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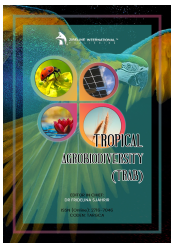

RESEARCH ARTICLE

ASSESSMENT OF MORPHO- MOLOECULAR VARIATION IN KENAF

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ABSTRACT

Germplasm plays an important role for developing new varieties of crops to the plant bredders. In Bangladesh, kenaf is geeting more attention as the substitute of jute and breeding new varieties of kenaf is time demanding. In the present research, morphological and genetic variation of 51 kenaf germplasms were analysed. Analysis revealed that genotype HC-92 had the higer plant height (3.4 m) with base diameter (27.3 mm), higher number of node in each plant, plant weight with or without leaves and fiber yield (37.9 g) compare to the other kenaf accessions. However, internode length, number of fruit and seeds and dry stick weight was higher in kenaf accession 5018, 5083, 1623 and 3746, respectively. Analysis also found the higher phenotypic variation than the genetic variation. During genotypic variation analysis, three primers among the sixteen primers produced polymorphism in kenaf accessions. The dendrogram had indicated segregation of 51 germplasms of Kenaf into 8 clusters where cluster II consisted 18 and cluster III consisted 20 accessions respectively. The information obtained in this study can be used for further breeding programs for the improvement of kenaf in Bangladesh.

KEYWORDS

Germplasm, kenaf, fiber yield, genetic variation and RAPD markers.

1. INTRODUCTION

Kenaf (*Hibiscus cannabinus* L.) is a fiber yielding annual crop under the Malvaceae family, cultivated as the substitute of jute for fiber production (Bhaskara et al., 2012). Kenaf is native to Africa however, it is cultivated worldwide (Li, 1990). Kenaf grows very fast with high production of biomass and a source of multipurpose fiber (Arbaoui et al., 2016). In Bangladesh, kenaf is getting quick attention as a fiber crop and reports showed that kenaf is cultivated in around 0.04 million hectares of land (Mostafa, 2012). Kenaf normally grow up to 14 to 18 feet tall within six months during the growing season which producing 5 to 10 tons of dry fiber per acre (bast and core fibers) (Islam, 2019). Kenaf has the potentiality to give high yield even in less care in the fallow and char lands having lower soil health (Hiron, 2007).

In Bangladesh, fiber of kenaf is mixed with jute fiber for making bags, sacks, twines, ropes, cordages and carpet and (Maiti et al., 2010). In addition, leaves of kenaf has numerous uses livestock (Lipa and Dam, 2013). Report also showed that manufacturing of kenaf products has been reported to evaluate for the textiles and found as additional prospective uses that can have positive impact on national economy of Bangladesh (Paridah, 2017). However, yield of kenaf and composition of plant components are affected through many factors including cultivar, plant date photosensitivity etc. (Webber and Bledsoe, 2002). Although Bangladesh Jute Research Institute has identified several new genotypes of kenaf but the yield varies in different agro-ecological zone of Bangladesh (Islam, 2019). So, breeding of kenaf is time demanding to strength the Bangladesh national economy of Bangladesh. Genetic variability is the pre-requisite for any plant breeding program (Bhandari et al., 2017).

Information about genetic diversity facilitates the selection of parental genotypes from random populations. Briefly, accurate assessment of the levels and patterns of genetic diversity can serve the analysis of genetic variability in germplasm (Smith, 1984, Cox et al., 1986). Identification of diverse parental combinations to create segregating progenies with maximum genetic variability for further selection (Barrett and Kidwell, 1998). And finally, introgression of desirable genes from wild germplasm into the adapted high yielding germplasm resource (Thompson et al., 1998). Such information is particularly useful to assess the potential of heterotic combinations before attempting crosses and hence saving time and resources (Hallauer and Miranda, 1988). Genetic diversity can be recognized through morphological, biochemical and molecular markers (Mamatha et al., 2017).

Molecular marker technology provides information that can help to define the distinctiveness of germplasm and their ranking according to the number of close relatives and their phylogenetic position (Winter and Kahl, 1995). DNA marker is an innovative approach based on DNA polymorphism among tested genotypes which is applicable to biological research (Nadeem et al., 2018). Several molecular markers are presently available to assess the variability and diversity at molecular level Random Amplified Polymorphic

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DNA (RAPD) marker is one of the popular and widely adopted markers where ten base primer amplify the random portion of genome (Williams et al., 1990). In the present research a total 51 kenaf accessions was used to assess their morphological as well as molecular variations by using RAPD markers.

2. Materials and Methods

2.1 Morphological characterization of Kenaf

The soil of the experimental site was clay loam in texture with p^H 5.5 to 6.0 belonging to the Agro-Ecological Zone 16 (AEZ-16): Middle Meghna River Flood Plain (UNDP, 1988). The land is medium high with uniform topography and almost homogenous with respect to soil fertility. A total 48 accessions and 03 varieties of kenaf was used for this study. The experiment was conducted in the Randomized Complete Block Design (RCBD) with three replications. Each experimental plot had a double row of 4m length having space 0.3m between rows and plot to plot distance was 1m. Seeds of the kenaf accessions and varieties were sown on 1st April, 2010 maintaining the standard production technology (Islam, 2008). The data on different morphological parameters from 51 germplasm of Kenaf were recorded from 10 randomly selected plants of each genotype from each replication. Different morphological data were recorded based on the standard and reported procedure (IJO, 1991). Range, mean, genotypic and phenotypic coefficients of variation of quantitative characters were calculated. The collected data on 17 characters were analyzed statistically for ANOVA using computer based statistical package MSTAT-C (Gomez and Gomez, 1984). Mean data of 51 accessions of each character were used. Genetic parameters such as genotypic and phenotypic coefficients of variation were estimated. Genotypic and phenotypic coefficients of variation were calculated according to the formula suggested by Burton (Burton, 1951).

