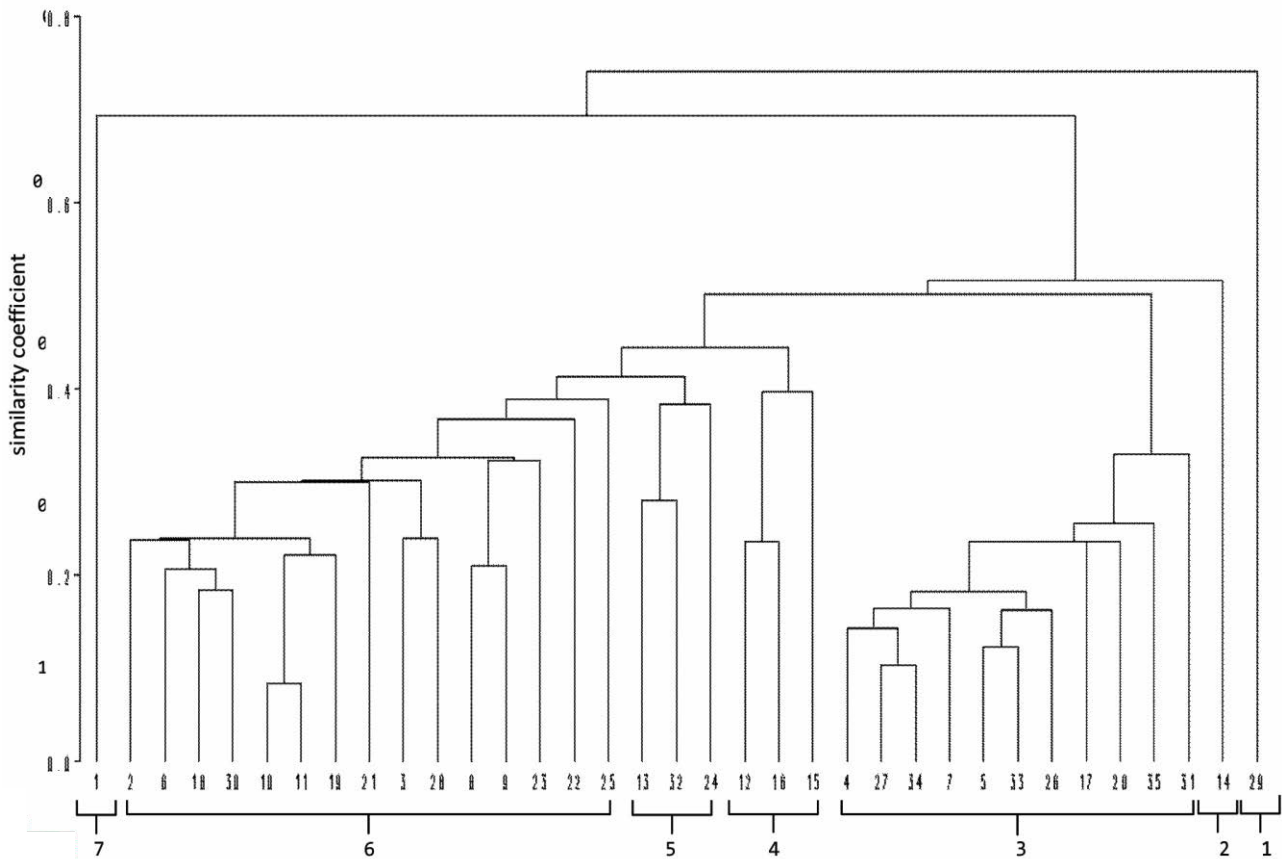
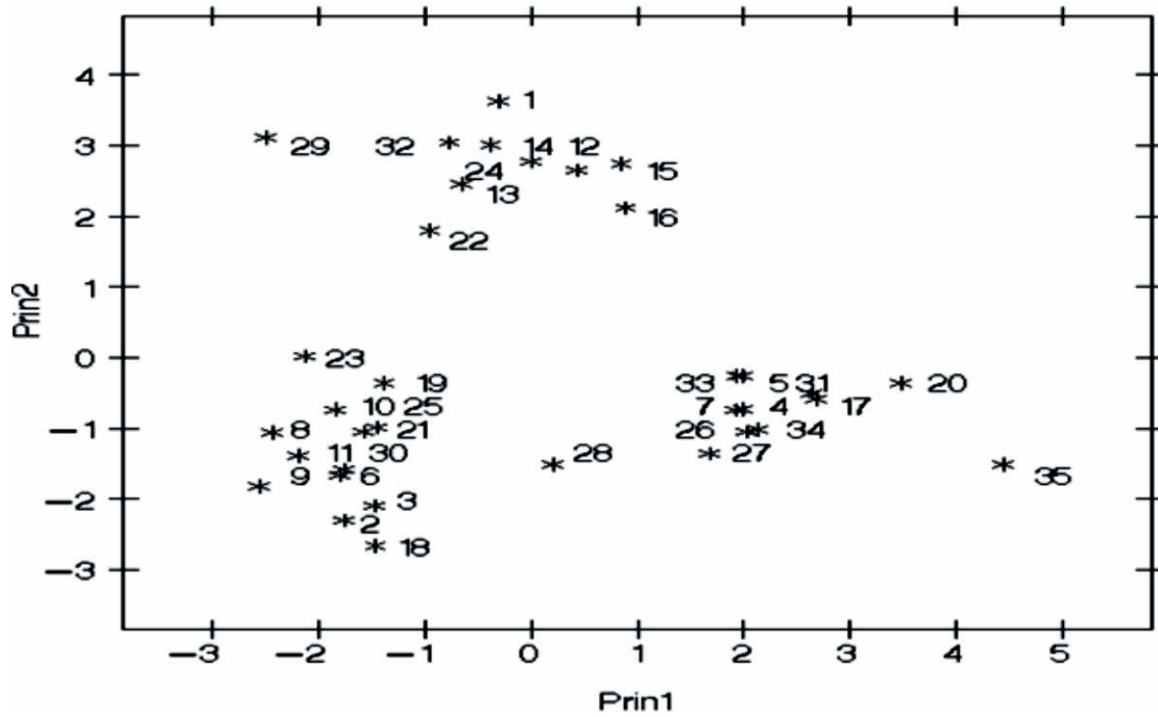
loaded with basal stem diameter, number of pods per plant, pod length and seed yield per plant while the characters that were mostly loaded on the third axis were number of branches, stalk weight and 100 seed weight.

