Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2582-2845.8096

ISSN: 2582 – 2845

Ind. J. Pure App. Biosci. (2020) *8*(3), 37-47

Research Article

Indian Journal of Pure & Applied Biosciences

Peer-Reviewed, Refereed, Open Access Journal

RAPD Analysis of Genetic Diversity and Relationships among Kenaf (*Hibiscus cannabinus* L.) Germplasm

Mohammad Golam Mostofa*, Lutfur Rahman, Abu Saleh Muhammad Yahiya, Mohammad Harun-or-Rashid and Mohammad Mukul Mia

Breeding Division, Bangladesh Jute Research Institute, Manik Mia Avenue, Dhaka-1207, Bangladesh

*Corresponding Author E-mail: mostofabjri@gmail.com

Received: 15.04.2020 | Revised: 21.05.2020 | Accepted: 27.05.2020

ABSTRACT

To analyse the genetic diversity and relationships among kenaf germplasm, two cultivars and twenty three accessions from different geographical regions were assessed using RAPD markers. Six RAPD primers generated 38 polymorphic bands with an average of 6.33 polymorphic bands per primer. A high level of genetic variation (proportion of polymorphic loci 79.17%) and high genetic distance (0.0426 to 0.6523) were found among 25 indigenous and exotic kenaf germplasm. The UPGMA dendrogram based on Nei's genetic distance segregated the genotypes into two major, two minor and one single genotype clusters. The distribution pattern of genotypes from different geographical regions into five clusters was random, indicating that geographical isolation may not be only factors causing genetic diversity. The minor cluster III comprised two accessions collected from same origin but they are distantly related to each other. The genetic distance between two cultivars was lower (0.0870) and they were found in the same cluster. These results indicate that studied kenaf germplasm not only exhibit a high level of genetic diversity but also have a different genetic background.

Keywords: *Diversity, Germplasm, RAPD, Kenaf*

INTRODUCTION

Kenaf (*Hibiscus cannabinus* L.) is a fast growing annual plant that is harvested for its bast fibre. It is closely related to cotton and bhendi (*H. esculentus*) and is mostly grown over a wide range of latitude from 16°S to 41°N (Kumar, 1999). Kenaf, a jute substitute, has capacity to produce a huge amount of biomass and hence it is considered presently as the main renewable source of raw materials for paper pulp production in many countries of the world (Wood, 1981; Hazra & Singh, 1997; Sinha and Day, 2008). Being tolerant to moisture stress, it can be grown in drought-prone areas and in low fertile soils where jute cannot be grown. It is a plant all parts of which have extensive uses.

Cite this article: Mostofa, M.G., Rahman, L., Muhammad Yahiya, A.S., Harun-or-Rashid, M., & Mukul Mia, M. (2020). RAPD Analysis of Genetic Diversity and Relationships among Kenaf (*Hibiscus cannabinus* L.) Germplasm, *Ind. J. Pure App. Biosci. 8*(3), 37-47. doi: http://dx.doi.org/10.18782/2582-2845.8096

Kenaf fibres are used by mills for making cordage, yarn, sacks and hessian cloth and also in combination with other synthetic fibres or natural fibres like jute (Maiti, 1997). Its leaves are a part of human diet in some parts of India and Africa (Killinger, 1969). Kenaf twigs and seeds are good feed for milch cows and dry stem is used for fuel, fencing, match sticks, climbing sticks of vegetables field, cattle shed and other domestic purposes. Moreover, it is biodegradable and environment friendly crop.

Kenaf is believed to be originated in Africa, more particularly in East Africa, where it is found in wild form. It was domesticated in Indian sub-continent as bast fibre crop. Later on, it is distributed throughout the tropical and sub-tropical parts of the world (Karmakar et al., 2008). Kenaf has a good potential of becoming an excellent source of various products such as poultry litter and cattle feed (Perry et al., 1993). It can be processed into acoustic tile, animal bedding, soil-less potting mixes, composite board for construction, mats for erosion control and absorbents for cleaning up chemicals or oils spills (Banuelos, 2000). Kenaf sticks contain around 38% cellulose, 25% hemi-cellulose and 20% lignin (Pandey & Krishnan, 1990). Being cheap and easily available in large quantities, it is an ideal ligno-cellulosic substrate for producing industrial raw materials for much higher values. Kenaf seeds contain about 21% oil, which has various industrial uses like manufacture of soaps, linoleum paints and for lubricating purposes.

Kenaf can absorb CO_2 and NO_2 3-5 times faster than forests. It can clean the environment efficiently (Lam, 2000). In some Japanese cities, kenaf was planted by government to improve the air quality. In Bangladesh, kenaf is one of the most important bast fibre crops and the area under its cultivation is increasing day by day. Around 50 thousand hectares of land is now being under kenaf cultivation and the fibre production is 100-110 thousand tons per annum with average yields of 2.0-2.5 tons/ha. From the beginning of crop improvement program in Bangladesh on kenaf, four high yielding varieties *viz.* HC-2 (popularly known as Joli kenaf), HC-95, BJRI Kenaf-3 and BJRI Kenaf-4 were developed through pure line selection from a few common accessions. These varieties, however, have some undesirable characters. They contain very limited genetic variability with respect to adaptability to different agro-climatic zones, fibre yield and quality. They are susceptible to root-knot nematode (*Meloidogyne* spp.), spiral borer (*Agrilus acutus*), yellow mosaic disease and have prickles on stems and bristles on fruits (a character disliked by farmers due to irritating to the skin). These undesirable characters limit their large-scale adaptation and extension. Therefore, systematic collection, characterization and utilization of diverse kenaf genetic resources are needed to overcome such problems.