$G.C.V = (\sqrt{V_g} / X) \times 100$

$P.C.V = (\sqrt{V_p} / X) \times 100$

Where, G.C.V = genotypic coefficient of variation,

$\sqrt{V_g}$ = genotypic standard deviation,

X = population mean,

P.C.V = phenotypic coefficient of variation,

$\sqrt{V_g}$ = phenotypic standard deviation

2.2 Molecular characterization of Kenaf germplasm through RAPD marker

2.2.1 Genomic DNA extraction

Genomic DNA was isolated from leave tissues of all kenaf accessions and varieties using phenol, chloroform and isoamylalcohol extraction and ethanol precipitation method (Ausubel et al., 1995). In brief, approximately 50 mg of fresh, vigorous, young actively growing leaf tissues were taken into a microcentrifuge tube, cut into small pieces, homogenized and digested with RNAse in extraction buffer (50 mM Tris-HCl, 25 mM EDTA, 300 mM NaCl, pH=8.0 and 1% SDS). DNA was purified by successive extraction with 600 µl of phenol: chloroform: isoamylalcohol (V: V: V = 25: 24: 1). DNA was precipitated first using about 0.1 volume of sodium acetate with 2.5 volumes of absolute ethanol and peletted by centrifugation. DNA was reprecipitated by adding two volumes of 70% ethanol and peletted by centrifugation. The pellets were then air-dried and resuspended in an appropriate volume of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH= 8.0). DNA quality was checked by electrophoresis and quantified using a spectrophotometer (Spectronic^R Genesis^{TM}).

2.2.2 PCR amplification and agarose gel electrophoresis

PCR amplification was done following the procedure (Williams et al., 1990) with little modifications. PCR reactions were performed on each DNA sample in a 10µl reaction mix containing: 1µl of 10x Ampli *Taq* polymerase buffer, 1µl of 2.5 mM dNTPs, 0.6 µl of 50 mM MgCl2, 1 unit of Ampli *Taq* DNA polymerase (Merck, India), 50 ng template DNA, 2 µl of 10 µM primer and a suitable amount of sterile deionized water. DNA was amplified in a thermal cycler (Eppendorf Mastercycler Gradient). The reaction mix was preheated at 94ºC for 3 min followed by 40 cycles of 1 min denaturation at 94ºC, 1 min annealing at 36ºC band elongation at 72ºC for 2 min. After the last cycle, a final extension of 7 min at 72ºC was added to allow complete extension of all amplified fragments. The amplified product from each sample was separated electrophoretically on 1.4% agarose gel in a 1XTBE buffer at 120V for 1½ h. Two molecular weight markers (1 kb and 20 bp DNA markers) were run alongside the RAPD reactions. The gels were stained with ethidium bromide and bands were observed and photographed by a gel documentation system (Bio-Rad).

2.2.3 RAPD data analysis

Fragments were scored as 1 if present and 0 if absent. The scores were then pooled for constructing a single data matrix. This was used for comparing the frequencies of all polymorphic RAPD markers and estimating Nei's (Nei, 1973) gene diversity (h), gene flow (Nm), coefficient of gene differentiation (GST), genetic distance (D) and constructing a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram among populations using POPGENE (version 1.31) (Yeh et al., 1999) computer program. The similarity index values (SI) between the RAPD profiles of any two individuals were calculated from RAPD markers according to the formula: Similarity index (SI) = 2 Nxy/Nx+Ny, Where, Nxy is the number of RAPD bands shared by individuals X and Y respectively, and Nx and Ny are the number of bands in individual x and y, respectively (Lynch, 1991).

3. Results

3.1 Comparison among kenaf accessions in respect of morphology and yield contributing characters

To understand the differences of different morphological and yield contributing characters, data of 51 kenaf genotypes were analyzed and summarized in Figure 1. Plant height varied from 2.50 m to 3.40 m and the genotype HC–2 showed the tallest height whereas genotype 5047 had the lowest height compare to the other genotypes (Figure 1A). Consistently, genotype HC-2 had the highest base diameter and number of node in each plant however, less base diameter and number of pod per plant was smaller in genotype 4111 and 5047, respectively (Figure 1B & 1C). Internode length was higher in genotype 4111 and smaller was in genotype 1627 (Table 1) whereas petiole length was lowest and highest in genotype 1662 and 5017, respectively (Figure 1D). Plant weight with and without leaf was smaller in the genotype 1662 whereas higher was in two different genotypes HC-2 and 4119, respectively (Figure 2A). Dry stick was found higher in genotype 3746 and lower was in genotype 4111 and fiber weight in each plant was higher in HC-2 and lower was in genotype 1664 (Figure 2B). Number of fruits in each plant was smaller in genotype 1564 and higher in genotype 5083 (Figure 2C). In case of seeds, highest number of seeds in each pod was higher in genotype 1623 whereas the lower was found in genotype 5081 (Figure 2C).

