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Full Length Article

Assessment of Genetic Variation in Different Kenaf (*Hibiscus cannabinus*) Genotypes using Morpho-agronomic Traits and RAPD Markers

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Abstract

Twenty five kenaf (*Hibiscus canabinus* L.) genotypes originated in different parts of the world were studied in Malaysian tropical environment to assess genetic variation using morpho-agronomic traits and random amplified polymorphic DNA (RAPD) markers. A total of 13 morpho-agronomic traits was selected for study and found that the genotypes varied significantly ($p<0.01$) in these traits. The major production traits such as stick weight (SW) and fiber weights (FW) were found highly correlated with other traits. Agglomerative hierarchical cluster analysis and principal component analysis (PCA) of the morpho-agronomic traits yielded four major cluster groups of the studied genotypes. The low fiber and stick yield producing early maturing genotypes were in cluster I, middle fiber weight, plant height and matured genotypes were in cluster II, and III and high fiber and stick weight producing late flowering genotypes were in cluster IV PCA of the phenotypic data using covariance matrix revealed that first three components accounted for 97.20% of total variation of the genotypes. The assessment of genetic diversity using RAPD marker revealed high genetic polymorphisms of the makers (7.2) with Jaccard's similarity coefficient of variation from 0.000 to 0.952. These findings of phenotypic and genetic variations in morpho-agronomic traits and polymorphism level at DNA expressed the usefulness of these genotypes as parent materials for future improvement in kenaf breeding programs. © 2015 Friends Science Publishers

Keywords: Kenaf genotypes; Morpho-agronomic trait; RAPD marker; Genetic diversity; Kenaf breeding program

Introduction

Kenaf (*Hibiscus canabinus* L.) is a traditional fiber producing plant belonging to the family Malvaceae. In the present global environmental needs and inadequate green fiber resources, kenaf is a potential crop with higher tensile strength fiber (Faruq *et al.*, 2013) and because of lower production cost and labor requirements it is now replacing jute plants traditionally used for fiber production (Golam *et al.*, 2011). To date, kenaf has been utilized for manufacturing various industrial products such as pulping and paper making. Good quality kenaf fiber can be utilized for producing industrial filter and the core can be utilized as a bio-remediation agent, animal bedding, and low-density particle board (Sellers and Reichert, 1999; Baldwin and Graham, 2006). In order to expand its industrial use and maintain the economic viability, it is important to study the genetic diversity of different kenaf genotypes for developing an effective breeding program that will yield high fiber or stick (Bitzer *et al.*, 2000).

The widespread method to define the variability of kenaf is the study of morpho-agronomic traits. Raw morphological properties play important roles to classify kenaf varieties. However, defining the kenaf genotype by common traits such as plant height, leaf shape and maturity etc. are sometimes difficult. For instance, morphological traits cannot be utilized in early selection of potential kenaf genotypes. In addition, genetic variability detection using morphological traits is not worthy when the target gene expression changes with environmental condition and plant development stages (Kalpana *et al.*, 2012). It is also important to define the circulating seeds in the market to secure farmer's interest from potential fraudulent admixtures (Cheng *et al.*, 2002). Traditional genetic variation analysis was on morphological and agronomical traits and due to the difficulty to identify cultivars based entirely on these traits effective recently molecular technologies are introduced (Islam *et al*., 2014).

For characterization of genetic variation in plants certain molecular DNA based markers, such as randomly amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSR)

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can be applied (Murtaza, 2006; Begum *et al*. 2013; Islam *et al*., 2014). Among these markers, RAPD is a widely used for diversity analysis in plant because of its advantage in rapid assessment of genetic composition in large number of individuals (Bhattacharya and Ranade, 2001). Besides, it can be utilized in any stage of the plants where other techniques such as isozyme analysis were found to be insignificant (Sreekumar and Renuka, 2006). Moreover, RAPD is single primer based marker and analysis with this marker is cheaper than other molecular techniques and it can detect variable multiple loci in the chromosome. This study was, therefore, conducted to determine the genetic diversity in different kenaf genotypes of diverse origin in the Malaysian tropical environment using morphological traits and RAPD markers.

