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Genetic Diversity Studies in Kenaf (*Hibiscus cannabinus* L.) through Multivariate Analysis

Jubayer Ahmed[1], Firoz Mahmud[1], Sharmin Sultana[2,*]

[1]Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, Bangladesh
[2]Department of Biochemistry, Sher-e-Bangla Agricultural University, Bangladesh

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Abstract Kenaf is a fast-growing plant of the Malvaceae family. It is also known for its bast fiber. An experiment was conducted with twenty-five kenaf genotypes with geographic origins at the central jute agricultural experiment station of Bangladesh Jute Research Institute (BJRI), Jagir, Manikganj using randomized complete block design with three replications to assess the genetic diversity of various morphological characters. Multivariate analyses were performed for this purpose. Twenty-five kenaf genotypes were grouped into five clusters while cluster II comprises maximum 7 genotypes followed by 5 genotypes in clusters III, IV, and cluster V. The first principal axis, plant height (m), accounted for the majority of the variation among genotypes, accounting for 74.07% of the total variation. The highest fiber weight was recorded by cluster V (19.00) while cluster I (10.33) showed the least fiber weight. The maximum and minimum inter-cluster distances were observed between clusters I and V (13.566) and I and III (2.602), respectively. Cluster I had the greatest intra-cluster distance (0.518), while Cluster V had the smallest (0.092). The inter-cluster distances were larger than the intra-cluster distances. Green bark thickness contributed maximum towards total divergence. The genotypes of clusters I (G5, G11, G12) and V (G1, G2, G22, G24, G25) were more diverse as they could be used as parents for future breeding programs. Recombination is enabled by genetic diversity, which is required for varietal development. Considering cluster distance, inter-genotypic distances G22 and G25 might be suggested for future breeding programs.

Keywords Genetic Diversity, Cluster Analysis, Principal Component Analysis, Kenaf, *Hibiscus cannabinus*

1. Introduction

Kenaf (*Hibiscus cannabinus* L.) is a fiber-yielding annual crop belonging to the Malvaceae family and it is grown mainly for its great air permeability, antibacterial characteristics and outstanding traits like tolerance to salinity, drought resistance, broad adaptability and high fiber output. There is a global commitment to "save the environment" rising global demand for Jute and Allied Fiber (JAF) because natural fibers, unlike synthetics, do not contaminate the environment. Kenaf, a jute replacement, can produce a significant quantity of biomass, thus, it is currently employed as a renewable raw material source for the manufacturing of paper pulp. [1,2]. Although it is indigenous to Africa, kenaf is grown all over the world [3]. There are more than 200 *Hibiscus* species found in tropical and subtropical conditions, [4] but only two of these species, kenaf (*H. cannabinus* L.) and Roselle (*H. sabdariffa L. var. altissima*) have economically importance for pulp and paper manufactures [5].

The bast (bark) and core of kenaf (wood) both contain fibers. The bast accounts for 40% of the plant. When extracted from the bast, "crude fiber" is multicellular in nature made up of numerous distinct cells that have been

adhered together [6]. Edible vegetable oil is produced by kenaf seeds. Additionally, kenaf seed oil is utilized in the creation of biofuels, industrial lubricants, and cosmetics. Numerous omega polyunsaturated fatty acids can be found in kenaf oil (PUFAs). Linoleic acid (Omega-6), a polyunsaturated fatty acid, is abundant in kenaf seed oil (PUFA). Linoleic acid (C18:2) is the most prevalent PUFA, followed by oleic acid (C18:1). There is 2 to 4% alpha-linolenic acid (C18:3) in the whole [7]. Kenaf is rapidly gaining popularity in Bangladesh as a fiber crop, and according to statistics, it is grown on approximately 0.04 million hectares of land [8]. Kenaf generally grows to a height of 14 to 18 feet in six months during the growing season and yields 5 to 10 tons of dry fiber per acre [9].

In Bangladesh, kenaf fiber is blended with jute fiber to make bags, sacks, ropes, cordages and carpets [10]. The ancient use of kenaf as a cordage crop has given way to a variety of new uses, including paper goods, building materials, absorbents, and livestock feed, and the article also disclosed that the production of kenaf goods has reportedly been evaluated for textiles and considered to be another promising usage that can benefit Bangladesh's national economy [6]. The Bangladesh Jute Research Institute has access to more than 6031 unique Jute, Kenaf, Tosha, and Mesta germplasm samples from both foreign and native origins [11].