Table 1: Estimation of genetic parameters of 51 *H. cannabinus* germplasm on eleven morphological characters

Characters	Genotypic variance (σ^2g)	Phenotypic variance (σ^2p)	Grand mean	Heritability (%) h^2_b	GCV (%)	PCV (%)	GA	GA (%)
Plant height (m)	0.02	0.03	3.07	55.79	4.33	5.79	0.20	6.66
Basal diameter (mm)	6.60	6.74	20.12	97.88	12.76	12.90	5.23	26.01
Number of node plant^{-1}	98.04	100.19	95.98	97.86	10.32	10.43	20.18	21.02
Internode length (cm)	0.15	0.23	4.49	63.76	8.50	10.65	0.63	13.99
Petiole length (cm)	2.53	2.72	11.60	92.95	13.72	14.23	3.16	27.25
Green weight with leaves (g plant^{-1})	13569.98	13713.22	418.99	98.96	27.80	27.95	238.71	56.97
Green weight without leaves (g plant^{-1})	6436.52	6534.51	298.65	98.50	26.86	27.07	164.03	54.92
Dry stick weight (g plant^{-1})	204.32	210.25	52.41	97.18	27.27	27.67	29.03	55.39
Number of fruits plant^{-1}	169.96	170.76	27.64	99.53	47.17	47.28	26.79	96.93
Number of seeds fruit^{-1}	15.89	16.18	12.96	98.18	30.76	31.04	8.14	62.78
Fibre weight (g plant^{-1})	41.43	42.00	20.39	98.65	31.57	31.78	13.17	64.58

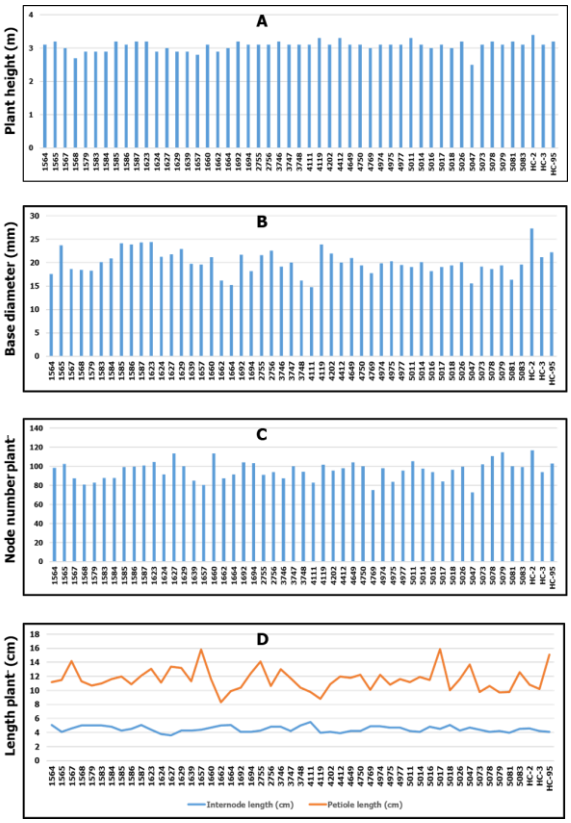

Figure 1: Morphological characters of kenaf accessions. (A) plant height, (B) base diameter, (C) node number in each plant, and (D) internode length and petiole length.

3.2 Evaluation of various genetic characteristics of kenaf accessions

Phenotypic variation of kenaf (*H. cannabinus*) germplasm were little bit higher than their genotypic variation but the genotypic and phenotypic variances were statistically more or less close among the studied whole characters (Table 1). However, all the characters were closer to their respective genotypic and phenotypic variances but green weight with leaves showed the higher variances (13569.98 and 13713.22) followed by green weight without leaves, dry stick weight, number of fruits plant^{-1}, number of node plant^{-1}, fibre weight, number of seeds plant^{-1}, basal diameter, petiole and internode length. Among the studied characters, plant height showed the least variance (0.02 and 0.03) in respect of genotypic and phenotypic variances, respectively.

3.3 Genotypic and phenotypic co–relation analysis of Hibiscus cannabinus

Correlation coefficients analysis revealed that plant height had positive correlation with the whole characters while internode and petiole length were shown negative correlation whereas plant height was highly positive correlated with node plant^{-1} (Table 2). Basal diameter also showed positive and significant correlation among the all characters except internode length. Petiole length was negatively correlated with plant height, node plant and internode length while other studied characters had positive correlation whereas all the relationships were lower and petiole length had low positive co–relationship with seeds fruit^{-1} (Table 2). A negative correlation was also found between green weight with leaves and internode length while green weight with leaves showed positive correlation with other all characters. In case of correlation study between dry stick weight with other characters, it showed negative correlation with internode length (–0.242 and 0.228) and positive with other characters in respect of both genotypic and phenotypic levels, respectively. Statistically similar co–relationships were obtained for the characters of fruits plant^{-1} and seeds fruit^{-1} with other characters in case of both characters were negatively correlated with only internode length at both genotypic (–0.369** and –0.064, respectively) and phenotypic (–0.342** and –0.039, respectively) levels.