Materials and Methods

Location of Experiment and Soil Condition

The experiment was conducted in the field of Genetics and Molecular Biology, Institute of Biological science, University of Malaya, Kuala Lumpur, Malaysia during October 3, 2012 to January 8, 2013. The research field was located at 3.20°N and 101.40°E with elevation of 22 m from sea level and soil type was sandy loam. Weather was hot and humid during the course of experimentation (Table 1).

Experimental Material and Design

Twenty five kenaf genotypes were collected from Bangladesh Jute Research Institute (BJRI) Gene Bank, through IJSG (International Jute Study Group), Dhaka, Bangladesh. The genotypes had fifteen different geographic origins (Table 2). The experiment was conducted following randomized complete block design with three replications. Individual experimental plots were 2.5 m long and 2.4 m wide, with 40 cm spaced 6 rows.

Field Managements

Malaysian Agricultural Development Research Institute recommended crop management practices were followed for this experiment. Experimental plots were ploughed and leveled properly. Drainage channel around the plots were used to drain out excess rain water. The plots were fertilized with the N, P_2O_5, and K_2O at the rate of 122, 122 and 144 kg ha^{-1}, respectively. Nitrogen was applied in three equal splits. One third of N, and whole P_2O_5 and K_2O were applied as basal dose; whereas remaining N was applied in two equal splits each at 20 and 35 days of sowing. To adopt insect-pests and disease control measurement fungicide 80% w/w Mancozeb was used @ 2 kg/ha (40 gm with 10 L water) and Diazinon 50% WP @ 2 lbs/ha together with 100 gallon of water.

Table 1: Weather condition during the study (Monthly mean)

Month	Temperature (°C)	Humidity (%)	Rainfall (mm)
Oct, 2012	27.7	79.9	459.0
Nov, 2012	27.2	83.7	684.0
Dec, 2012	27.1	83.5	455.2
January, 2013	27.6	83.8	464.1

Source: Department of Metrology, Ministry of Science, Technology and Innovation, Malaysia

Table 2: Country of origin and Bangladesh Jute Research Institute (BJRI) code of 25 different kenaf genotypes

Entry	BJRI Code	Origin	Entry	BJRI Code	Origin
E4	1585	USA	E41	4408	South Africa
E5	1593	USA	E42	4410	El Salvador
E7	1627	Iran	E43	4414	El Salvador
E12	1693	USA	E44	4432	France
E15	2922	Netherland	E50	4443	Egypt
E19	3746	Kenya	E51	4444	Egypt
E21	3748	Kenya	E53	4625	Cuba
E24	3834	Kenya	E54	4626	USA
E25	4119	Kenya	E56	4628	USA
E31	4283	Tanzania	E61	4649	Australia
E33	4335	Tanzania	E72	5026	Pakistan
E36	4372	Poland	E74	5073	Nepal
E37	4383	Sudan			

Observations

Morpho-agronomic data were collected from 10 randomly selected plants from each plot. The plant was cut and height was measured from ground level to the top of the plants. After completion of proper retting, kenaf fibers were stripped from stick manually and washed in clean water. The complete drying of the fiber was done by keeping in direct sun light for 4-5 days. To get dry sticks weight, kenaf sticks were dried for seven continuous days. The yields of fiber and stick were recorded from each of the individual plant.

RAPD Analysis

For RAPD analysis, five OPA primer sets described by Cheng *et al*. (2002) were used in this study. The primers were synthesized by medigene Sdn Bhd, Malaysia. The PCR reaction mixture contained 10 mM Tris-HCl (pH 9.0), 10 mM KCl, 20 mM $MgCl_2$, 200 µM dNTPs, 0.2µM primer, 1.25 units of YEAtaq DNA polymerase (Yeastern-biotech, Taiwan) and 25 ng template DNA in a total volume of 25 µL. Polymerase Chain Reaction (PCR) amplification for each primer set was performed in C1000 Thermal Cycler (Bio-Rad, USA). The PCR amplifications were carried out with an initial denaturation at 94°C for 5 min, followed by 45 cycles of denaturation at 94°C for 30 s, and annealing at 39.9°C for 30 s and elongation at 72°C for 2 min, and the final extension at 72°C for 10 min. Following amplification, the presence of PCR products were verified via electrophoresis with 1.0% agarose gel.