Genetic diversity is essential to meet the diverse goals such as producing cultivars with increased yield (Joshi & Dhawan, 1986), wider adaptation, desirable quality, pest and disease resistance (Nevo et al., 1982). For the study of genetic diversity, the plant scientists have used traditionally morphological and physiological features of plants. But in most cases, plant genomes have large amounts of repetitive DNA which are not expressed and do not contribute to the physiological or morphological appearance of the plants. In the case of very closely related plant varieties and species, there are very few morphological differences, which as a matter of fact, do not represent the true genetic differences at the DNA level. So, there is always a need to study polymorphism at DNA level, which can be indicative of genetic diversity. RAPD technology has been used for assessing germplasm for species identification (Welsh & McClelland, 1990) and molecular characterization of many crop species, such as soybean, rice, rose and mustard (Fujishiro & Sasakuma, 1994). Similarly, varietal identification of kenaf has been done through RAPD (Rahman et al., 2007) but large scale germplasm characterization has yet been rare. Zhou et al. (2002) studied with 14 kenaf varieties and reported that identification of

individual kenaf variety through morphological characterization was difficult but it was clearly separated by RAPD analysis. They concluded that RAPD analysis is an effective tool for identifying kenaf varieties and determining their genetic relationship to a certain extent.

In this study, we investigated the genetic diversity and relationships among kenaf accessions and cultivars based on RAPD markers, and also examined its efficiency in the light of the known origin of the study materials.

MATERIALS AND METHODS

Plant materials

Twenty three kenaf accessions and two cultivars were obtained from the Genebank of Bangladesh Jute Research Institute (BJRI), Dhaka (Table 1). These germplasm were collected from different geographical regions (16 countries) and coded herein G_1 through G_{25}. The two cultivars – HC-2 and HC-95 are widely used as commercial variety and also used as parental material for kenaf breeding program in Bangladesh.

Table 1: Kenaf accessions and cultivars analysed

Sl. No.	Country of origin	Accession and cultivar name
01	Bangladesh	G_1 (HC-2), G_2 (HC-95), G_4 (Acc. 2731), G_{10} (Acc. 2103)
02	Java	G_3 (Acc. 1993)
03	Netherlands	G_5 (Acc. 2922)
04	Thailand	G_6 (Acc. 4634)
05	Australia	G_7 (CPI-72126), G_8 (Acc. 4659)
06	USA	G_9 (Acc. 4718), G_{14} (Acc. 4628), G_{23} (Acc. 4750)
07	Poland	G_{11} (Acc. 4372)
08	Sudan	G_{12} (Acc. 4383), G_{21} (Acc. 4389)
09	El-Salvador	G_{13} (Acc. 4410)
10	China	G_{15} (Acc. 4922), G_{24} (Acc. 5017)
11	Pakistan	G_{16} (Acc. 5050), G_{25} (Acc. 5030)
12	Nepal	G_{17} (Acc. 5080)
13	Cuba	G_{18} (Acc. 1983)
14	Kenya	G_{19} (Acc. 4197)
15	Tanzania	G_{20} (Acc. 4348)
16	France	G_{22} (Acc. 4432)

DNA extraction and RAPD analysis

Total genomic DNA was isolated from a bulk of young leaf tissues from five plants grown in greenhouse, following the modified Doyle and Doyle’s (1990) protocol of CTAB (Cetyltrimethyl ammonium bromide) method (Murray and Thompson, 1980). The experiment was carried out at Genetic Fingerprinting Laboratory of the Department of Genetics and Plant Breeding, and Central Laboratory of Bangladesh Agricultural University, Mymensingh. Nineteen primers of random sequence were screened on a sub sample of 4 randomly chosen individuals from 25 different kenaf genotypes to evaluate their suitability for amplifying DNA sequences that could be accurately scored. Primers were evaluated on the basis of band resolution or intensity, repeatability of markers, presence of smearing, consistency within individuals and the potential to differentiate polymorphism. The details of the primers are given in Table 2. A final subset of 6 primers exhibiting good quality banding patterns and sufficient variability were selected for the analysis of whole sample set of genotypes.