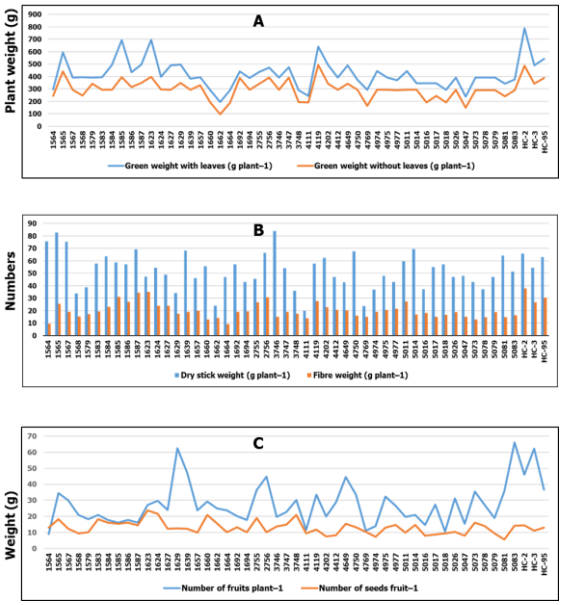

Figure 2: Yield contributing characters of kenaf accessions. (A) plant weight with or without leaves, (B) dry stick or fiber weight in each plant, and (C) number of fruit in each plant and number of seeds in each seed .

Table 2: Genotypic and phenotypic correlation coefficients among the studied morphoagronomic, yield and yield contributing characters of 51 *H. cannabinus* germplasm

Characters		Basal diameter (mm)	Number of node plant^{-1}	Internode length (cm)	Petiole length (cm)	Green weight with leaves (g plant^{-1})	Green weight without leaves (g plant^{-1})	Dry stick weight (g plant^{-1})	Number of fruits plant^{-1}	Number of seeds fruit^{-1}	Fibre weight (g plant^{-1})
Plant height (m)	Rg	0.44**	0.70**	-0.50**	-0.27	0.50**	0.53**	0.29*	0.19	0.10	0.41**
	Rp	0.45**	0.70**	-0.30*	-0.21	0.49**	0.51**	0.29*	0.20	0.12	0.41**
Basal diameter (mm)	Rg		0.50**	-0.41**	0.16	0.87**	0.82**	0.41**	0.31*	0.37**	0.82**
	Rp		0.50**	-0.38**	0.16	0.87**	0.82**	0.41**	0.31*	0.37**	0.82**
Number of node plant^{-1}	Rg			-0.50**	-0.14	0.47**	0.44**	0.21	0.20	0.23	0.33*
	Rp			-0.50**	-0.13	0.47**	0.44**	0.22	0.21	0.24	0.34*
Internode length (cm)	Rg				-0.26	-0.41**	-0.48**	-0.24	-0.36**	-0.06	-0.30*
	Rp				-0.20	-0.39**	-0.46**	-0.23	-0.34*	-0.04	-0.28*
Petiole length (cm)	Rg					0.15	0.15	0.20	0.10	0.01	0.15
	Rp					0.15	0.15	0.21	0.10	0.02	0.15
Green wt + leaves (g plant^{-1})	Rg						0.90**	0.34*	0.30*	0.25	0.83**
	Rp						0.89**	0.34*	0.30*	0.25	0.83**
Green wt – leaves (g plant^{-1})	Rg							0.44**	0.40**	0.21	0.72**
	Rp							0.44**	0.40**	0.21	0.72**
Dry stick weight (g plant^{-1})	Rg								0.10	0.12	0.29*
	Rp								0.10	0.13	0.29*
Table 2 continued											
Number of fruits plant^{-1}	Rg									0.17	0.20
	Rp									0.17	0.20
Number of seeds fruit^{-1}	Rg										0.27
	Rp										0.27

** = Significant at 0.01% and * = Significant at 0.05% level of probability

3.4 Genotypic and phenotypic path analysis of different accessions

In the present investigation, fibre yield plant^{-1} of *H. cannabinus* was considered as the dependent variable, and the genotypic and phenotypic correlation of the character with other ten quantitative characters, respectively was partitioned into their corresponding direct and indirect effect (Table 3). Path coefficient analysis at both genotypic and phenotypic levels found the highest fibre weight was exerted by green weight with leaves (Rg: 0.5824 and Rp: 0.5813) among the studied characters and which was owing to its indirect contribution via base diameter and plant height (Table 3). Basal diameter showed the considerable direct effect (Rg: 0.5249 and Rp: 0.5251) next to green weight with leaves on fibre yield at both genotypic and phenotypic levels, respectively followed by plant height, petiole length and seeds fruit^{-1} (Table 3).

