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ASSESSMENT OF VARIATION AMONG ELEVEN KENAF GERMPLASM USING MOLECULAR MARKERS

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ABSTRACT

Genetic variation of 11 kenaf (*Hibiscus cannabinus* L.) germplasm was analyzed through SSR primers. Out of 25 primers 8 polymorphic primers were detected among the kenaf germplasm. A total of 22 alleles were detected in 11 kenaf germplasm. The highest number of alleles (4) was detected by marker JMBD2064. The highest level of gene diversity value (0.640) was observed for primer 2064. The study revealed that markers having the highest number of alleles showed higher gene diversity. The PIC values ranged from a low of 0.253 (JMBD880) to a high of 0.581 (JMBD2064). PIC values also showed a significant positive correlation with the number of alleles and allele size range for microsatellites. The highest genetic distance (0.857) was observed between the accessions 4626, 3827 and 4626, 5029. For dendrogram the Unweighted Pair Group Method with Arithmetic Means (UPGMA) cluster tree analysis led to the grouping of the 11 germplasm into three clusters. Cluster 1, 2, and 3 contain 5, 2 and 4 germplasm respectively. Overall, this study indicated that germplasm having the highest genetic variation can be used as parental source for improved breeding lines to develop kenaf varieties.

Key words: Kenaf, molecular marker, genetic variation

Introduction

Kenaf (*Hibiscus cannabinus* L.) is a fibre yielding crop cultivated as substitute of jute. It is a bast fibre crop like jute having versatile applications. In Bangladesh, the fibre from kenaf is primarily mixed with bast fibre obtained from jute for making bags, sacks, twines, ropes, cordages and carpets. Kenaf is rapidly replacing jute, because the crop has less labour requirements, is cheaper to produce, may be grown on a wide range of soils under varied climatic conditions, and is not necessarily competitive with food crops. Kenaf also produces more biomass in poor soil where even jute cannot be grown. Genetic variability is the pre-requisite for any plant breeding program. Wild, weedy, primitive cultivar, land races and advanced breeding lines of crops are the major components of genetic resources. There is a germplasm depository (Gene Bank) at Bangladesh Jute Research Institute (BJRI). Before these resources can be exploited, they should be systematically evaluated to assess genetic diversity. DNA fingerprinting is an important approach to identify duplicates in the germplasm collections. Core collection or a subset of germplasm that might comprise up to 10 percent of the total and represent genetic diversity of a large collection can be evaluated through molecular markers. Such well characterized collection would be easy to maintain and serve as a source of material for use in breeding programs for introducing desired variability to develop improved varieties of crop plants. With increasing production and utilization of kenaf breeding for desired agronomic traits such as high fiber yield and disease-resistance become important. There are many local varieties and germplasm of kenaf. Their qualitative traits need to be improved. Before hybridization, selection of parents is an important step. Morphological characters have provided very limited information for varietal identification of kenaf germplasm (Deng *et al*., 1994; Siepe *et al*., 1997; Cheng *et al*., 2002).

This selection procedure can be practiced based on polymorphism in DNA level. Random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR) have been developed (Zhang *et al*., 2013). The detection of polymorphism by DNA-based methods such as Random Amplification of Polymorphic DNA (RAPD) and microsatellite markers (SSR) is more accurate than morphological methods (Rajora and Rahman 2003). SSRs are the most important genetic markers for the analysis of genetic diversity, high-

density genetic mapping, and marker-assisted breeding (Wei *et al*., 2011). The detection of polymorphism by DNA-based methods such as Random Amplification of Polymorphic DNA (RAPD) and microsatellite markers (SSR) is more accurate than either morphological or biochemical methods.

Materials and Methods

Eleven kenaf genotypes (Table 1) were studied in this experiment collected from the Gene Bank of Bangladesh Jute Research Institute (BJRI). The experiment was carried out in the laboratory of Cytogenetics Department of Genetic Resources and Seed Division, BJRI during the period of 2020-21.