Table 2: List of random primers used in the study for screening

Primer code	Sequence (5' - 3')	GC content (%)
GLA05	AGGGGTCTTG	60
GLA07	GAAACGGGTG	60
GLA09	GGGTAACGCC	70
GLA10	GTGATCGCAG	60
OPG1	TGCCGAGCTG	70
OPG2	AGTCAGCCAC	60
OPG3	GAAACGGGTG	60
OPG4	GGGTAACGCC	70
OPG5	GTGATCGCAG	60
OPG6	CAATCGCCGT	60
OPG7	CAGCACCCAC	70
OPG8	CCGCCCAAAC	70
OPG9	AGCGAGCAAG	60
OPG10	GAACACTGGG	60
OPG11	CCCTACCGAC	70
OPG12	AATGGCCCAG	60
OPG13	CTCCTGCCAA	60
OPG14	CCCAGCTGTG	70
OPG15	GTGTCGCGAG	70

Primers marked with bold letter and underline showed polymorphism and were selected for RAPD analysis

DNA amplification was conducted as described by Williams et al., (1990) with some modifications. PCR reactions were performed in a 25µl reaction mix consisting of

- *Taq* DNA polymerase buffer (10 x) = 4.00µl
- Primer (10µM) = 3.00µl
- dNTPs (250µM) = 0.50µl
- *Taq* DNA polymerase = 0.20µl (1 unit)
- Genomic DNA (25 ng/µl) = 3.00µl
- Sterile deionized water = 14.30µl

Amplification was carried out in a thermal cycler (Master Cycler Gradient, Eppendorf) that was programmed as an initial denaturation or preheating at 94 ^{o}C for 3 minutes (first cycle), followed by 40 cycles of the procedure at 94 ^{o}C for 1 minute (denaturation), 36 ^{o}C for 1 minute (annealing), 72 ^{o}C for 2 minutes (extention/elongation), and adding a final elongation at 72 ^{o}C for 5 minutes (last cycle). After completion of cycling program, reactions were held at 4 ^{o}C followed by cooling. The PCR products were then separated electrophoretically on 1.5% agarose gel containing ethedium bromide in 1 X TBE buffer at 100V for 1 hour. DNA bands were observed under UV-light on a Transilluminator and photographed by a Gel Cam Polaroid camera (Type 667).

Data analysis

Polymorphic RAPD markers were manually scored as binary data: present (1) or absent (0) for each individual and each primer. Only clearly distinguishable bands were scored. The scores obtained using all primers in RAPD analysis were then pooled for constructing a single data matrix. This was used to estimate polymorphic loci, Nei's (1973) gene diversity and Nei's (1972) genetic distance (D) using a computer program, POPGEGE (version 1.31) (Yeh et al., 1999). To investigate the genetic relationships among accessions, genetic distances between all pairs of individual accession were estimated. A dendrogram was constructed based on genetic distance using unweighted pair group method with arithmetic averages (UPGMA).

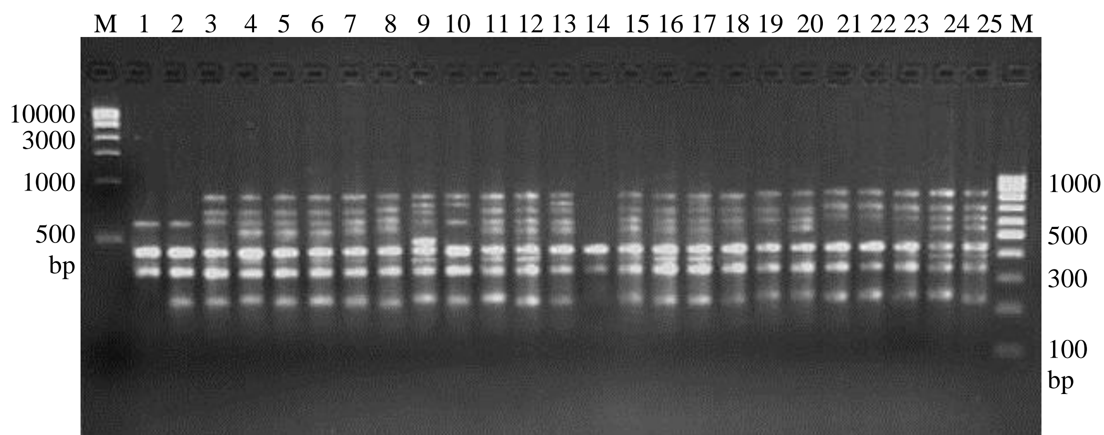
RESULTS AND DISCUSSION

Nineteen arbitrary decamer primers were initially surveyed for their ability to produce polymorphic patterns. Out of them, six primers viz. GLA09, GLA10, OPG1, OPG2, OPG7 and OPG9 yielded comparatively higher number of amplification products with higher intensity, minimal smearing and good resolutions with clear bands. Hence, these six primers were selected for evaluation of

diversity across all the genotypes. Amplification of the isolated genomic DNA from each of 25 germplasm using selected primers revealed a variety of RAPD patterns (Plate 1). Clearly detectable and reproducible bands ranged from 220 to 850 bp and 270 to 650 bp in size in case of primer GLA09 and OPG1. A total of 48 scorable bands produced from six primers of random sequence and their size ranges (bp) are presented in Table 3.