There are great efforts being made to improve the fiber quality and output of kenaf due to the plant's enormous economic potential. Recently, researchers from various disciplines have expressed an interest to exploit kenaf for multiple purposes and develop into a more promising crop. Therefore, before starting a successful breeding program, it is necessary to understand the genetic basis for kenaf planting materials. Morphological evaluation is the most economical and most measurable of the several techniques, making it the greatest substitute for plant breeders in a crop improvement program. However, morphological analysis is labor-intensive, highly dependent on the environment, has a low rate of polymorphism, and raises the possibility that estimates will be biased [12]. Despite the limitations of using morphological assessment to measure genetic diversity, the method gives adequate crop characterization information and identifies sources of desirable genotypes for crop improvement [13]. The selection of suitable genetic resources that are adapted to certain conditions prior to the start of a breeding program necessitates knowledge of the ability to discern variances among the available germplasm. Therefore, this study was designed to assess the genetic diversity and relationship between kenaf genotypes in order to identify genotypes with desirable features.

2. Materials and Methods

2.1. Plant Materials

Twenty-five genotypes of kenaf were collected from the gene bank of Bangladesh Jute Research Institute (BJRI), Dhaka. The seeds were healthy and genetically pure. The name and origin of these genotypes are presented in Table 1, where G is denoted to genotype ID of kenaf accessions. For examples, G1= BJRI Kenaf 3 (HC-3), G22= Acc-1607 and G25= Acc-1876.

Table 1. Name and origin of 25 selected genotypes of kenaf

Genotype No.	Accession	Origin/Country name
G1	BJRI Kenaf 3 (HC-3)	Check (Australia)
G2	Acc-1653 (HC 95)	Check (Iran)
G3	Acc-1583	USA
G4	Acc-1585	USA
G5	Acc-1589	USA
G6	Acc-1592	USA
G7	Acc-1593	USA
G8	Acc-1594	USA
G9	Acc-1611	Iran
G10	Acc-1612	Iran
G11	Acc-1626	Iran
G12	Acc-1633	Iran
G13	Acc-3741	Kenya
G14	Acc-3746	Kenya
G15	Acc-4622	USA
G16	Acc-4623	USA
G17	Acc-4627	USA
G18	Acc-4718	USA
G19	Acc-4750	USA
G20	Acc-4823	Kenya
G21	Acc-1575	Pakistan
G22	Acc-1607	Iran
G23	Acc-4415 (PI-329192)	Elsalvador
G24	Acc-1576	Pakistan
G25	Acc-1876	Kenya

2.2. Design and Layout of the Experiment

The study followed a randomized complete block design (RCBD) with three replications. The genotypes were assigned to each plot in each block based on the experiment plan. Each plot was measured 3 m or 1 m in size. The experiment's twenty-five genotypes were distributed randomly among plots for each replication. Rows and plants are spaced apart by 30 cm and 5-7 cm, respectively. Three meters were kept between the two lines. Data were collected on nine morphological characters viz., base diameter (mm), no. of nodes per plant, internode length (cm), green weight with leaves (gm), green weight without leaves (gm), and green bark thickness (mm). Dry stack weight (gm) and dry fiber weight (gm) were recorded from 5 randomly selected plants of each genotype from each replication during the experiment.

2.3. Statistical Analyses

The general distance (D2) statistic and its auxiliary analyses, developed by Mahalanobis [14], were used to determine the genetic diversity among the genotypes. Mahalanobis' D2 statistic is used to select the parents in the hybridization procedure, and this method is more reliable because the required information on the parents' variety of features is accessible prior to crossing. Multivariate analysis, including Principal Component Analysis (PCA), Principal Coordinate Analysis (PCA), Cluster Analysis (CA), and Canonical Vector Analysis (CVA), which quantifies the differences between a number of quantitative variables, is an effective way to assess genetic diversity.