The gel electrophoresis were carried out at 100V, 200 mA using 1xTBE Running Buffer for 45 min. The gels were stained with ethidium bromide (10 mg/mL) before being visualized under ultraviolet light using gel documentation system (Siber Hegner, Germany).

Statistical Analysis

XLSTAT Version 2013 and SAS 9.2 were used for Duncan's New Multiple Range Test (DNMRT) (Gomez and Gomez, 1984). Analysis of variance and correlation studies were conducted determining the Pearson's correlation coefficient described by Hollander and Wolfe (1973) and Best and Roberts (1975). Principal Component Analysis (PCA) was done by using covariance matrix (Jolliffe, 2005) and clustering was done by Agglomerative Hierarchical Clustering following by Ward's method (Ward Jr., 1963). For molecular analysis, the binary data matrix was applied for the computations of Jaccard's coefficient of genetic similarity between all possible pairs of accessions. Estimated similarity coefficient values were used to construct a dendogram (cluster diagram) according to the method of un-weighted pair group with arithmetic averages (UPGMA) and principal component analysis (PCA) were executed with the software package NTSYS-pc, version 2.02 (Rohlf, 2002).

Results

Morpho-Agronomic Traits

Genetic variations and correlation study: A total of 13 morpho-agronomic traits, was studied for the genetic variability, all of which showed significant differences ($P<0.01$) among the genotypes (Table 3 and 4). The two major production traits such as stick weight (SW) and fiber weights (FW) were found highly positively correlated with plant height (PH), base diameter (BD), middle diameter, core diameter (CD), node number (NN) and days of 50% flowering (DF) (Table 4). Significant ($p<0.05$) negative correlation was observed between these two traits and leaf width. Days of flowering were negatively correlated with top diameter (TD), leaf length (LL), leaf angel (LA) and petal length (PL) (Table 5).

Cluster and Principal component analysis (PCA): Agglomerative hierarchical cluster analysis with 13 of morpho-agronomic traits using Euclidian distance yielded 4 groups from the 25 genotypes (Fig. 1, Fig. 2 and Table 6). Cluster 1, 2, 3 and 4 composed of 9, 7, 6, and 3 kenaf genotypes respectively and revealed distance within the genotypes by forming clusters with more homogenous group. The low fiber and stick yield producing early maturing genotypes were in cluster I, middle fiber weight, plant height and matured genotypes were in cluster II, and III and high fiber and stick weight producing late flowering genotypes were in cluster IV (Table 6).

Table 3: Mean values, minimum, maximum, range, standard deviation, coefficients of variation and F value for 13 agronomic traits of 25 kenaf genotypes (*Hibiscus cannabinus* L.)

Traits	Minimum	Maximum	Range	Mean	Standard deviation	CV	F-value
PH	130.21	226	95.79	169.2	24.914	4.25	32.29
BD	7.4	14.25	6.85	10.87	1.795	5.02	28.92
MD	5.05	9.09	4.04	7.05	1.015	7.63	8.27
TD	2.49	4.74	2.25	3.48	0.501	7.71	8.06
CD	6	11.87	5.87	9.18	1.575	8.73	9.16
LL	6.49	11	4.51	8.81	1.085	7.05	6.85
LW	5.13	10.86	5.73	7.81	1.351	6.97	15.7
LA	50	72	22	63.31	5.331	3.11	19.09
PL	4	10.675	6.68	7.48	1.308	9.47	7.86
NN	21	44.1	23.1	32.47	6.12	8	13.92
DF	47	60	13	52.68	3.411	8.09	6.67
SW	8.45	23.98	15.53	15.3	5	14.27	23.88
FW	1.6	6.7	5.1	3.88	1.591	13	42.665

PH =Plant height (cm), BD =Base diameter (mm), MD =Middle diameter (mm), CD =Core diameter (mm), TD= Top diameter, LL= leaf length (cm), LW =leaf width (cm), LA= leaf angle, PL =Petal length (mm), NN=Number of nodes, DF =50% Flowering date (Days), SW= Stick weight (gm), FW = Fibre weight (gm)