Table 1. Eleven accessions of Kenaf with their origin

Sl. no.	Accession number	Country of Origin
1	Accession# 4626	USA
2	Accession# 2048	Pakistan
3	Accession# 4334	Tanzania
4	Accession# 4426	France
5	Accession# 1597	Iran
6	Accession# 2047	Pakistan
7	Accession# 4119	Kenya
8	Accession# 4213	Tanzania
9	Accession# 5029	Pakistan
10	Accession# 2003	France
11	Accession# 3827	Kenya

The seeds were placed on wet blotting paper in petri dishes and kept in a dark place. DNA was isolated from 4 days old seedlings using the mini preparation CTAB method (Doyle and Doyle, 1987) with some modifications. Approximately 1.5 g tissue was grinded to fine powder with mortar and pestle in liquid nitrogen and was taken in 2 ml centrifuge tube and 1ml extraction buffer (2% CTAB, 1.4M NaCl, 20mM EDTA, 100 mM Trisbase, 100 mM β-mercaptoethanol, 2% Polyvinylpolypyrrolidon) was added. The centrifuge tubes were centrifuged with 8500×g for 10 minutes. The supernatants were collected and transferred to fresh 1.5 ml centrifuge tubes. DNA was purified by Phenol: Chloroform:Isoamyl alcohol(25:24:1) and was precipitated using ice cold isopropanol in presence of 0.3 M sodium acetate. Finally, DNA was pelleted and washed with 70% and 100% ethanol. The pellets were dried with vacuum freeze dryer and dissolved in 100 µl TE buffer (10 mM Tris-HCl, l mM EDTA pH-8.0) and stored at -20°C. For making working solutions extracted DNA solutions were diluted to 10 times in new centrifuge tubes. A total of SSR primers 25 (table 2) were used in this experiment.

PCR was performed in 10 µl reactions containing around 25 ng of DNA template (3 µl DNA with 10X dilution factor), 1 µl 10X TB buffer (containing 200 mM Tris–HCl pH 8.3, 500 mM KCl), 1.35 µl 25 mM $MgCl_2$, 0.2 µl of 10 mM dNTP, 0.5 µl each of 10 µM forward and reverse primers and 0.1 µl of Taq DNA polymerase (5 U/µl) using thermal cycler. After initial denaturation for 5 min at 94°C, each cycle comprises 45 sec denaturation at 94°C, 45 sec annealing at 55°C, and 2 min extension at 72°C with a final extension for 7 min at 72°C at the end of 35 cycles. 2.5 µl of 10x loading buffer (Bromophenol Blue 0.4%, Xylene cyanol 0.4%, Glycerol 50%) was added to each PCR product.

PCR products were run on 8 % polyacrylamide gel following standard lab protocols. Vertical gel electrophoresis system with 24 wells was used. Electrophoresis was conducted at 100 volt for 90 minutes. DNA ladder (1 kb plus) were electrophoresed alongside the PCR products. After completion of the electrophoresis the gel was stained in ethidium bromide solution for 25 minutes. Then the gel was rinsed carefully with the tap water and placed on gel documentation system for visualization of DNA bands and the images were taken and saved in computer.