Figure 1 shows the configuration of the genotypes on principal axes 1 and 2, both of which accounted for 55.90% of the total variation. The genotypes were grouped into seven clusters. Genotypes AU-245 2<sup>5</sup> (29), AC 313 24<sup>4</sup> (28), AU 2452 3 (20) and 2QQ17<sup>3</sup> (35) were the most distinct and each formed separate cluster. Using the character variation, cluster analysis summarized the relationship among the

35 genotypes into a dendrogram (Figure 2). At 100% level of similarity, all the genotypes were distinct from each other, while all had formed a single cluster at 27.0% level of similarity. For the entire cluster, genetic similarity level ranged from 27.0% to 94.0%. At the 94.0% level, genotypes AU 2452<sup>4</sup> (10) and Local Line 35 (11) were the closest related genotypes in the population. The dendrogram revealed seven different clusters at similarity level of 60.0%. Clusters 1, 2, and 7 had one genotype each and were the most distinct of the genotypes, AU 245 2<sup>5</sup> (29), HC-583 31<sup>1</sup> (14) and Ex-Giwa34<sup>1</sup>(1), respectively. Cluster 3

**Table 2:** Eigen values, total variance and the eigen vectors of the major characters of the three principal components that described the variation of 13 characters measured on 35 genotypes of kenaf