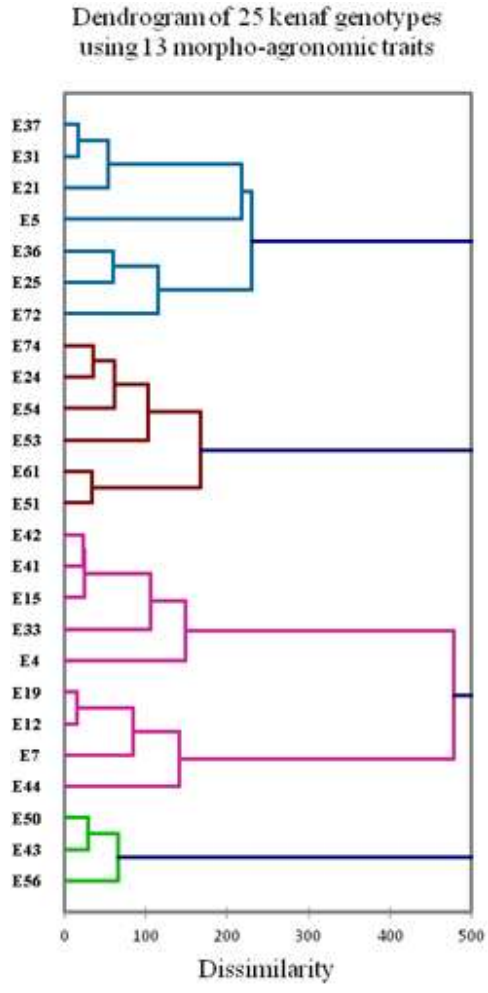

Fig. 1: Cluster analysis showing the genetic relationships relationships of 25 kenaf genotypes using ward's method

Table 4: Mean squares of sources of variation of 13 morpho-agronomic traits of 25 genotypes of kenaf (*Hibiscus cannabinus* L.)

Source	DF	PH	BD	MD	TD	CD	LL	LW	LA	PL	NN	DF	SW	FW
Genotype	24	1810*	9.2*	2.5*	0.6*	6.3*	2.7*	4.8*	79.5*	4.2*	100*	27.3*	70.9*	7.4*
Error	50	50	0.34	0.30	0.07	0.62	0.44	0.37	3.9	0.51	7.3	4.09	2.97	0.17

*Significant at 1% probability levels, PH =Plant height (cm), BD =Base diameter (mm), MD =Middle diameter (mm), CD =Core diameter (mm), TD= Top diameter, LL= leaf length (cm), LW =leaf width (cm), LA= leaf angle, PL =Petal length (mm), NN=Number of nodes, DF =50% Flowering date (Days), SW= Stick weight (gm), FW = Fibre weight (gm)

Table 5: Pearson correlation coefficient matrix for 13 agronomic traits of 25 different kenaf genotypes

Traits	PH	BD	MD	TD	CD	LL	LW	LA	PL	NN	DF	SW	FW
PH	1.00												
BD	0.59	1.00											
MD	0.30*	0.54	1.00										
TD	-0.06	0.25	0.24	1.00									
CD	0.53*	0.79*	0.60**	0.28	1.00								
LL	0.02	-0.12	0.19	0.03	0.20	1.00							
LW	-0.02	-0.15	0.19	-0.01	0.08	0.79	1.00						
LA	0.07	-0.06	-0.10	-0.13	0.11	0.07	-0.21	1.00					
PL	0.05	0.02	0.26*	0.31**	0.23*	0.68*	0.66*	-0.22	1.00				
NN	0.61*	0.65*	0.60*	0.17	0.55*	0.02	0.07	-0.19	0.11	1.00			
DF	0.59*	0.47*	0.29	-0.07	0.52*	-0.01	-0.11	0.12	-0.02	0.24	1.00		
SW	0.72*	0.70*	0.26	0.10	0.68*	0.03	-0.06	0.28	-0.02	0.46*	0.48*	1.00	
FW	0.75**	0.77*	0.51*	0.01	0.66*	-0.08	-0.03	-0.04	0.02	0.67*	0.57*	0.72*	1.00