Table 2. SSR Primers used for diversity analysis of 11 kenaf germplasm

Sl.	Primer	Types	Sequence	Status
1	JMBD880	Forward Primer Reverse Primer	GCTCCTACTTTCATTGAATGGC CCTGTTCTTGTTGCTGCTGA	Polymorphic
2	MJM618	Forward Primer Reverse Primer	CGTTATCAAGCAAATCCAACC CATCTGGTGACTGCTTCGTCT	Not amplified
3	JMBD563	Forward Primer Reverse Primer	TGGGCTTGTAACCAAGGAAG CAAACAAATGTGCCATTCCA	Polymorphic
4	JMBD2045	Forward Primer Reverse Primer	GGACAGAAGTTCGAGCCAAG GTTTCCCACCAGTAGTCCGA	Monomorphic
5	JMBD598	Forward Primer Reverse Primer	CCTAATTTCCACCACCAACG CGGGTTAAGGGTCTTGTTGA	Polymorphic
6	JMBD1061	Forward Primer Reverse Primer	TGTAGCCTGCATAGTGCCTG CCCAAAGCAGACAACCTCAT	Monomorphic
7	JMBD2032	Forward Primer Reverse Primer	AAAGCATTGGATCTTCGTGG GTTGCATACTGGTGCATTGG	Polymorphic
8	JMBD2064	Forward Primer Reverse Primer	ACGAGATGGATTCTGATGCC CTCCAGCTTTGCTTGGAAAC	Polymorphic
9	MJM606	Forward Primer Reverse Primer	GGTACTGGTGCATGCTGATTT TTCTGTGGAACCTGAGCATCT	Not amplified
10	MJM467	Forward Primer Reverse Primer	CATGAATTGAGTGAGCATCCA ATCTTCAAGCCCAAATATGCC	Not amplified
11	MJM1140	Forward Primer Reverse Primer	GCTGTCACCTGCCATCTTTT TGCTTGCTGTTGCTGATAGG	Not amplified
12	MJM592	Forward Primer Reverse Primer	CGAACGTTTCGGCAAATATAA CGAACGTTTCGGCAAATATAA	Not amplified
13	JMBD492	Forward Primer Reverse Primer	AACCAAAGCACCACCACTTC CGCTGACGACGATATCTTGA	Monomorphic
14	JMBD57	Forward Primer Reverse Primer	CCTTCCAACTCCTAATGCCA CCGAGGGATCAGGATAGTCA	Monomorphic
15	MH4	Forward Primer Reverse Primer	AGTGACTTATAGTCTAATTAGTGA ACAGATAGGATGTTAACGGGA	Monomorphic
16	MJM467	Forward Primer Reverse Primer	CATGAATTGAGTGAGCATCCA ATCTTCAAGCCCAAATATGCC	Monomorphic
17	MTIC3	Forward Primer Reverse Primer	TGGTGACGACATACAAGAAAAGA CCCGGTGGTTTAGGAAGTTT	Monomorphic
18	MH10	Forward Primer Reverse Primer	TTAGGAGTCATTTCTAACAAGAC AATCCCTCCAGCTTTCTCGA	Not amplified
19	MJM457	Forward Primer Reverse Primer	AAAGGTCGGTGTTGGTCAAG AAATCCGTCCCGTTTTCTTC	Not amplified
20	MJM1195	Forward Primer Reverse Primer	GAGGCTGACAGCGAGTGTTA CCTAAAACCCAGACGAACCA	Polymorphic
21	MJM562	Forward Primer Reverse Primer	GAAGAACAGGCGGTTGACAT CTTCCTTGGTTACAAGCCCA	Not amplified
22	MJM1305	Forward Primer Reverse Primer	ACTACAAAAGACAGAGAAATAGGAAAA ATGTGGGACCAAATTAATGC	Not amplified
23	MJM1401	Forward Primer Reverse Primer	CAGAAACAAGTTCAACAACATCA GAC TCC TTG GTG GTG TCC TC	Polymorphic
24	MJM1042	Forward Primer Reverse Primer	GCATACCACGTGTCGTTTTG GCAGAAGAAATTAGAACAGAAGAGAGA	Not amplified
25	JMBD148	Forward Primer Reverse Primer	ACCCACCAAGTTCATGCTTC GAAGGAAGTGAGCAAGCCAG	Polymorphic

Data analysis: Molecular weight for each amplified allele was measured in base pair using AlphaEaseFC 4.0 software. The allele frequency data from Power Marker version 3.25 (Liu and Muse. 2005) was used to export the data in binary format (allele presence=1 and allele absence= 0) for analysis with NTSYS-pc version 2.2. The summery statistics including the number of alleles per locus, major allele frequency, genetic diversity, polymorphism information content (PIC) values were determined using Power Marker version 3.25. A similarity matrix was calculated with the Simqual subprogram using the DICE coefficient, followed by cluster analysis with the SAHN subprogram using the UPGMA clustering method and implemented in NTSYS-pc to construct a dendogram showing relationship among the genotypes.

Results and Discussion

Twenty-five SSR primers were used and a total number of 22 loci were amplified by these SSR primers. Out of 25 primers 8 primers were found polymorphic (Table 2) for the germplasm and they amplified 22 loci (Table 4). Seven primers were monomorphic (Table 2) for the germplasm and 10 primers did not amplify properly. The SSR profile of 22 jute accessions using SSR primers JMBD2064 is shown in Fig. 1. Major allele frequency of 10 polymorphic primers and genetic diversity values are shown in Table 3.