Table 3: RAPD primers with corresponding bands score and their size range together with polymorphic bands observed in 25 kenaf genotypes

Primer code	Sequences (5´-3´)	Total number of bands scored	Size ranges (bp)	Number of polymorphic bands
GLA09	GGGTAACGCC	10	220 – 850	8
GLA10	GTGATCGCAG	9	210 – 1200	7
OPG1	TGCCGAGCTG	7	270 – 650	7
OPG2	AGTCAGCCAC	7	180 – 1800	5
OPG7	CAGCACCCAC	6	270 – 1500	3
OPG9	AGCGAGCAAG	9	330 – 800	8
Total		48		38
Average		8		6.33



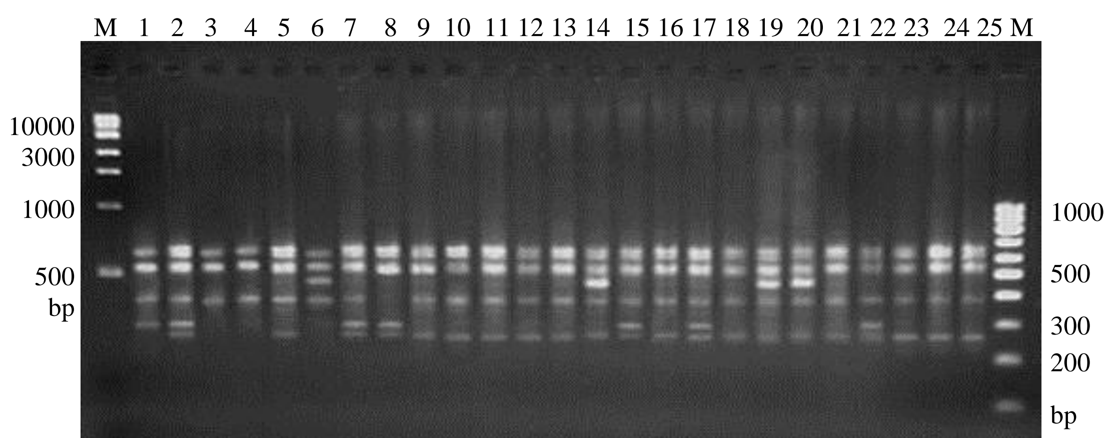


Plate 1: RAPD profiles of 25 kenaf genotypes amplified with primers GLA09 and OPG1. M: Molecular weight marker (1 kb DNA ladder in left side and 100 bp DNA ladder in right side)

Copyright © May-June, 2020; IJPAB

Lane	Genotypes	Lane	Genotypes	Lane	Genotypes	Lane	Genotypes	Lane	Genotypes
1	HC-2	6	Acc. 4634	11	Acc. 4372	16	Acc. 5050	21	Acc. 4389
2	HC-95	7	CPI-72126	12	Acc. 4383	17	Acc. 5080	22	Acc. 4432
3	Acc. 1993	8	Acc. 4659	13	Acc. 4410	18	Acc. 1983	23	Acc. 4750
4	Acc. 2731	9	Acc. 4718	14	Acc. 4628	19	Acc. 4197	24	Acc. 5017
5	Acc. 2922	10	Acc. 2103	15	Acc. 4922	20	Acc. 4348	25	Acc. 5030

The efficiency of molecular marker technique depends upon it polymorphism level in the set of accessions tested. In this study, RAPD markers amplified a total of 48 bands of which 38 (79.17%) were found to be polymorphic and the rest 10 (20.83%) were monomorphic in nature. On an average, each primer generated 6.33 polymorphic bands (Table 3). This is in accordance with the findings of Geo-Anping et al. (2002) who worked with different *Hibiscus* species using RAPD primers and showed that 16 primers of arbitrary sequence out of 80 amplified 192 bands of which 149 bands were polymorphic and 43 were monomorphic.

The number of amplified fragments against genotypes and primers varied widely. Size of PCR amplification products scored for each individual of 25 kenaf genotypes, and for each primer is presented in Table 4.

Table 4: Number of amplified fragments scored against genotypes and primers

Genotypes \ Primers	GLA 09	GLA 10	OPG 1	OPG 2	OPG 7	OPG 9	Total band	Polymorphic band
G_1 (HC-2)	3	5	2	4	4	3	21	11
G_2 (HC-95)	4	5	3	6	4	3	25	15
G_3 (Acc. 1993)	6	5	2	3	4	3	23	13
G_4 (Acc. 2731)	7	4	2	3	5	4	25	15
G_5 (Acc. 2922)	7	5	2	5	4	4	27	17
G_6 (Acc. 4634)	7	5	2	4	5	4	27	17
G_7 (CPI-72126)	7	6	3	6	3	2	27	17
G_8 (Acc. 4659)	7	5	4	5	4	3	28	18
G_9 (Acc. 4718)	9	7	2	5	5	5	33	23
G_{10} (Acc. 2103)	6	7	3	5	5	5	31	21
G_{11} (Acc. 4372)	8	4	5	5	4	3	29	19
G_{12} (Acc. 4383)	8	6	2	5	4	2	27	17
G_{13} (Acc. 4410)	8	6	4	5	4	4	31	21
G_{14} (Acc. 4628)	2	6	4	6	3	5	26	16
G_{15} (Acc. 4922)	7	6	4	6	4	5	32	22
G_{16} (Acc. 5050)	8	4	2	5	3	3	25	15
G_{17} (Acc. 5080)	8	3	4	6	4	3	28	18
G_{18} (Acc. 1983)	6	6	4	5	4	4	29	19
G_{19} (Acc. 4197)	6	7	2	6	4	4	29	19
G_{20} (Acc. 4348)	7	6	2	6	4	5	30	20
G_{21} (Acc. 4389)	6	5	2	5	4	5	27	17
G_{22} (Acc. 4432)	6	4	4	6	4	4	28	18
G_{23} (Acc. 4750)	6	4	3	5	4	4	26	16
G_{24} (Acc. 5017)	8	4	4	5	5	2	28	18
G_{25} (Acc. 5030)	8	4	4	5	4	3	28	18
Total	165	129	75	127	102	92	690	440