*Significant at 1% probability levels. PH =Plant height (cm), BD =Base diameter (mm), MD =Middle diameter (mm), CD =Core diameter (mm), TD= Top diameter, LL= leaf length (cm), LW =leaf width (cm), LA= leaf area, PL =Petal length (mm), NN=Number of nodes, DF =50% Flowering date (Days), SW= Stick weight (gm), FW = Fibre weight (gm)

PCA of 13 morpo-agronomic traits of the kenaf genotypes using covariance matrix and Pearson's correlation coefficient revealed that the first three components accounted for 97.20% of the total variation (Table 7). The first component explained 90.18% of the total variation and was characterized by plant height, base diameter, core diameter, number of nodes, days of 50% flowering, stick weight and fiber weight. The second component was characterized by leaf angel, middle diameter and top diameter.

RAPD Analysis

Marker analysis: Five selected primers generated 36 polymorphic bands (data not shown). The number of bands generated per primer varied from 4 to 14. The lowest number of bands was generated by primer OPA16, while the primer OPA3 produced the highest band. Primer OPA3 produced the maximum number of polymorphic bands in all the genotypes, followed by OPA7, OPA12 0r OPA20 and OPA16 (Table 8). The percentages of polymorphisms for OPA3, OPA7, OPA12, OPA16, and OPA20 were 92.9%, 92.3%, 80.0%, 75.0%, 60.8% and 80.0%, respectively. The average RAPD markers amplification with polymorphism was 7.2.

Cluster analysis and PCA: The UPGMA cluster analysis of the Jaccard's similarity coefficient generated dendogram demonstrating the overall genetic relationship among the genotypes but showed little explanation according to the origin of the genotypes (Fig. 3, Table 2).

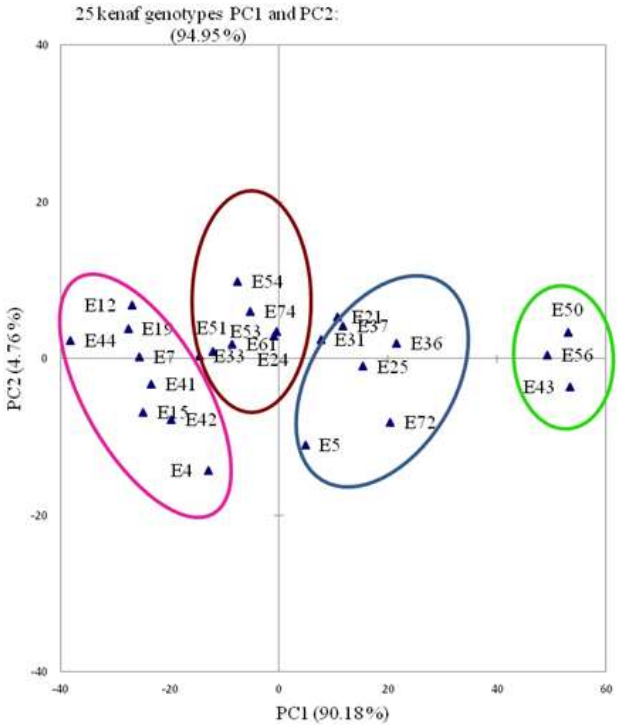

Fig. 2: Scatter plot using 13 morpho-agronomic traits from first two components of PCA analysis showing the variation of 25 kenaf genotypes

Genotypes were clustered into six major clusters. Cluster I composed of ten genotypes namely E4, E24, E7, E36, E21,

E31, E15, E25, E33 and E5. Each of Cluster II and V consisted of two genotypes E12, E19 and E44, E51 respectively. Cluster III includes three genotypes E37, E74 and E61. Genotype E43 was separate and formed an individual cluster VI and rest seven genotypes were grouped under cluster IV. Based on Jaccard's similarity coefficient, the genetic variation among the Kenaf genotypes ranged from 0.000 to 0.952 (data not shown).

Three principal components (PCs) accounted for 66.90% of the total variation in the 25 genotypes, where the first three PCs exhibited variations of 40.20, 15.80 and 10.90%. In the two-dimensional graph of PCA from RAPD marker analysis, 25 kenaf genotypes were clustered into seven groups (Fig. 4).