Table 3. Summary of the genetic variation statistics for major alleles

Marker	Sample size	No. of obs.	No. of allele	Highest frequency allele		Gene Diversity	Polymorphism information content
				size (bp)	frequency		
JMBD598	11	11	3	340	**0.818**	0.314	0.292
JMBD880	11	11	2	210	**0.818**	0.298	0.253
JMBD 2032	11	11	3	310	0.80	0.340	0.314
JMBD563	11	11	3	315	0.750	0.406	0.371
JMBD2064	11	11	**4**	230	0.50	**0.640**	**0.581**
MJM1401	11	11	2	230	0.80	0.320	0.269
MJM1195	11	11	2	145	0.636	**0.463**	0.356
JMBD148	11	11	3	220	0.70	0.460	**0.410**

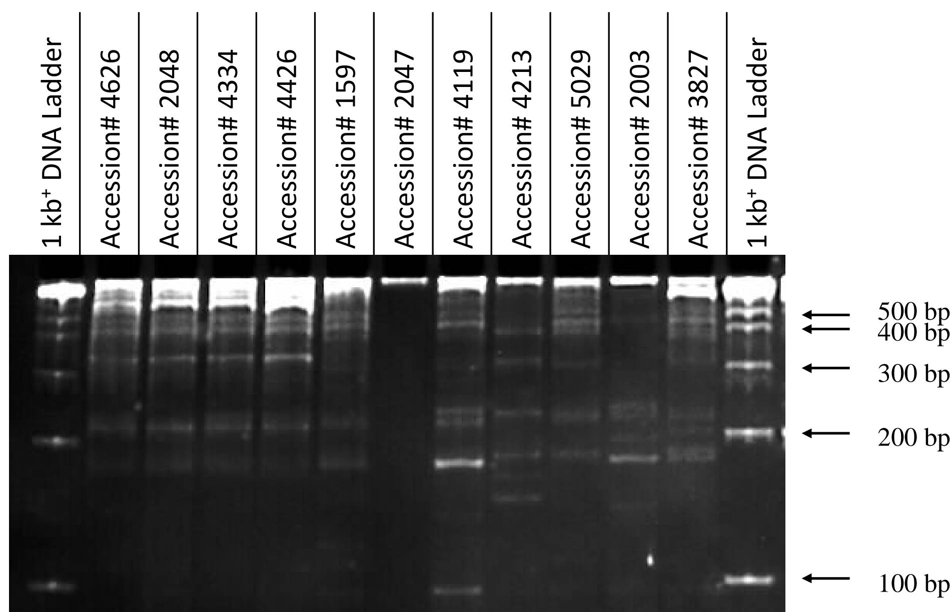

Fig. 1. Microsatellite profiles of 11 Kenaf germplasms for SSR primer JMBD2064

The highest number of allele (4) was found from primer JMBD2064 for the studied germplasm. The highest gene diversity value (0.640) and PIC (Polymorphism information content) value (0.581) were also found from JMBD2064. The second highest gene diversity value (0.0.463) was found for primer MJM1195 and the second PIC value (0.410) was found for primer JMBD148. The result suggests that genetic variations exist among the studied kenaf germplasm. The highest (0.818) major allele frequency was found for allele size 340 bp by JMBD598 and allele size 210 by JMBD880 (Tables 3-4).

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Table 4. Overall Allele size and their frequency of 8 polymorphic markers

Sl. no.	Marker	Allele Size (bp)	Frequency	Variance	SD
1	JMBD598	155	0.091	0.008	0.087
2	JMBD598	340	**0.818**	0.014	0.116
3	JMBD598	360	0.091	0.008	0.087
4	JMBD880	210	**0.818**	0.014	0.116
5	JMBD880	260	0.182	0.014	0.116
6	JMBD 2032	290	0.100	0.009	0.095
7	JMBD 2032	300	0.100	0.009	0.095
8	JMBD 2032	310	0.800	0.016	0.126
9	JMBD563	280	0.125	0.014	0.117
10	JMBD563	315	0.750	0.023	0.153
11	JMBD563	480	0.125	0.014	0.117
12	JMBD2064	150	0.100	0.009	0.095
13	JMBD2064	190	0.300	0.021	0.145
14	JMBD2064	200	0.100	0.009	0.095
15	JMBD2064	230	0.500	0.025	0.158
16	MJM1401	175	0.200	**0.032**	**0.179**
17	MJM1401	230	0.800	**0.032**	**0.179**
18	MJM1195	145	0.636	0.021	0.145
19	MJM1195	180	0.364	0.021	0.145
20	MJM148	220	0.700	0.021	0.145
21	MJM148	235	0.200	0.016	0.126
22	MJM148	290	0.100	0.009	0.095