The primer GLA09 is able to produce a total of 165 bands in 25 genotypes (average 6.6 bands per genotype) and the highest number (9 bands) was recorded in G_9 (Acc. 4718). On the contrary, the lowest number of bands (75 or 3 bands/genotype) was recorded in primer OPG1. From the polymorphic loci point of view, G_9 produced the highest number of polymorphic bands (23) across all primers and G_1 produced the least (11 polymorphic bands). It means, none of the primers alone was able to identify all genotypes. Similar results were obtained by Vilarinhos et al. (2000) in Citrus, who identified 12 hybrids with six of 20 primers tested. None of 20 primers used by them was useful singly to identify all 12 hybrids.

The values of Nei's (1973) gene diversity and Shannon's information index for different kenaf genotypes across all loci are shown in Table 5. The estimate of Nei's (1973) genetic diversity for entire genotypes was 0.2118 and Shannon's information index was 0.3363.

Table 5: Estimation of genetic variability

No. of polymorphic loci	Proportion of polymorphic loci (%)	Nei's (1973) gene diversity (h)	Shannon's information index (i)
38	79.17	(0.2118 ± 0.1668)	(0.3363 ± 0.2349)

The high level of polymorphism revealed by the proportion of polymorphic loci (79.17%) indicated that there was a high level of genetic variation among the studied genotypes. Estimates of Nei's (1973) gene diversity (0.2118) and Shannon's information index (0.3363) across all loci (Table 5) also support the existence of high level of genetic variation in all studied materials.

The amplified products were scored and used for construction of a dendrogram (Figure 1) as well as for determining their genetic distances. Table 6 shows the distance between kenaf genotypes. The values of pair-wise comparison of Nei's (1972) genetic distance (D) between genotypes computed from combined data sets for six primers ranged from 0.0426 to 0.6523.

Table 6: Five of each higher and lower Nei's (1972) genetic distance (D) between pairs of genotype based on RAPD markers

Five higher D values	Genotype combination	Five lower D values	Genotype combination
0.6523	G_{14} x G_{10}, G_{23} x G_{10}	0.0426	G_{25} x G_{17}, G_{25} x G_{24}
0.5754	G_{11} x G_{10}, G_{15} x G_9, G_{17} xG_{10}	0.0645	G_{21} x G_{20}
0.5390	G_{10} x G_1, G_{10} x G_4	0.0870	G_2 x G_1, G_3 x G_1, G_4 x G_3, G_5 x G_4, G_6 x G_4, G_6 x G_5, G_{18} x G_5, G_{19} x G_5, G_{19} x G_{18}, G_{24} x G_{11}, G_{24} x G_{17}, G_{25} x G_{11}, G_{25} x G_{16}
0.5039	G_{10} x G_8, G_{14} x G_9, G_{16} x G_{10}, G_{22} x G_{10}, G_{24} x G_{10}, G_{25} x G_{10}	0.1100	G_{16} x G_5, G_{16} x G_{12}, G_{24} x G_{12}, G_{25} x G_5, G_{25} x G_{12}, G_{25} x G_{18}
0.4700	G_{10} x G_3, G_{10} x G_5, G_{10} x G_9, G_{12} x G_{10}, G_{20} x G_{14}, G_{23} x G_{20}	0.1335	G_3 x G_2, G_5 x G_3, G_6 x G_3, G_7 x G_5, G_{12} x G_3, G_{12} x G_5, G_{13} x G_7, G_{16} x G_{11}, G_{17} x G_{11}, G_{17} x G_{16}, G_{18} x G_7, G_{18} x G_{13}, G_{19} x G_3, G_{19} x G_6, G_{19} x G_9, G_{22} x G_8, G_{22} x G_{11}, G_{22} x G_{17}, G_{24} x G_{16}, G_{24} x G_{22}, G_{25} x G_{22}

The highest genetic distance (0.6523) was observed between the genotypes G_{10} and G_{14}, and G_{10} and G_{23}. While the lowest genetic distance (0.0426) was observed between the genotypes G_{17} and G_{25}, and G_{24} and G_{25}. The distance between the highest and the lowest values indicated the presence of variability among 25 kenaf genotypes. The dendrogram shows five different clusters designated as I, II, III, IV and V, in which cluster I, II and IV were further divided into ten sub-clusters. The distribution of cluster members is shown in Table 7.