Table 3: Genotypic and phenotypic path coefficient analysis showing direct and indirect effect on different characters of Kenaf

Characters		Plant height (m)	Basal diameter (mm)	Number of node plant^{-1}	Internode length (cm)	Petiole length (cm)	Green wt. + leaves (g plant^{-1})	Green wt – leaves (g plant^{-1})	Dry stick weight (g plant^{-1})	Number of fruits plant^{-1}	Number of seeds fruit^{-1}	Fibre weight (g plant^{-1})
Plant height (m)	Rg	**0.1760**	0.2314	-0.1445	-0.0033	-0.0120	0.2912	-0.1044	-0.0136	-0.0100	0.0014	0.412**
	Rp	**0.1680**	0.2347	-0.1500	0.00695	-0.0057	0.2819	-0.0997	-0.0131	-0.0116	0.0017	0.413**
Basal diameter (mm)	Rg	0.0776	**0.5249**	-0.1012	-0.0030	0.0069	0.5078	-0.1629	-0.0191	-0.0162	0.0052	0.820**
	Rp	0.0750	**0.5251**	-0.1065	0.0087	0.0044	0.5057	-0.1601	-0.0184	-0.0184	0.0054	0.821**
Number of node plant^{-1}	Rg	0.1228	0.2566	**-0.2070**	-0.0037	-0.0061	0.2749	-0.0870	-0.0099	-0.0107	0.0032	0.333*
	Rp	0.1164	0.2583	**-0.2165**	0.0110	-0.0035	0.2732	-0.0854	-0.0097	-0.0122	0.0034	0.335*
Internode length (cm)	Rg	-0.079	-0.2125	0.1049	**0.0074**	-0.0114	-0.2370	0.0953	0.0112	0.0189	-0.0009	-0.303*
	Rp	-0.050	-0.1969	0.1026	**-0.0232**	-0.0055	-0.2284	0.0907	0.0102	0.0203	-0.0005	-0.281*
Petiole length (cm)	Rg	-0.048	0.0813	0.0285	-0.0019	**0.0447**	0.0844	-0.0290	-0.0093	-0.0044	0.0001	0.147
	Rp	-0.035	0.0861	0.0279	0.0047	**0.0273**	0.0842	-0.0287	-0.0092	-0.0052	0.0002	0.152
Green wt. + leaves (g plant^{-1}	Rg	0.0880	0.4577	-0.0977	-0.0030	0.0064	**0.5824**	-0.1780	-0.0159	-0.0155	0.0035	0.828**
	Rp	0.0814	0.4568	-0.1017	0.0091	0.0039	**0.5813**	-0.1748	-0.0153	-0.0175	0.0036	0.827**
Green wt. – leaves (g plant^{-1})	Rg	0.0925	0.4304	-0.0906	-0.0035	0.0065	0.5218	**-0.1986**	-0.0204	-0.0189	0.0029	0.722**
	Rp	0.0856	0.4300	-0.0946	0.0107	0.0040	0.5196	**-0.1955**	-0.0197	-0.0213	0.0030	0.722**
Wt of dry stick (g plant^{-1})	Rg	0.0515	0.2157	-0.0443	-0.0018	0.0089	0.1997	-0.0874	**-0.0465**	-0.0047	0.0017	0.293*
	Rp	0.0493	0.2163	-0.0469	0.0053	0.0056	0.1988	-0.0860	**-0.0448**	-0.0054	0.0018	0.294*
Table 3 continued												
Number of fruits plant^{-1}	Rg	0.0336	0.1621	-0.0422	-0.0026	0.0038	0.1712	-0.0713	-0.0042	**-0.0527**	0.0023	0.200
	Rp	0.0327	0.1627	-0.0443	0.0079	0.0024	0.1709	-0.0702	-0.0041	**-0.0595**	0.0024	0.201
Number of seeds fruit^{-1}	Rg	0.0177	0.1947	-0.0482	-0.0004	0.0004	0.1473	-0.0411	-0.0057	-0.0087	**0.0140**	0.270
	Rp	0.0201	0.1964	-0.0513	0.0009	0.0004	0.1470	-0.0406	-0.0056	-0.0100	**0.0146**	0.272

Correspondingly, the lowest and negative direct effect was found by node plant^{-1}, green weight without leaves, dry stick weight, and fruits plant^{-1} on fibre yield whereas node plant^{-1} had higher negative direct effect (Rg: -0.2070 and Rp: -0.2165) on fibre yield followed by green weight without leaves, fruits plant^{-1} and dry stick weight at both genotypic and phenotypic levels, respectively. Although, internode length showed positive direct effect (0.0074) at genotypic level, it was negative (-0.0232) at phenotypic level. The variation in perspective variables were found due to differences in germplasm at both genotypic and phenotypic levels. Considering the direct and indirect effect on fibre yield, the important characters were green weight with leaves and basal diameter in *H. cannabinus*. Both genotypic and phenotypic residual effect (Rg: 0.2432 and Rp: 0.2429) indicated that almost all the contributors on fibre yield have been considered in the present investigation (Table 3).

3.4 Molecular characterization of kenaf accessions using RAPD markers

A total sixteen primers were used to observe ability of producing polymorphisms in the 51 kenaf accessions and three primers viz. OPAB-02, S-1063 and S-1265 produced distinct polymorphic amplified products (Table 1 and Figure 3-5). A total 33 RAPD bands were found where OPAB-02, S-1063 and S-1265 primers produced 12, 11 and 10 bands, respectively (Table 4). The size of PCR bands ranged from 118 bp to 3863 bp. In addition, selected three primers (OPAB-02, S-1063 and S-1265) produced comparatively maximum number of bands with high intensity.