	PC1	PC2	PC3
Eigen value	3.78	3.49	1.39
Proportion of Variance (%)	29.05	26.85	10.70
Cumulative Variance (%)	29.05	55.90	66.60
Number of branches	0.21	0.09	0.32
Plant height (cm)	0.47	0.10	-0.17
Stalk height (cm)	0.48	0.09	-0.16
Basal stem diameter	0.28	0.40	0.01
Internode distance	0.40	-0.23	0.01
Days of flowering	-0.05	0.23	0.24
Number of pods/Plant	0.18	-0.38	0.02
Pod length (cm)	-0.23	0.33	0.02
Pod weight (g)	0.21	-0.09	0.17
Number of seeds/Pod	-0.33	-0.32	0.07
Stalk weight (g)	-0.17	0.27	-0.51
100 seed weight (g)	-0.01	0.19	-0.70
Seed yield/Plant (g)	-0.02	0.49	-0.01



comprised of eleven genotypes, with 2QQ1<sup>3</sup> (31) and B1 100 10<sup>1</sup> (4) inclusive; clusters 4 and 5 had three genotypes each. Cluster 4 had AU-71 9<sup>3</sup>(15), AU-75 41<sup>4</sup>(16) and AU 72 4<sup>8</sup>(12) while cluster 5 had Cuba-comprised of eleven genotypes, with 2QQ1<sup>3</sup> (31) and B1 100 10<sup>1</sup> (4) inclusive; clusters 4 and 5 had three genotypes each. Cluster 4 had AU-71 9<sup>3</sup>(15), AU-75 41<sup>4</sup>(16) and AU 72 4<sup>8</sup>(12) while cluster 5 had Ovate 5<sup>1</sup> (24), A-60-282 5<sup>1</sup>(32) and A-60-282 15<sup>3</sup>(13). However, cluster 7 had the largest number of genotypes, fifteen, with Cuba 19<sup>3</sup> (25) and Purple Flower(2) inclusive.

The characteristic means of the eight cluster groups in the 35 kenaf genotypes generated by the FASTCLUS technique are presented in Table 3. The range of means revealed that plant height, stalk height, days to flowering, number of pods/ plant, stalk weight, 100seed weight and seed yield/ plant contributed the largest proportion of morphological variations observed among the cluster groups. The other traits did not contribute any significant variation between the clusters. Clusters II and III contained one genotype each, while clusters IV and V had three genotypes each. Clusters I, VI, VII and VIII contained 9, 6, 4 and 8 genotypes, respectively. Members of cluster IV recorded the highest value for plant height, stalk height and number of pods/ plant while the only member of cluster III recorded the highest value for 100seed weight and seed yield/ plant. The earliest

flowering genotypes were found in cluster I and they also had the highest value for number of seeds/pod. The only member of cluster II had the shortest plant height and stalk height, but recorded the highest value for stalk weight.