Table 5. Summary of Nei's (1983) genetic distance values for different pairs of Kenaf germplasm

Accession no.	Accession# 1597	Accession# 2003	Accession# 2047	Accession# 2048	Accession# 3827	Accession# 4119	Accession# 4213	Accession# 4334	Accession# 4426	Accession# 4626
Accession# 2003	0.400									
Accession# 2047	0.250	0.250								
Accession# 2048	0.250	0.800	0.571							
Accession# 3827	0.286	0.400	0.333	0.500						
Accession# 4119	0.167	0.250	0.400	0.333	**0.000**					
Accession# 4213	0.429	0.400	0.500	0.714	0.500	0.500				
Accession# 4334	0.286	0.800	0.667	0.286	0.667	0.500	0.714			
Accession# 4426	0.143	0.600	0.500	**0.125**	0.500	0.333	0.571	0.143		
Accession# 4626	0.444	0.800	0.625	0.333	**0.857**	0.667	**0.857**	0.143	0.250	
Accession# 5029	0.286	0.600	0.333	0.500	0.143	0.200	0.500	0.667	0.500	**0.857**

The frequency and variance of all 22 allele from 8 polymorphic markers for 11 kenaf genotypes are presented in Table 4. Alleles of MJM1401 showed the highest variance and SD value for the kenaf genotypes. The values of pair wise Nei's genetic distance between accessions were computed from combined data for 8 polymorphic SSR primers. For the studied kenaf germplasm, the values were ranged from 0 to 0.857 (Table 5). Accession no. 4626 showed the highest distance for both accession no. 3827 and accession no. 5029. The lowest genetic distance between accessions 3827 and 4119 means that the used primers could not differentiate them or their genetic make-up is very much similar. These results are similar with the findings of Li *et al.* (2016) in 28 kenaf cultivars using 72 EST-SSR markers. Results from this study confirmed the results obtained by Cheng *et al*. (2004), where low levels of genetic diversity were also detected. The second lowest genetic distance (0.125) was found between accessions 4426 and 2048.

In the dendrogram kenaf germplasm are grouped into 3 clusters. Cluster 1 is consisted of accessions 1597, 2047, 3827, 5029 and 4119. Accessions 2003 and 4213 formed cluster 2. In cluster 3 the accessions are 2048, 4426, 4334 and 4626 (Fig. 2). Marker assisted selection can be very helpful to improve the efficiency of plant breeding because of its increased accuracy. However, more polymorphic information from more SSR primer will be needed for practical application especially for marker assisted selection (Varshney and Tuberosa, 2007; Zhang *et al.* 2013)