3. Results and Discussion

3.1. Nonhierarchical Clustering

The clustering of plant genotypes into distinct groups is an essential aspect of plant breeding and selection programs. In this study, we utilized nonhierarchical clustering techniques based on the covariance matrix to classify twenty-five kenaf (*Hibiscus cannabinus* L.) genotypes into distinct clusters. Kenaf genotypes with more or less identical desirable features were put together into one cluster, while those with more dissimilar desirable traits were divided into different groups. Our results showed that the genotypes were grouped into five clusters, with cluster II comprising the highest proportion of genotypes (28%), followed by clusters III, IV, and V (20% each), while the remaining genotypes (12%) were assigned to cluster I. Previous studies have shown the effectiveness of nonhierarchical clustering techniques based on the covariance matrix for grouping individuals with similar characteristics in kenaf [15, 16]. Our study is consistent with these findings and provides valuable information for plant breeding and genetic improvement programs. The composition of clusters with different genotypes was presented in Table 2, cluster II had the maximum number of genotypes (G3, G6, G7, G8, G15, G19, G21) followed by cluster III (G9, G10, G13, G14, G23), cluster IV (G4, G16, G17, G18, G20) and cluster V (G1, G2, G22, G24, G25) having five genotypes. Cluster I comprised three genotypes (G5, G11, G12). Similar findings in rice and oil palm were reported by Myint et al. [17] and Sarif et al. [11], respectively. According to Jui et al.'s [18] study of morphological features, all white jute accessions exhibit genetic divergence and five cluster groups were created using the cluster analysis to organize the experimental 95 white jute accessions. The identification of distinct clusters can facilitate the selection of desirable traits for future breeding efforts in kenaf [19]. The genotypes in the same cluster are likely to deviate very little from one another. Effective segregants are unlikely to be produced when genotypes from the same cluster are crossed [20]. The success of crop improvement depends on the identification and application of new significant variability in a population. Allelic differences between any two parents can be determined via divergence. Our study demonstrates the effectiveness of nonhierarchical clustering techniques based on the covariance matrix for grouping kenaf genotypes into distinct clusters. The identified clusters provide valuable information for plant breeding and selection programs aimed at improving specific traits in kenaf.

Table 2. Distribution of twenty-five genotypes of kenaf in different clusters

Cluster no.	No. of genotypes	Name of genotypes
I	3	G5, G11, G12
II	7	G3, G6, G7, G8, G15, G19, G21
III	5	G9, G10, G13, G14, G23
IV	5	G4, G16, G17, G18, G20,
V	5	G1, G2, G22, G24, G25,

3.2. Principal Component Analysis (PCA)

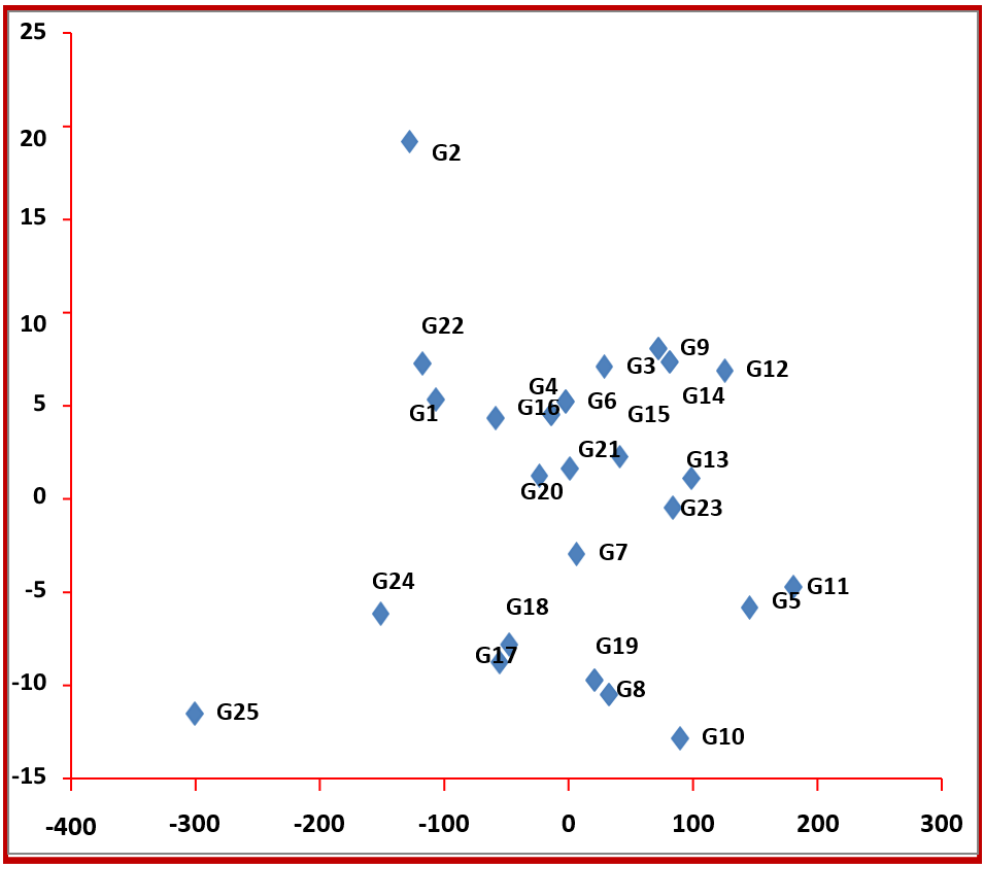
Eigenvalues of the principal component axis, percent of the total variation, and cumulative variation obtained from principal component analysis were presented in Table 3. The results revealed that the first principal axis, plant height (m), accounted for the majority of the variation among genotypes, accounting for 74.07% of the total variation. The first two main component axes (eigenvalues > 1 (01) unity) account for 88.78% of the total variation across the nine characters, suggesting that identified variables within these axes had a significant effect on phenotypic kenaf genotype and may be used successfully for selection. According to Zaman et al. [21], the first three axes explained 94.00% of the overall variation whereas the first main components explained