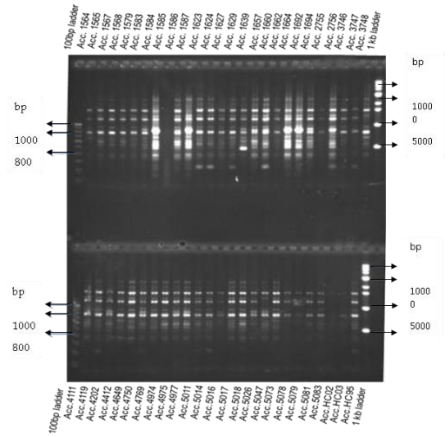

Figure 3: RAPD profiles of 51 Kenaf germplasm using primer OPAB02.

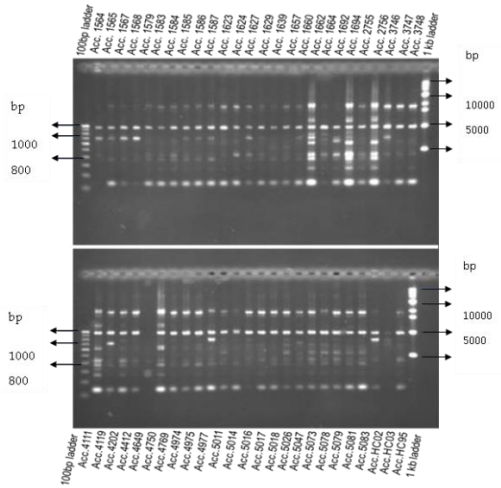

Figure 4: RAPD profiles of 51 Kenaf germplasm using primer S1063.

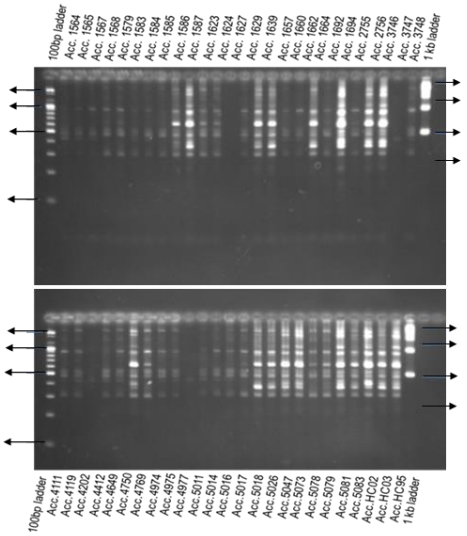

Figure 5: RAPD profiles of 51 Kenaf germplasm using primer S1265.

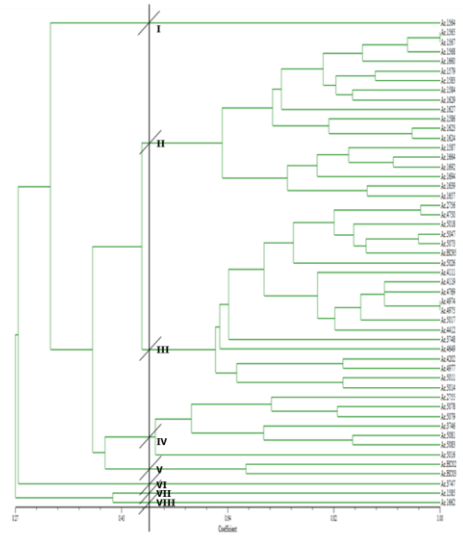

Figure 6: Unweighted pair group method of arithmetic mean (UPGMA) dendrogram based on Nei's (1972) genetic distance, summarizing data on differentiation in 51 Kenaf germplasm according to RAPD analysis.

Table 4: RAPD primers with corresponding band scores together with polymorphic bands observed in fifty-one Kenaf germplasm

Primer name	Number of bands	Band size	Number of polymorphic bands	Proportion of polymorphic loci (%)
OPAB02	12	190-3863	12	100
S1063	11	118-3074	11	100
S1265	10	288-1665	10	100
Total	**33**	**-**	**33**	**100**
Average	**11**	**-**	**11**	**100**

Table 5: Summary of genetic variation statistics for all loci in 51 Kenaf germplasm