Discussion

Development of plant breeding is the forwarding step for increasing yield and quality. It involves analysis of the variation and is associated with plants morpho-agronomic data of different traits along with the major production traits of the plants. An improved production and quality attribute can be achieved by measuring phenotypic characteristics of the plants using rigorous statistical procedures (Lynch and Walsh, 1998). Analysis of the genetic variations and phenotypic performance has been used successfully in practical plant breeding since the last century to improve certain crops (Hammer *et al*., 2006). Morpho-agronomic variation among genotypes depends on the different geographical origin, planting date, plant maturity period, length of growing season (Webber and Bledsoe, 2002; Faruq *et al*., 2013). Therefore, Malaysian tropical environment and different genotypes originated from different countries influenced to vary in the morpho-agronomic traits among the genotypes. In addition, the significant differences in the morpho-agronomic traits such as 50% flowering day (DF), stick weight (SW) and fiber weights (FW) among the kenaf genotypes were also supported by the similar previous reports (Balogun *et al*., 2008; Golam *et al*., 2011; Faruq *et al*., 2013). The two major production traits FW and SW were highly positively correlated with plant height, diameter and maturity period (Table 4). But these major traits were significantly negative correlated with leaf width (Table 4), as also reported the similar result by Balogun *et al*. (2008). Days of flowering were negatively correlated with kenaf top diameter, leaf size and petal length. These results were supported by the previous reports, as early maturity reduces vegetative growth and associated with shorter internodes and petiole lengths of plants (Webber and Bledsoe, 2002; Faruq *et al*., 2013).

PCA is one of the important multivariate techniques utilized for the objectives to create groups of individuals or objects on the basis of similar characteristics they possess (Hair *et al.,* 1995). It facilitates to combine the individuals with similar characteristics by mathematically

Table 6: The eigenvalues of the covariance matrix for 13 morpho-agronomic traits of 25 kenaf genotypes

Traits	PC1	PC2	PC3
Eigenvalue	641.09	33.87	16.00
Variability (%)	90.18	4.76	2.25
Plant height	0.969	0.009	-0.203
Base diameter	0.044	-0.037	0.210
Middle diameter	0.012	-0.047	0.124
Top diameter	-0.002	-0.011	0.035
Core diameter	0.034	0.018	0.167
Leaf length	-0.001	0.023	-0.022
Leaf width	-0.003	-0.046	-0.035
Leaf angle	0.015	0.814	0.338
Petal length	0.000	-0.041	-0.032
Node number	0.155	-0.475	0.771
Days of 50% flowering	0.086	0.096	-0.072
Stick weight	0.152	0.305	0.365
Fibre weight	0.047	-0.018	0.144

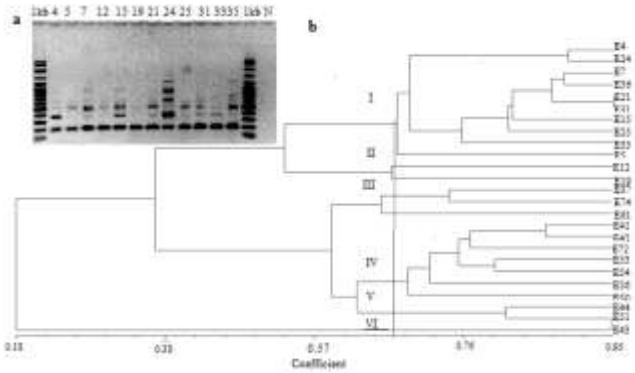

Fig. 3: Amplification pattern of RAPD marker of certain genotypes (a); Dendrogram exploring the genetic relationships of 25 kenaf genotypes using Jaccard index and (b) UPGMA clustering method

gatherings into one cluster. The successful cluster analysis classifies closer individuals in one cluster and separate the others forming different cluster by representing in geometrical plots (Hair *et al.,* 1995). In the present work, the low fiber and stick yield producing early maturing genotypes were differentiated from high fiber weight stick weight producing late flowering genotypes by applying this analysis (Fig. 1, Table 6). Therefore, these kenaf genotypes forming clusters with different flowering stages can be utilized for the selection of high yielding kenaf breeding program. The similar recommendation has been made in previous studies with different kenaf genotypes obtained from different environmental origin (Golam *et al.,* 2011; Faruq *et al.,* 2013).