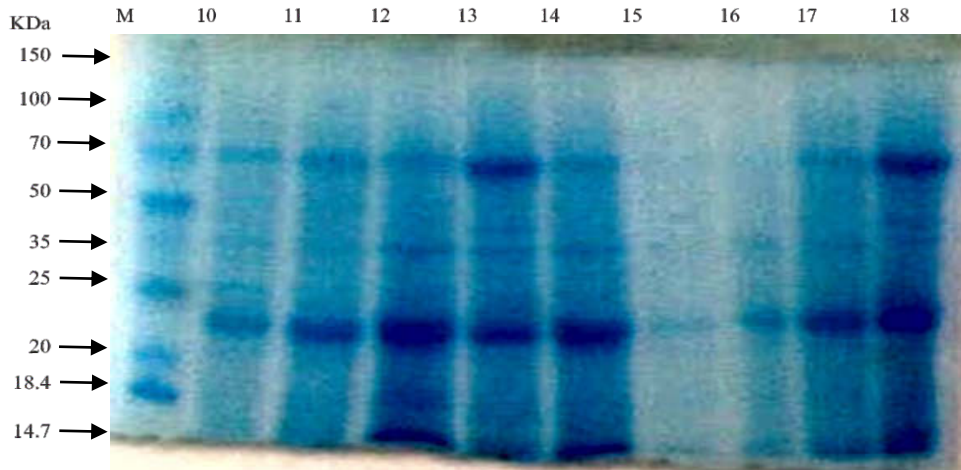
*Seed Proteins*

Ten protein bands from ProteoLadder were used to assess the genetic variability among the 35 kenaf genotypes. Polymorphic bands were detected showing high degree of variation among the genotypes (Plate 1). Eight of the protein bands were polymorphic across genotypes and were able to differentiate at least any two of the 35 kenaf genotypes at a time. Genotypes HC-58331<sup>1</sup>, AU 719<sup>2</sup>, Tainung 221<sup>2</sup> and 2QQ1<sup>3</sup> had the highest (8 bands) and genotypes

Table 3: Major characteristics pattern of eight clusters of the kenaf genotypes

Characters	I	II	III	IV	V	VI	VII	VIII	Min	Max
	2,3,6,8,9,18, 21,23,30	29	1	17,20,31	13,14,32	10,11,22,25, 28,34	12,15,16,24	4,5,7,19,26,27, 33,35		
Number of branches	3.28 (1.13)*	3.17(0.00)	4.50(0.00)	3.69 (1.79)	3.28 (0.63)	3.28 (1.08)	3.92 (0.79)	4.31 (1.02)	3.17	4.50
PH height(cm)	131.05 (4.34)	129.33(0.00)	143.00(0.00)	177.47 (4.47)	152.11 (6.58)	138.17 (4.53)	161.46 (5.75)	163.67 (4.00)	129.33	177.47
Stalk height(cm)	129.20 (4.51)	127.67(0.00)	141.00(0.00)	177.01 (4.43)	151.44 (5.74)	136.64 (5.08)	158.52 (5.46)	162.90 (3.96)	127.67	177.01
Basal stem diameter(cm)	1.25 (0.09)	1.52(0.00)	1.76(0.00)	1.57 (0.12)	1.66 (0.03)	1.30 (0.14)	1.73 (0.05)	1.52 (0.06)	1.25	1.76
Internode dist(cm)	5.15 (0.51)	4.37(0.00)	4.53(0.00)	7.20 (0.47)	4.52 (0.34)	5.04 (0.32)	4.85 (0.54)	6.67 (0.84)	4.37	7.20
Days to flowering	49.48 (1.03)	51.00(0.00)	52.33(0.00)	49.44 (0.51)	51.67 (3.21)	49.61 (0.49)	49.75 (1.71)	49.29 (0.49)	49.29	52.33
No of pods/plant	20.07 (4.55)	11.80(0.00)	16.00(0.00)	24.28 (1.46)	16.45 (0.69)	19.64 (3.28)	13.52 (0.85)	22.40 (0.77)	11.80	24.28
Pod length (cm)	1.62 (0.10)	1.70(0.00)	1.71(0.00)	1.58 (0.05)	1.70 (0.03)	1.63 (0.11)	1.70 (0.04)	1.53 (0.04)	1.53	1.71
Pod weight(g)	1.21 (0.08)	1.13(0.00)	1.10(0.00)	1.29 (0.05)	1.10 (0.04)	1.20 (0.16)	1.14 (0.04)	1.93 (2.07)	1.10	1.93
No. of seeds/pod	15.74 (0.81)	13.00(0.00)	13.33(0.00)	13.28 (0.12)	13.33 (0.34)	14.72 (1.79)	12.75 (0.57)	13.27 (0.44)	12.75	15.74
Stalk weight(g)	41.23 (5.29)	68.23(0.00)	28.97(0.00)	41.08 (3.36)	58.05 (7.50)	40.80 (3.84)	46.15 (5.67)	36.81 (2.59)	28.97	68.23
100 seed weight(g)	5.32 (0.56)	5.23(0.00)	20.94(0.00)	5.32 (0.07)	5.34 (0.02)	5.06 (0.46)	5.31 (0.02)	5.26 (0.12)	5.06	20.94
Seed yield/plant(g)	26.77 (3.50)	45.90(0.00)	48.40(0.00)	31.65 (1.98)	44.02 (0.36)	35.52 (6.43)	41.94 (4.94)	30.90 (1.73)	26.77	48.40