Table 7: Distribution of 25 kenaf genotypes under different clusters using RAPD data

Cluster number	Total no. of genotypes in		Genotypes included in different clusters
	Cluster	Sub-cluster	
I	8	$Sc_I = 2$	G_1(HC-2), G_2(HC-95)
		$Sc_{II} = 4$	G_3(Acc.1993), G_4(Acc. 2731), G_5(Acc. 2922), G_6(Acc. 4634)
		$Sc_{III} = 2$	G_{18} (Acc. 1983), G_{19} (Acc. 4197)
II	10	$Sc_I = 2$	G_7 (CPI-72126), G_{13} (Acc. 4410)
		$Sc_{II} = 2$	G_8 (Acc. 4659), G_{22} (Acc. 4432)
		$Sc_{III} = 4$	G_{11}(Acc. 4372), G_{17}(Acc.5080), G_{24}(Acc. 5017), G_{25}(Acc.5030)
		$Sc_{IV} = 2$	G_{12} (Acc. 4383), G_{16} (Acc. 5050)
III	2	-	G_{14} (Acc. 4628), G_{23} (Acc. 4750)
IV	4	$Sc_I = 1$	G_{10} (Acc. 2103)
		$Sc_{II} = 1$	G_{15} (Acc. 4922)
		$Sc_{III} = 2$	G_{20} (Acc. 4348), G_{21} (Acc. 4389)
V	1	-	G_9 (Acc. 4718)

Dendrogram based on Nei's (1972) genetic distance using UPGMA indicated segregation of 25 kenaf genotypes into two major, two minor and one single genotype clusters. Identical and closely related genotypes were clustered together. The last single one genotype cluster appeared as outlier in the dendrogram and distantly related with the rest of the genotypes. The minor clusters had 2 to 4 genotypes. The two major clusters consisted of 8 to 10 genotypes. The first major cluster formed three separate sub-clusters and the second major cluster was subdivided into four sub-clusters (Table 7). The first minor cluster composed of two genotypes and the second minor cluster grouped together four genotypes. Similarly, Suvakanta et al. (2006) studied with two species of *Hibiscus* (*H. sabdariffa* and *H. schizopetalus*) and 16 varieties of *Hibiscus rosa-sinesis* through RAPD markers and reported that the studied materials formed a single cluster. The first major cluster consisted of three varieties; and a second major cluster consisted of two species and 13 varieties. The genetic distance was very close within the varieties and also among the species.