81.94% in mustard. Sawarkar et al. [22], examined the genetic diversity across thirty tossa jute genotypes and found that fiber yield contributed most to divergence (43.68%), followed by plant height (20.69%), base diameter (9.56%), and green weight (5.28%). The remaining seven characters accounted for the remaining 11.22% of the total variation.

A two-dimensional scatter diagram (Z1-Z2) with component score 1 as the X-axis and component score 2 as the Y-axis was made based on the principal component scores I and II obtained from the principal component analysis, as shown in Figure 1. The genotype positions in the scatter plot appeared to be spread into five groups, indicating that the genotypes were somewhat diverse (Fig 2). Rameeh [23] reported 4 clusters in rapeseed. The contribution of several traits to genotypic divergence is an essential concern. The traits that contributed the most to the divergence are given preference when selecting on the cluster for further selection and the parents for hybridization.

Table 3. Eigen values and percentage of variation for corresponding nine component characters in 25 Kenaf genotypes

Principal component axes	Eigen values	variation Percent	Cumulative % of variation
I	6.666	74.07	74.07
II	1.324	14.71	88.78
III	0.486	5.40	94.18
IV	0.186	2.07	96.25
V	0.150	1.67	97.92
VI	0.123	1.36	99.28
VII	0.045	0.50	99.78
VIII	0.015	0.16	99.94
IX	0.005	0.06	100.00



Z1-Z2 Graph

Figure 1. Scatter pattern of Kenaf genotypes on the basis of their principal component scores