\* Standard deviation

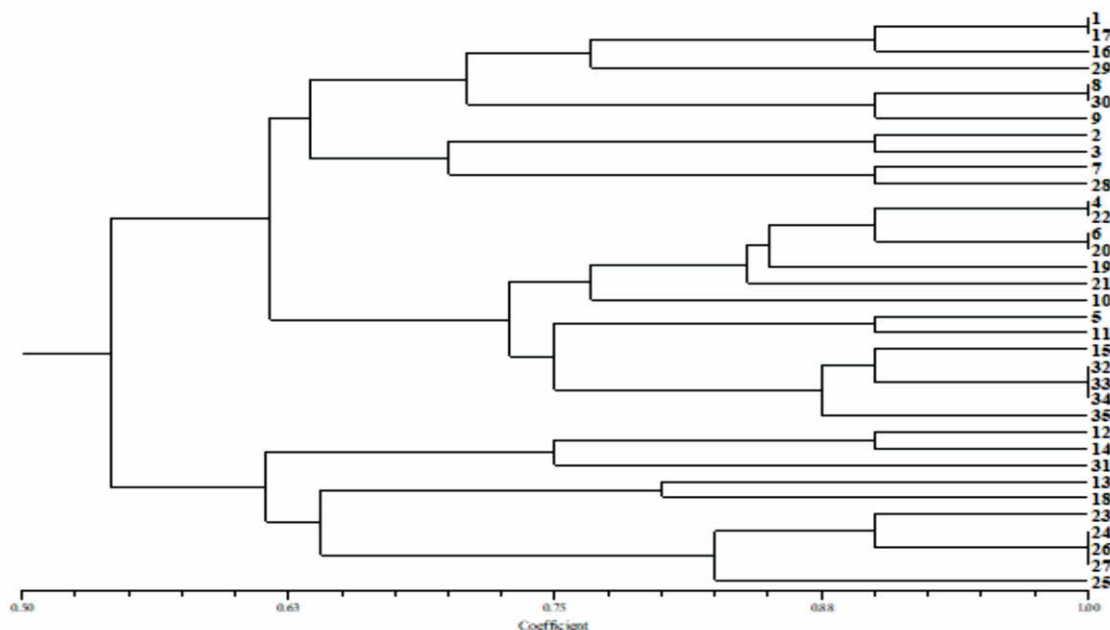


S108 14 47<sup>3</sup> and AU 2452 3 had the lowest (2 bands). The dendrogram based on Nei's similarity index using UPGMA is presented in Fig 3. Genotype Ex-Giwa 34<sup>1</sup>, AU-7541<sup>3</sup> (1, 17), 25 Krad 3<sup>1</sup>, Ex-Shika 24<sup>2</sup> (8, 30), B1 100 10<sup>1</sup>, Tainung 2 21<sup>4</sup> (4, 22), S 108 14 47<sup>3</sup>, AU-2452-3 (6, 20), A-60 282 5<sup>1</sup>, G422, AU-2452 (32, 33, 34), and Cuba-Ovate 5<sup>1</sup>, Cuba 19<sup>2</sup>, AC-313 30<sup>2</sup> (24, 26, 27) had the highest genetic similarity level of 100%. However, at 50% level of similarity all the genotypes had formed a single cluster. At 78% level of similarity, genotypes AU-245 2<sup>5</sup>(29) and 2QQ1<sup>3</sup> (31) were distinct from the rest of the population. It was however observed that a high level of genetic variability was identified among the genotypes within a range of 63.0 - 90.0% genetic distance. At an agglomerative coefficient of 63% similarity level, the genotypes were clustered into four groups. The first cluster was made up of eleven genotype with Ex-Giwa 34<sup>1</sup> (1) and AC-313 24<sup>4</sup> (28) inclusive; cluster 2 had the largest number of genotypes, fourteen, with B1 100 10<sup>1</sup> (4) and 2QQ17<sup>3</sup> (35) inclusive. Cluster 3 had the lowest number of genotypes, three, AU 72 4<sup>8</sup>, HC-583 31<sup>1</sup> and 2QQ1<sup>3</sup> (12, 14 and 31, respectively) while cluster 4 had seven genotypes, A-60-282 15<sup>3</sup> (13) and Cuba 19<sup>3</sup> (25).

**Discussion**

Germplasm evaluation must be considered the first step in the selection of cultivars for a breeding programme and it is commonly based on agronomic and morphological characters (Faruq et al. 2013). Principal component analysis revealed the

importance of plant height, stalk height, internode distance, number of seeds per pod, basal stem diameter, number of pods per plant, pod length, number of branches per plant, stalk weight, 100 seed weight and seed yield per plant to identification of these genotypes. This indicated that both vegetative and reproductive



phases of the plant are important in discriminating genotypes for future breeding exercise. Ghafoor et al. (2003) reported the same findings on agronomic traits in chickpea.