PCA is referred to a "data reduction method" for explanation of the relationships between two or more characters and to split the total variance of the original characters into a limited number of uncorrelated new variables (Wiley, 1981). Application of PCA is useful for preliminary data classification, unsupervised pattern recognition and determination of relationship among different genotypes (Šamec *et al*., 2014). Thus, the PCA

Table 7: Numbers of the kenaf (*Hibiscus cannabinus* L.) genotypes and means forming four clusters

No	NG	PH	BD	MD	TD	CD	LL	LW	LA	PL	NN	DF	SW	FW
1	9	146.58	9.51	6.84	3.13	8.02	9.58	8.58	59.67	7.67	29.75	51	11.36	2.85
2	7	184.00	11.45	7.57	3.42	9.95	10.88	10.56	62.67	10.35	35.50	54	18.44	3.69
3	6	164.00	10.55	5.64	3.69	8.76	8.93	7.69	70.67	7.89	32.13	53	14.63	2.99
4	3	221.27	14.11	6.05	2.83	9.87	7.68	6.40	65.03	6.36	36.03	57	24.31	6.40

NG= Number of germplasms, PH =Plant height (cm), BD =Base diameter (mm), MD =Middle diameter (mm), CD =Core diameter (mm), TD= Top diameter, LL= leaf length (cm), LW =leaf width (cm), LA= leaf angle, PL =Petal length (mm), NN=Number of nodes, DF =50% Flowering date (Days), SW= Stick weight (gm), FW = Fibre weight (gm)

Table 8: Number of patterns that can be distinguished within the 25 kenaf varieties with different RAPD primers and total number of polymorphic fragments in the data set

Primers	Sequence	Total fragment	Polymorphic fragments	Polymorphism (%)
OPA-3	AGTCAGCCAC	14	13	92.9
OPA-7	GAAACGGGTG	13	12	92.3
OPA-12	TCGGCGATAG	5	4	80.0
OPA-16	AGCCAGCGAA	4	3	75.0
OPA-20	GTTGCGATCC	5	4	80.0

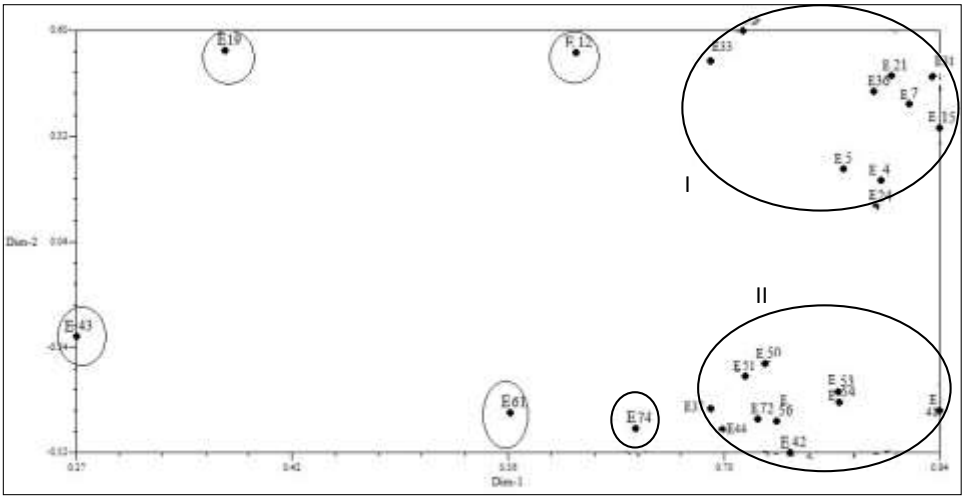

Fig. 4: Principal component analysis of 25 genotypes using RAPD marker showing two major clusters in 2D-graph

results obtained from morpho-agronomic traits represented 97.20% variations of the kenaf germplasm which could be explained by plant height, plant diameter, leaf shape and maturity (Table 7). Furthermore, supporting the cluster analysis-PCA of 13 of morpho-agronomic traits divided 25 genotypes into 4 groups (Fig. 2).