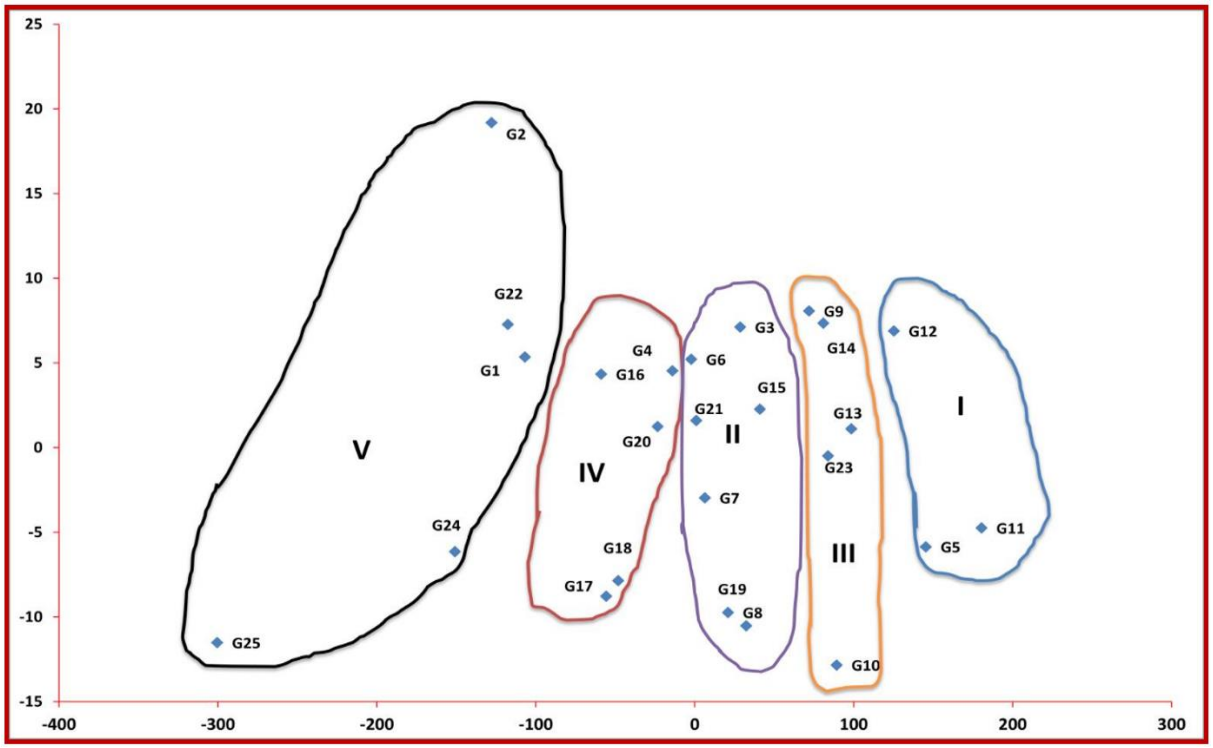


Figure 2. Scatter diagram of Kenaf genotypes based on their principal component scores

3.3. Cluster Mean Analysis

There were significant differences between clusters when the cluster means of nine different characters (Table 4) were examined. Observed in cluster V (2.66) was the highest plant height, whereas Cluster I had the lowest plant height (2.13). The maximum (22.02) and minimum (16.18) base diameters were observed in clusters V and I respectively. Cluster I genotypes had the fewest nodes per plant (57.11), while cluster V genotypes had the most nodes per plant (77.00). The highest internode length was measured in cluster I, whereas cluster V had the shortest internode length (3.49). Clusters V and I had the highest (382.80) and lowest (140.11) green weight with leaves, respectively. Cluster V had the highest green weight without leaves at 310.00, while cluster I had the lowest at 118.67. Cluster IV had the highest value for green bark thickness (2.20), while Cluster V had the least (2.07). Stick weight was highest in cluster V (50.60) and lowest in genotypes in cluster I (22.67). Cluster V (19.00) had the highest fiber weight, while cluster I (10.33) had the lowest fiber weight. Jui et al. [18], reported that plant height, base diameter, green weight, and fiber output were all higher in cluster II among jute accessions. Cluster V showed the lowest mean value. The genotypes of clusters (I, II, I, IV) have higher means than their respective grand centroids. As a result, it would be considered nearly as good genotypes as the genotypes of the cluster (V) with lower means. However, there was a wide range in the magnitude of the mean values among the various clusters, which also suggests a greater degree of genetic variation among the nine characters. Similar findings were reported by Arpita and Kumar [24] and Jatothu et al. [25] while researching the genetic diversity of *Corchorus spp*.

Table 4. Cluster mean for nine different characters of 25 genotypes of Kenaf

Characters	I	II	III	IV	V
PH	2.13	2.39	2.26	2.41	2.66
BD	16.18	19.36	17.17	20.00	22.02
NPP	57.11	64.81	62.00	70.07	77.00
IL	4.44	3.90	4.23	3.83	3.49
GWL	140.11	243.90	190.27	290.80	382.80
GWWL	118.67	199.19	160.53	232.93	310.00
GBT	2.09	2.12	2.15	2.20	2.07
SW	22.67	33.43	25.87	39.53	50.60
FW	10.33	13.09	11.27	15.33	19.00

PH = Plant height (m), BD = Base diameter (mm), NPP = Number of nodes per plant, IL = Internode length (cm), GWL = Green weight with leaves per plant (gm), GWWL = Green weight without leaves per plant (gm), GBT = Green bark thickness (mm), SW = Stick weight (gm) and FW = Fibre weight (gm).

3.4. Inter and Intra Cluster Distance

The inter-cluster distances were calculated using Canonical Variable Analysis (CVA). The inter-cluster distances were greatest in this experiment (13.566) (Table 5) between cluster I and cluster V, followed by III and V

(11.164), IV and I (9.398), and II and V (8.275). Higher inter-cluster distances suggest that population variability spans a wide spectrum. The fact that clusters I and V had the maximum inter-cluster distance revealed that their genotypes were more diversified than those of the other clusters. The minimal distance noticed between clusters I to III (2.602) indicated a close relationship between the genotypes present. Consequently, the desired outcome will be obtained from crossing the genotypes resulting from clusters I and V. Because cross pairings involving parents from the most diverse clusters exhibit the largest proportion of heterosis. More emphasis should be placed on clusters I and V to achieve stronger heterosis and segregatants have a wide range of variability due to a new recombination of desired characteristics.