Locus	Locus Size (bp)	Sample Size	na*	ne*	h*	I*
OPAB02-1	3863	51	2.000	1.262	0.208	0.362
OPAB02-2	3094	51	2.000	1.710	0.415	0.606
OPAB02-3	2326	51	2.000	1.562	0.360	0.546
OPAB02-4	1863	51	2.000	1.125	0.111	0.224
OPAB02-5	1590	51	2.000	1.460	0.315	0.495
OPAB02-6	1020	51	2.000	1.215	0.177	0.321
OPAB02-7	899	51	2.000	1.310	0.237	0.400
OPAB02-8	614	51	2.000	1.169	0.145	0.275
OPAB02-9	524	51	2.000	1.460	0.315	0.495
OPAB02-10	434	51	2.000	1.841	0.457	0.649
OPAB02-11	316	51	2.000	1.800	0.444	0.637
OPAB02-12	190	51	2.000	1.999	0.500	0.693
S1063-1	3074	51	2.000	1.310	0.237	0.400
S1063-2	2518	51	2.000	1.800	0.444	0.637
S1063-3	1634	51	2.000	1.981	0.495	0.688
S1063-4	1252	51	2.000	1.993	0.498	0.691
S1063-5	840	51	2.000	1.878	0.468	0.660
S1063-6	666	51	2.000	1.878	0.468	0.660
S1063-7	404	51	2.000	1.410	0.291	0.466
S1063-8	342	51	2.000	1.613	0.380	0.568
S1063-9	280	51	2.000	1.963	0.491	0.684
S1063-10	222	51	2.000	1.911	0.477	0.670
S1063-11	118	51	2.000	1.999	0.500	0.693
S1265-1	1665	51	2.000	1.710	0.415	0.606
S1265-2	1541	51	2.000	1.940	0.484	0.678
S1265-3	1074	51	2.000	1.710	0.415	0.606
Table 5 continued						
S1265-4	944	51	2.000	1.999	0.500	0.693
S1265-5	675	51	2.000	1.800	0.444	0.637
S1265-6	564	51	2.000	1.963	0.491	0.684
S1265-7	496	51	2.000	1.310	0.237	0.400
S1265-8	403	51	2.000	1.841	0.457	0.649
S1265-9	364	51	2.000	1.841	0.457	0.649
S1265-10	288	51	2.000	1.963	0.491	0.684
Mean		**51**	**2.000**	**1.689**	**0.389**	**0.570**
St. Dev			**0.000**	**0.286**	**0.119**	**0.138**

na* = Observed number of alleles
ne* = Effective number of alleles
h* = Nei's (1973) gene diversity
I* = Shannon's Information index

Table 6: Overall gene frequencies and locus size for three primers in fifty one Kenaf germplasm

Locus name	Gene frequency	Locus name	Gene frequency
OPAB02-1_{3863}	0.882	S1063-6_{666}	0.373
OPAB02-2_{3094}	0.706	S1063-7_{404}	0.177
OPAB02-3_{2326}	0.765	S1063-8_{342}	0.255
OPAB02-4_{1863}	0.941	S1063-9_{280}	0.569
OPAB02-5_{1590}	0.804	S1063-10_{222}	0.392
OPAB02-6_{1020}	0.902	S1063-11_{118}	0.510
OPAB02-7_{899}	0.863	S1265-1_{1665}	0.706
OPAB02-8_{614}	0.922	S1265-2_{1541}	0.588
OPAB02-9_{524}	0.804	S1265-3_{1074}	0.294
OPAB02-10_{434}	0.353	S1265-4_{944}	0.490
OPAB02-11_{316}	0.333	S1265-5_{675}	0.667
OPAB02-12_{190}	0.490	S1265-6_{564}	0.431
S1063-1_{3074}	0.137	S1265-7_{496}	0.863
S1063-2_{2518}	0.667	S1265-8_{403}	0.647
S1063-3_{1634}	0.549	S1265-9_{364}	0.353
S1063-4_{1252}	0.471	S1265-10_{288}	0.569
S1063-5_{840}	0.628		

N.B: The size of each of the locus is shown as subscript

Genetic diversity among the kenaf accessions for the selected three primers was analyzed and summarized in table 5. Analysis revealed the average gene diversity was 0.389 for all loci among the all kenaf accessions. The high value of genetic diversity and Shannon's information index was found in locus OPAB02-12, S1063-11 and S1265-4 (0.500 and 0.693, respectively). The lowest values of gene diversity and Shannon's information index were found 0.111 and 0.224, respectively in OPAB02- 4. In addition, gene frequency of all kenaf accessions were determined and the result was showed in table 6. The highest value of gene frequency (0.941) was found for primer OPAB02- 4_{1863} and the lowest value was (0.137) for primer S1063-1_{3074}.

3.5 Unweighted pair group method of arithmetic mean (UPGMA) dendrogram analysis

A UPGMA dendrogram was constructed based on genetic distance (Nei's, 1973). A clear divergence among the germplasm was found from the cluster analysis (Figure 6). The 51 germplasm of Kenaf grouped into 8 clusters namely cluster I, II, III, IV, V, VI, VII and cluster VIII (Fig 4.4). Cluster I, VI, VII and VIII consisted of only one accession which is accession 1564, accession 3747, accession 1585 and accession 1662. In this dendrogram cluster II and III was formed large cluster.

4. Discussion

4.1 Morphological characterization of Kenaf

The improvement of any crop is highly depended on the extent of genetic variation and magnitude of available beneficial genetic variability (Clegg, 1968). Identification of desirable genes is important to increase crop yield through breeding program. The improvement of a crop mainly depends upon the magnitude of genetic variability and the degree to which the yield and its components are heritable (Dudley, 1969). Yield is a character which is governed by several factors. Fifty-one Kenaf germplasm were evaluated in field condition to observe the agronomic tratis including genetic characteristics, genotypic correlation coefficient, genotypic and phenotypic path coefficient etc.

The variations in agronomic traits resulted due to the variation in genetic make-up of germplasm and also the adaptability with the agro-climatic and condition of the research area (Mudher et al., 2020, Zhao et al., 2020). It was also reported that appropriateness of cultivars, photoperiodism and day light increases plant height leading to the higher yield of crops (Shukor et al., 2009). In addition, significant variations in base diameter, node plant^{-1}, green weight with leaves and dry stick weight also resulted by the kenaf germplasm (Faruq, 2013). In this research, genotype HC–2 was significantly the privileged yielder than other germplasm regarding plant height, higher basal diameter, maximum nodes, higher green weight with leaves and fiber weight per plant (Figure 1 & 2).