Recently, RAPD has been used as a promising marker system for determining the genetic diversity in population and conservation genetics (Cruzan, 1998; Qian *et al.*, 2001). As descried earlier, the RAPD analysis has certain advantages over other relevant techniques. For example, sampling of relatively unbiased portion of the genome, lower cost, simplicity in use, can be performed with a small amount of plant material (Fritsch and Rieseberg, 1996). To date, RAPD analysis has been effectively utilized for determining the genetic diversity in many species, for example, tomato (Joshi *et al*., 2013) and oat (Ruwali *et al*., 2013). It has also been reported that the superiority of the molecular analysis data than the morpho-agronomic analysis for identification of different kenaf varieties (Cheng *et al*., 2002). Therefore, we have used this technique for detection of genetic diversity in different kenaf genotypes originated from 15 countries (Table 2), but cultivated in single tropical environment (Malaysia). The analysis of 36 polymorphic RAPD markers in this study revealed a considerable genetic variation among the kenaf genotypes obtained from different geographic origins (Table 8). The analysis of RAPD marker application showed its due potentiality for distinguishing different kenaf genotypes with utilization of a small numbers of primers with high genetic polymorphisms (7.2). In this present work, the average RAPD markers amplification was higher in polymorphism than described by Cheng *et al*. (2002) (2.2 vs 7.2). This result of genetic polymorphism had the similarity of genetic variability study described by RAPD analysis, where higher genetic variations were obtained in base line

genotypes than the commercial variety (Leite *et al*., 2002). Thus the resolution of the RAPD markers described here showed its capability for differentiation of the above kenaf genotypes. Thus, the presented primers with DNA fingerprinting technology may be an effective mean for genetic diversity study of kenaf.

Cluster analysis of the 25 kenaf genotyped originated from 15 countries revealed six major groups with a similarity coefficient level 0.67 (Table 2, Fig. 3). However, the present RAPD markers showed little relationship between genetic variationa and geographical origins of the genotypes. Similar finding have been reported by Nejatzadeh-Barandozi *et al*. (2012) while studying with genetic diversity of accessions in Iranian Aloe Vera using RAPD markers. Furthermore, similar result was also reported by Amini *et al*. (2008) and they explained the reason of the exchange of plant materials across the origin of the plants. Several reports have been made for the complex phenotypic and genotypic relationship among the eukaryotes where phenotypic traits were significantly controlled by non-genetic or environmental factors (Wong *et al*., 2005; Bonduriansky and Day, 2009). The phenotypic appearance of plant may be altered due to the complex genetic interaction where the expression of dominant alleles would be suppressed by other genes that minimize the appearance of the phenotype (Miko, 2008). In addition, phenotypic appearance of the eukaryotes is the result of inherited genotypic interaction (the individual's genetic makeup), non-hereditary environmental variation and epigenetic factors transmission (changes of the genome function without alteration the nucleotide sequence within the DNA). There were high genetic variations among the Kenaf genotypes based on Jaccard's similarity. The highest genetic similarity coefficient (0.952) was computed between the genotype E21 and E31. The lowest coefficient was observed between E19 and E41, E42, E50. The high genetic coefficient of diversity among these genotypes opens up an opportunity for their utilization in effective breeding program. In the present study, the PCA analysis using RAPD markers well supported the cluster results, where one of the major groups of 2D-graph of PCA composed most of the genotypes of grouped accordingly to the cluster I and IV (Fig. 4). Thus to obtain greater heterosis, in the kenaf breeding program genotypes from different clusters (Fig. 4) with high genetic variability proven by the RAPD markers could be used for effective hybridization program, which has been proposed by many researchers (Punitha and Ganesamurthy, 2010; Latif *et al.*, 2011; Rafii *et al.*, 2012).

Conclusion

There was significant variability among the tested genotypes of diverse origin. High positive relationship of the major agronomic traits such as fiber and stick yield with other traits will help for the selection of better kenaf plant. Based on cluster analysis of the morpho-agronomic traits, four major cluster groups were found in the studied genotypes giving the clear picture of flowering and maturating stage, fiber and stick yields and plant height of each group. These findings will provide good information to kenaf the breeders and could be used as powerful tools for future breeding program.

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