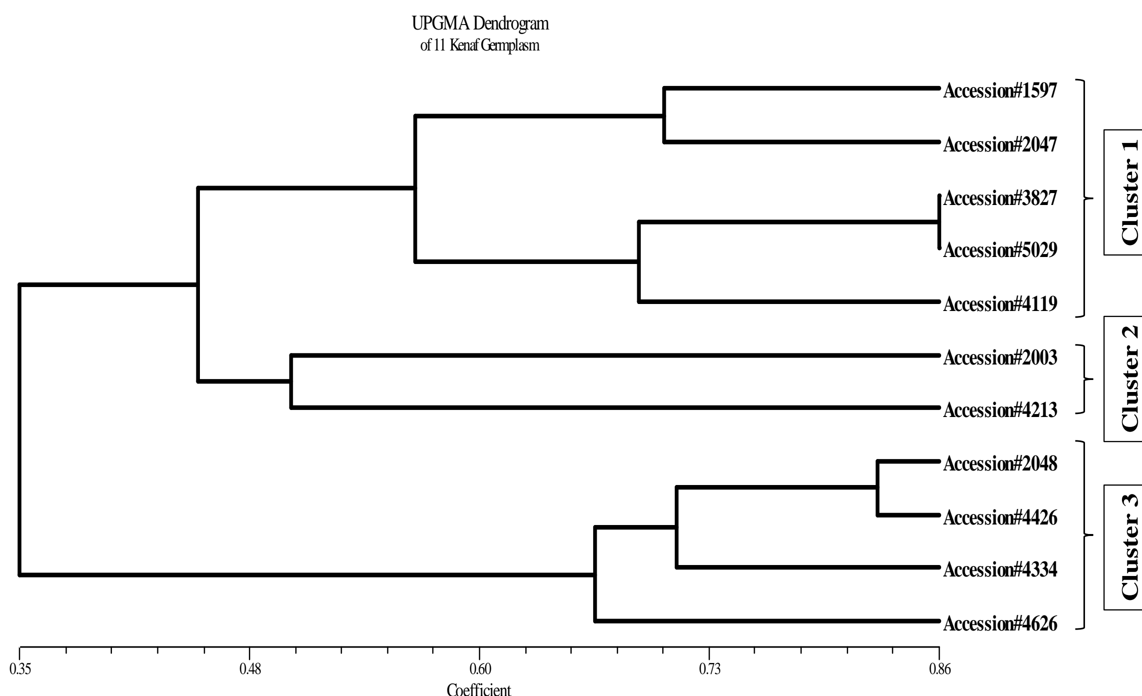

Fig.2. UPGMA dendrogram based on Nei's (1983) genetic distance summarizing the data on differentiation among 11 kenaf germplasm according to SSR analysis

Conclusion

Genetic variability within germplasm indicates rich genetic material of a species which could be used as parental source for breeding to improve Kenaf varieties. Germplasm that showed the highest genetic variation can be used as parental source for breeding line to improve Kenaf varieties. However, the ongoing development of SSR and SNP markers may be a better tool for construction of a genetic linkage map, QTL mapping and marker assisted selection in kenaf germplasm.

References

Cheng, Z., Lu, B., Baldwin, B. S., Sameshima, K. and Chen, J. 2002. Comparative studies of genetic diversity in kenaf (*Hibiscus cannabinus* L.) varieties based on agronomic and RAPD data. *Hereditas*, 136: 231-239.

Cheng, Z., Lu, B., Sameshima, K., Fu, D. and Chen, J. 2004. Identification and genetic relationships of kenaf (*Hibiscus cannabinus* L.) germplasm revealed by AFLP analysis. *Genetic Reso.Crop Evo*., 51: 393–401.

Deng, L-Q., Li, J-Q. and Li, A-Q. 1994. Studies on the agronomic characters of kenaf germplasm and their utilisation. *China's Fibre Crops*, 4: 1-4.

Doyle, J. J. and Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull.,* 19:11-15.

Li, H. L., Chen, A., Tang, H., Li, J. and Huang, S. 2016. Characterization of the kenaf (*Hibiscus cannabinus* L) global transcriptome using illumina paired-end sequencing and development of EST-SSR markers. *PLoS ONE*, 11(3): e0150548

Rajora, O. P. and Rahman, M. H. 2003. Microsatellite DNA and RAPD fingerprinting, identification and genetic relationships of hybrid poplar (Populus 3 canadensis) cultivars. *Theor. Appl Genet.,* 106,470-477

Siepe, T., Ventrella, D. and Lapenta, E. 1997. Evaluation of genetic variability in a collection of *Hibiscus cannabinus* (L.) and *Hibiscus* spp (L.). Industrial Crops and Products, 6, 343-352.

Varshney, R. K. and Tuberosa, R. 2007. Genomics-assisted crop improvement. Springer Dordrecht, Netherlands.

Wei, W., Qi, X., Wang, L., Zhang, Y., Hua, W., Li, D., Lv, H. and Zhang, X. 2011. Characterization of the sesame (*Sesamum indicum* L.) global transcriptome using Illumina paired-end sequencing and development of EST-SSR markers. *BMC Genomics*, 12,451.

Zhang, L., Li, A., Wang, X., Xu, J., Zhang, G., Su, J., Qi, J. and Guan, C. 2013. Genetic Diversity of Kenaf (*Hibiscus cannabinus* L.) Evaluated by Inter-Simple Sequence Repeat (ISSR). *Biochem. gene.,*51, 800-810.