Table 5. Average intra (Bold) and inter cluster distances (D^2) for 25 genotypes of Kenaf

Cluster	I	II	III	IV	V
I	**0.518**	5.557	2.602	9.398	13.566
II		**0.076**	3.443	4.214	8.275
III			**0.067**	7.194	11.164
IV				**0.087**	4.752
V					**0.092**

Cluster I has the greatest intra-cluster distance (0.518), followed by Cluster V (0.092), and Cluster III (0.067). (Table 5). All five of the clusters' intra-cluster distances were less than their inter-cluster distances, indicating that the genotypes within each cluster were closely connected. The greater genetic variety among the genotypes of various groups was evidenced by the fact that the inter-cluster distances were greater than the intra-cluster distances. Pandey et al. [26] reported the greatest inter-cluster distance between clusters II and III, indicating a considerable genetic diversity among Indian mustard genotypes in these groupings. Jui et al. [18], studied white jute accessions and found that the higher inter-cluster distance (6.66) was recorded between clusters IV and V; on the other hand, clusters II and III had the shortest distance (1.14). For the relevant characters, the genotypes of a cluster group exhibited greater or less similarity.

3.5. Contribution of Characters towards Divergence

Characters' contributions to the divergence determined via canonical variates analysis are shown in Table 6. The attribute that provided a large absolute magnitude for vector 1 was thought to be the key differentiator. The features that provided a higher absolute magnitude for vector 2 were also thought to be in charge of secondary differentiation. The character was thought to be in charge of both primary and secondary differentiation if it were given identical magnitudes for both vectors.

Because all of these parameters had positive signals, the significant characters responsible for genetic divergence in the axis of differentiation in vector 1 were green bark thickness (0.0299) and internode length (0.3339). On the other hand, the first axis of differentiation showed a negative sign for plant height, base diameter, number of nodes per plant, green weight with leaves, green weight without leaves, stick weight, and fiber weight. Except for plant height, the number of nodes per plant, and the thickness of the green bark, all the features showed negative indications in the second axis of differentiation, indicating that it played a limited influence in genetic diversity. Positive signals were found in both vectors for green bark thickness, indicating that this attribute was crucial and contributed significantly to the genetic divergence of the materials under study. According to Begum et al. [27] the number of seeds per siliquae and the number of branches per plant had the greatest impact on the divergence of the linseed germplasm that was already in existence. When examining the genetic diversity of tossa jute, Jatothu et al. [25] showed that plant height, base diameter, green weight, stick weight per plant and number of nodes all played major roles in genetic divergence.

Table 6. Relative contribution of nine different characters of 25 kenaf genotypes to the total divergence

Characters	Principal Component	
	Vector-1	Vector-2
Plant height (m)	-0.3510	0.1381
Base diameter (mm)	-0.3631	-0.0342
Number of nodes per plant	-0.3350	0.3323
Internodes length (cm)	0.3339	-0.2677
Green weight with leaves (gm)	-0.3687	-0.1125
Green weight without leaves (gm)	-0.3750	-0.0572
Green bark thickness (mm)	0.0299	0.8065
Stick weight (gm)	-0.3657	-0.0916
Fibre weight (gm)	-0.3318	-0.3505

Islam [28] and Mostofa, [29] also reported on the role of characters in divergence using principal component analysis (PCA) in tossa jute and kenaf, respectively. They found that most of the characters in the first axis of differentiation (PC1) were important for genetic divergence of which 1000 seeds weight, dry stick weight, number of nodes, dry fibre weight, and plant height were the major ones.

4. Conclusions

Investigating the genetic components of its morphological features is the first step in creating kenaf genotypes that might result in a sustainable fiber supply. This is due to the fact that genetic diversity allows for recombination, which is necessary for varietal development. The most diversity was found to be caused

by plant height. The greatest and smallest cluster distances were recorded between clusters I and V, as well as I and III. The inter-cluster distances were greater than the intra-cluster distances. So, the genotypes G5, G11, G12 from cluster I and G1, G2, G22, G24, G25 from cluster V were more diverse. So, the genotypes belonging to clusters I and V could be used as parents for future breeding programs to develop the kenaf variety.

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