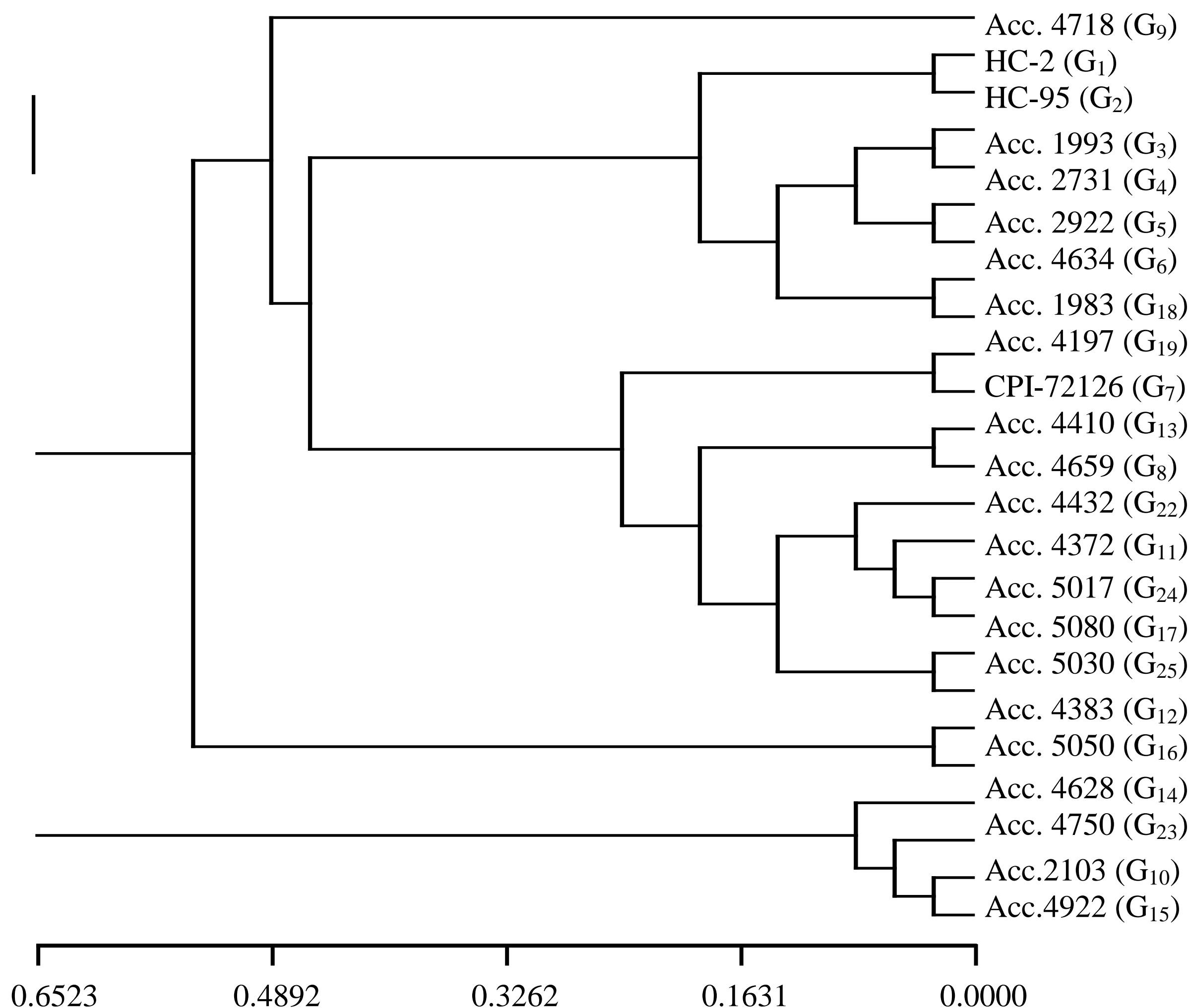


Fig. 1: Dendrogram of 25 kenaf genotypes using UPGMA based on Nei's (1972) genetic distance according to RAPD marker analysis

CONCLUSION

Diversity analysis is usually performed to identify the diverse genotypes for hybridization purposes. The genotypes grouped together are less divergent among themselves than those which are fall into different clusters. The crosses involving parents belonging to the maximum divergent clusters were expected to manifest maximum heterosis in F_1 and wide variation in F_2. In choosing parental materials for crossing work, selection criteria should be based on genetic distances between genotypes and information on their relationship in cluster analysis. Cox et al. (1985) proposed that crosses between distantly related lines in an inbred improvement program would increase the number of segregating loci in the F_2 and subsequent inbred generations. Cross combinations involving parents that are distantly related and coming from different clusters are more expected to produce heterotic offspring.

Molecular identity of kenaf genotypes, particularly for the released varieties, is very important to protect bio-piracy. In the present study, twenty five genotypes of kenaf were used for RAPD analysis using six decamer random primers. Amplification of the isolated genomic DNA from each of the 25 genotypes, revealed a variety of RAPD patterns. A total of 48 RAPD markers were generated of which 38 (79.17%) were considered as polymorphic and the rest were monomorphic in nature. Primers GLA09 and OPG9 individually amplified the maximum number of polymorphic bands (8)

and the minimum number (3) was recorded with primer OPG7. On an average each primer generated 6.33 polymorphic bands. The estimate of Nei's (1973) genetic diversity for 25 kenaf genotypes was 0.2118 and Shannon's information index was 0.3363 across all loci indicated the presence of high level of genetic variation among the studied genotypes.

The UPGMA dendrogram based on Nei's (1972) genetic distances indicated segregation of 25 kenaf genotypes into two major, two minor and one single genotype clusters. The first and second major cluster consisted of 8 and 10 genotypes, respectively; while the two minor clusters (III and IV) had 2 to 4 genotypes. The highest genetic distance (0.6523) was found between G_{10} and G_{14} and/or between G_{10} and G_{23} and they remain in different cluster though G_{14} and G_{23} formed same cluster. Likewise, the genetic distance between G_{17} and G_{25} and/or between G_{24} and G_{25} was lowest (0.0426) and they remain together in the same cluster.

However, the relationships observed using the RAPD-based dendrogram may provide detail information on the studied materials. Considering interrelationships and genetic distances the genotypes G_4, G_5, G_7, G_8, G_{19} and G_{25} may be selected as parental source for future breeding program of kenaf improvement.

Acknowledgements

The authors thank the Bangladesh Jute Research Institute (BJRI) for supplying the valuable seeds of kenaf accessions and cultivars used in this research. This research work was fully supported by the Bangladesh Agricultural Research Council, Ministry of Agriculture, Bangladesh. The authors expressed their deepest gratitude and appreciation to Dr. Asma Khatun, Dr. M. M. Hussain, Dr. C. K. Saha, Dr. M. S. Haque, Dr. M. S. Islam; and Director Agriculture of BJRI Dr. M. M. Rahman for their inspiration and kind support. The authors also expressed their heartiest gratitude to all personnel of Breeding Discipline, BJRI for their contribution regarding this study.

REFERENCES

Banuelos, G.S. (2000). Kenaf and Canola– Selenium Slurpers. *Agril. Res., June 2000*: 10-11.

Cox, T.S., Lookhart, G.L., Walker, D.E., Harrell, L.G., Albers, L.D., & Rodgers, D.M. (1985). Genetic relationships among hard red winter wheat cultivars as evaluated by pedigree analysis and gliadin polyacrylamide gel electrophoretic patterns. *Crop Sci., 25*, 1058-1063.

Doyle, J. J., & Doyle, J.L. (1990). Isolation of plant DNA from fresh tissues. *Focus, 12*, 13-15.

Fujishiro, T., & Sasakuma, T. (1994). Variety identification and molecular characterization of newly bred line by RAPD marker in *Brassica juncea*. *Breed. Sci., 44*(1), 132.