The ordinates of the first two principal axes grouped the 35 genotypes into seven clusters with genotypes 20, 28, 29 and 35 appearing in separate clusters. The character scores, in addition to the characteristic mean values of FASTCLUS grouping of the genotypes confirmed the contributions of the traits to the total variability among the 35 genotypes. Plant height, stalk height, days to flowering, number of pods per plant, stalk weight, 100 seed weight and seed yield per plant, however, contributed the largest proportion of morphological variation that existed between the cluster groups. There was no significant variation between clusters with respect to other traits. The FASTCLUS procedure grouped the genotypes into eight clusters; it however, still maintained different clusters for each of these four genotypes. This indicated the existence of large enough genetic divergence among these genotypes for the traits studied to classify them into different clusters and offered opportunities for using them as parents for their desired traits in future kenaf breeding programmes.

The dendrogram based on agromorphological characters revealed the extent of morphological relatedness among genotypes within each cluster group. However, such classification may not provide an accurate estimation of genetic diversity because of variations in environmental conditions (Ghafoor et al., 2003). The dendrogram obtained from SDS-PAGE molecular markers revealed a considerable intra-specific variation, in addition to duplicates among the kenaf genotypes that indicated the valid utilization of seed protein markers for germplasm classification in kenaf. The genotypes with the highest genetic similarity level of 100%, that is, similar banding patterns may be duplicated in the germplasm.

The study concluded that, both morphological and protein variability analyses clearly showed that there was genetic divergence among these genotypes with respect to the 13 characters studied; however, protein analysis showed higher levels of polymorphism than the morphological markers. The four major clusters of the SDS-PAGE dendrogram together with their internal groups demonstrated the highly polymorphic nature of the protein markers and

wide genetic base of the 35 kenaf genotypes investigated; this could be used to further broaden the genetic base of the crop for better utilization of its genetic potentials in the country. Furthermore, for better management of germplasm, a comprehensive knowledge of morphological and biochemical data (protein and DNA) is essential to eliminate duplicates from germplasm collections (Ghafoor et al., 2003). It is therefore, suggested that the duplicates be confirmed by the use of 2-D electrophoresis focusing as suggested by earlier researchers (Celis and Bravo, 1984).