The above findings of the present study were supported by other research (Balogun et al., 2008, Nwangburuka et al., 2012). Internode length and petiole

varied significantly while the longest internode was noted in the accession 4111 and longest petiole was observed in accession 5017. However, green weight without leaves of Kenaf was higher in the accession 4119 but the number of fruits plant^{-1} was highest in 5083 and the highest number of seed plant^{-1} was found in 1626. Early flowering and short duration occurred by climatic factors which ultimately reduced the yield of the genotype 1664. It has been reported that early flowering significantly reduced the fibre yield in crops (Alam et al., 2011, Islam et al., 2001).

Genetic characteristics mainly depends on genotypic and phenotypic variances and this variation may promote population persistence due to several reasons (Forsman, 2014). Evaluations of genetic characteristics, the characters were close to their respective genotypic and phenotypic variances but green weight with leaves showed the higher variances while plant height showed the lower variance in respect of both genotypic and phenotypic variances (Table 1). This suggests that the genotypic factor of the studied green weight with leaves had greater effect at phenotypic level and this finding was supported by several researchers (Nwangburuka et al., 2012, Alam et al., 2011, Ibrahim and Hussein, 2006). Hence selection based on the phenotypic performance of these characters seems to be reliable and effective.

In correlation study, the fibre yield was positively and significantly correlated among the studied characters with both genotypic and phenotypic levels while genotypic correlation was higher than their corresponding phenotypic correlation (Table 2). Similar results were also reported in different crops and it was revealed that a large proportion of the phenotypic variance for such traits was due to genetic effect, so selection for these traits will be effective which was also reported in Roselle (*H. sabdariffa* L.) (Ibrahim et al., 2013). In this research, fibre yield per plant was positively and significantly correlated with plant height, base diameter, green weight with leaves and stick weight (Table 2). Similar relationship was also reported in white jute and kenaf (Alam et al., 2011, Islam et al., 2001, Shakhes et al., 2009). However, fibre yield was negatively correlated with internode length and similar result was also observed in roselle (*Hibiscus sabdariffa* L.) (Ibrahim and Hussain, 2006). From this study it can be said that direct selection of traits will be effective in ensuring seed and fibre yield of Kenaf and this hypothesis is supported by different research (Adeniji and Aremu, 2007, Faruq et al., 2011).

DNA/Molecular markers like RAPD markers are nucleotide sequences have been widely used to quantify genetic variation in different species (Nadeem et al., 2018). DNA marker is an innovative approach based on DNA polymorphism among tested genotypes, and thus applicable to biological research. Several molecular markers are presently available to assess the variability and diversity at molecular level (Joshi et al., 2000). Little references were observed to identify variations among kenaf germplasm with the use of RAPD marker (Table 5). RAPD markers are technically simple and have good throughput with relatively low cost. In this study, selected 3 primers were used for identification based on the DNA polymorphism of the Kenaf germplasm (Table 4). All primers amplified multiple fragments in each germplasm (Figure 3-5). Assessment of genetic relationship among Kenaf (*H. cannabinus* L.) accessions was done using RAPD markers and found most of the fragments were polymorphic in nature (Omalsaad et al., 2001). Most of the fragments were also found polymorphic in this study. It has been reported that RAPD primers of arbitrary sequence amplified 192 bands of which 149 bands were polymorphic and 43 were monomorphic (Geo-Anping et al., 2002).

The dendrogram generated from cluster analysis showed eight major clusters of the Kenaf accessions. Cluster I, VI, VII and VIII consisted of only one accession (Figure 6). They had different geographic origins such as Bangladesh, Kenya, USA and Uganda. The cluster III contained 20 accessions which were Asian origin and mostly from China. Cluster II formed with 18 accessions which were a USA origin. Genotypes in the same cluster may be resulted due to their pedigree relationship or might be originated from the same population (Mamatha et al., 2017). Genotypic variations based on molecular characterization indicated that germplasm belonged to different clusters depend on their genetic components (Choudhary et al., 2013, Sundaram and Purwar, 2011). And the Therefore, it could be concluded that for further research program, especially for hybridization, genotype selected from different clusters will provide maximum heterosis regarding yield.

5. Conclusion

Genetic variation is an essential charcter for the development of new variety. Identification of potential gene/s is important for the breeding programmes. In the present research, 51 kenaf accessions were analysed in respect of morphological characters and genetic variation using RAPD markers. Phenotypical variation was more prominent than the genetic variation in the kenaf accessions. Moreover, dendrogram showed the origin of the kenaf accessions which were highly diverse in genetic components. Based on that breeding programme can be undertaken to maximize the kenaf yield.

Authors' Contributions

AKMSH, MSI, and MBM designed the experiment. AKMSH conducted the experiments. AKMSH, MSI, MSH and analysed the data. AKMSH, MAS and MZT wrote the manuscript. All authors read and approved the final manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

Ethical Issues

If applicable, declarations to be made regarding ethical issues.

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