Geo-Anping, Zhou-Peng, Su-Jian Gusng, Guo-AP, Zhou-P and Su-JG (2002). Random amplified polymorphic DNA (RAPD) analysis among *Hibiscus cannabinus* and related species. *J. Tropic., & Sub-tropic. Bot., 10*(4), 306-312.

Hazra, S.K., & Singh, D.P. (1997). Paper making from bast fibre crops- Aspects and Prospects. *Technical Bulletin Series No.4*. CRIJAF, p.56.

Joshi, A.B., & Dhawan, N.L. (1986). Genetic improvement of yield with special reference to self fertilizing crops. *Indian J. Genet., 26*(1), 101-113.

Karmaker, P.G., Hazra, S.K., Ramasubramanian, T., Mandal, R.K., Sinha, M.K., & Sen, H.S. (2008). *JUTE AND ALLIED FIBRE UPDATES: Production and Technology*. Central Research Institute for Jute and Allied Fibres (ICAR), Barrackpore, Kolkata-700 120, India. www.crijaf.org

Killinger, G.B. (1969). Kenaf (*Hibiscus cannabinus*), a multi-use crop. *Agron. J., 61*(5): 734-736.

Kumar, D. (1999). Possibility of commercial utilization of residual heterosis in

kenaf (*Hibiscus cannabinus* L.). *Indian J. Genet.*, 59(2),227-232.

Lam, T.B.T. (2000). Structural details of kenaf cell walls and fixation of carbon dioxide. *Proceedings of the 2000 international kenaf symposium*. Hiroshima, Japan, Oct.13-14, pp. 81-90.

Maiti, R.K. (1997). *World Fiber Crops*. Science Publishers Inc. New Hampshire, USA. pp. 41-61.

Murray, M.G., & Thompson, W.F. (1980). Rapid isolation of high molecular weight plant DNA. *Nucl. Acids Res., 8*, 4321-4325.

Nei, M. (1972). Genetic distance between populations. *Am. Nat., 106*, 283-292.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci., 70*, 3321-3323.

Nevo, E., Golenberg, E., Beilies, A., Brown, A.H.D., & Zohary, D. (1982). Genetic diversity and environmental association of wild wheat, *Triticum diococcoides* in Israel. *Theor. Appl. Genet., 62*, 241-254.

Pandey, S.N., & Krishnan, A. (1990). "*Fifty years of research (1939-1989)*", Jute Technological Research Institute, p.80.

Perry, R.C., Jones, D.E., & Bhangoo, M.S. (1993). "A preliminary study on kenaf as a feed for livestock (1992)", pp. 45-48 in proceedings. *Fifth Annual International Kenaf Association Conference*, March 3-5, 1993, Fresno CA.

Rahman, L., Molla, M.R., Sultana, S., Islam, M.N., Ahmed, N.U., Rahman M.S., & Nazim-ud-Dowla, M.A.N. (2007). Plant Varieties of Bangladesh: Morphological and molecular characterization. *Published by Seed wing, MoA, GoB, Khamarbari, Dhaka. 1*, 184-215.

Sinha, M.K., & Day, A. (2008). Pulp and Paper from Jute and Allied Fibres. In: P.G. Karmakar, S.K. Hazra, T. Ramasubramanian, R.K. Mandal, M.K. Sinha and H.S. Sen (ed), *JUTE AND ALLIED FIBRES UPDATES: Production and Technology*. CRIJAF, India. pp. 287-296.

Suvakanta, B., Senapati, S.K., Subhashree, A., Anuradha, M., & Rout, G.R. (2006). Identification and genetic variation among *Hibiscus* species (Malvaceae) using RAPD markers. *Biosciences, 61*(1/2), 123-128.

Vilarinhos, A.D., Viana, C.H.P., Soares, F.W.S., Nickel, O., Oliverra, R.P., & de Oliveira, R.P. (2000). RAPD markers for evaluating genetic diversity and identifying inter-specific hybrids in citrus. *Revista Brasileira de Fruticultura, 22*(21), 14-19. [Cited from CAB Abst., Vol. CIAC, 2000-2002].

Welsh, J., & McClelland, M. (1990). Fingerprinting genomes using PCR with arbitrary primers. *Nucl. Acids Res., 18*: 7213-7218.

Williams, J.G.K., Kubelik, A.R., Livac, K.J., Rafalski, J.A., & Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res., 18*(22), 6531-6535.

Wood, I.M. (1981). The utilization of field crops and crop residues for paper pulp production. Fields Crops Abstract. *Commonwealth Bureau of Pastures and Field Crops, 34*(7): 557-568.

Yeh, F.C., Yang, R.C., Boyle, T.B.J., Ye, Z.H., & Mao, J.X. (1999). POPGENE, the user-friendly software for population genetic analysis. Mol. Biol. Biotechnol. Centre, University of Alberta, Canada.

Zhou Cheng, Bao-Rong Lu, Brian S. Baldwin, Kazuhiko Sameshima and Jia-Kuan Chen (2002). Comparative studies of genetic diversity in kenaf (*Hibiscus cannabinus* L.) varieties based on analysis of agronomic and RAPD data. *Hereditas, 136*, 321-239.