The multivariate analysis was able to identify plant height, stalk height, days to flowering, number of pods/ plant, stalk weight, 100seed weight and seed yield/ plant as characters that contributed the largest proportion of morphological variations observed among the cluster groups.

## References

- Abd El-Hady, E. A. A., Haiba, A. A. A., Abd El-Hamid, N. R. and Rizkalla, A. A. (2010). Phylogenetic diversity and relationships of some tomato varieties by electrophoretic protein and RAPD analysis. *J. Amer. Sci.* 6 (11): 434-441.
- Alexopoulou, E., and Monti, A. (2013). *Kenaf: A Multi-Purpose Crop for Several Industrial Applications: New Insights from the Biokenaf Project*. Springer-Verlag, London, p. 203. DOI: 10.1007/978-1-4471-5067-1
- Ayo-Vaughan, M. A., Ariyo, O. J., Ojo, D. K., Alake, C. O. and Oduwaye, O. A. (2010). Random Amplified Polymorphic DNA (RAPD) Characterisation of some Nigerian cowpea genotypes. *Nigerian J. of Genet.* 23/24: 101 – 112. URL: <http://ajol.info/index.php/njg>
- Ayo-Vaughan, M. A., Alake, C. O., Ariyo, O. J and Adeniyani, O. N. (2013). Classification of variation patterns in kenaf. *11th African Crop Science Conference Proceedings, Uganda.* 11: 559 – 562. URL : <http://www.ajol.info/andwww.bioline.org.br/cs>
- Celis, J. E. and Bravo, R. (1984). *Two-dimensional gel electrophoresis of proteins: methods and applications*. Academic Press, New York, p. 478



- Falasca, S. L., Ulberich, A. C. and Pitta-Alvarez, S. (2014). Possibilities of growing kenaf (*Hibiscus cannabinus* L.) in Argentina as biomass feedstock under dry sub-humid and semi-arid climate conditions. *Biomass Bio-energy* 64:70–80.
- Food and Agricultural Organization (2015). Jute, kenaf, sisal, abaca, coir and allied fibers Statistics. p. 40. [www.fao.org/3/a-i5609e.pdf](http://www.fao.org/3/a-i5609e.pdf)
- Faruq, G., Alamgir, M. A., Rahma, M. M., Motior, M. R., Zakaria, H. P., Marchalina, B. and Mohamed, N. A. (2013). Morphological charcterisation of kenaf (*Hibiscus cannabinus* L.) in Malaysain tropical environment using multivariate analysis. *J. of Animal and Plant Sci.* 23 (1):60–67.
- Firoozi, B., Sofalian, O. and Seyyed Masoomi, S. Y. (2010). Evaluation of genetic diversity of promising cotton genotypes by seed protein markers. *Plant Ecophysiology* 2: 81-85.
- Ghafoor, A., Gulbaaz, F. N., Afzal, M., Ashraf, M. and Arshad, M. (2003). Inter-relationship between SDS-PAGE markers and agronomic traits in Chickpea (*Cicer arietinum* L.) *Pak. J. Bot.*, 35 (4): 613-624.
- Ojo, D. K., Ajayi, A. O. and Oduwaye, O. A. (2012). Genetic Relationships among Soybean accessions based on morphological and RAPDs Techniques. *Pertanika J. Trop. Agric. Sci.* 35 (2): 237-248.
- Rabbani, M. A., Qureshi, A. A., Afzal, M., Anwar, R. and Komatsu, S. (2001). Characterization of mustard (*Brassica juncea* (L.) Czern. & Coss) germplasm by SDS-PAGE of total seed protein. *Pak. J. of Bot.* 33 (2): 173-179.
- Wood, I. (2003). Kenaf: the forgotten fiber crop. *The Australian New Crops Newsletter* 10. <http://www.newcrops.uq.edu.au/newslett/ncn10212.htm> USDA, FAS