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红麻及其近缘种的 RAPD 分析

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摘要: 利用随机扩增多态性 DNA (RAPD) 技术分析了木槿属 (*Hibiscus*) *Furcaria* 组中纤维作物红麻 (*H. cannabinus*) 及其 6 个近缘种植物的 25 份材料。用筛选出的 16 个引物扩增出 192 个 RAPD 条带, 它们表现出丰富的多态性。根据得到的 RAPD 指纹图谱, 计算其 Nei 氏相似系数和遗传距离, 并构建了它们的系统树。结果表明: 25 份材料可划分为 7 个组, *H. penduriformis* 和 *H. calyphyllus* 两个种为一组; 红麻种分为两个组, 一组为栽培品种, 另 1 组为野生型材料; 其余 4 个种各成为 1 组。玫瑰麻 (*H. sabdariffa*) 和金线吊芙蓉 (*H. radiatus*) 的关系较近, 而且两者与红麻的亲缘关系也较近, 而其它四个种与红麻的关系较远。*H. trionum* 与其它六个种的关系较远。

关键词: 红麻; 近缘种; RAPD; 亲缘关系

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Random Amplified Polymorphic DNA (RAPD) Analyses among *Hibiscus cannabinus* and Related Species

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Abstract: RAPDs were used to study the relationships among species in *Hibiscus* sect. *Furcaria*. Twenty-five accessions of 7 species were examined. Sixteen primers of arbitrary sequences were screened from 80 primers for DNA amplification. A total of 192 bands were generated, of which 149 bands were polymorphic markers. Dendrogram was constructed using Nei's genetic similarity value and MINTS program. The result showed that 25 accessions were clustered in 7 clades, *H. penduriformis* and *H. calyphyllus* formed a clade, and 15 accessions of *H. cannabinus* were clustered in two clades, one being the cultivars, and the other, the wild type materials. The rest 4 species formed 4 individual clades. The most parsimonious dendrogram shows that *H. radiatus* is closely related to *H. sabdariffa*. The closely related species to the cultivars of *H. cannabinus* are *H. sabdariffa* and *H. radiatus*, whereas *H. trionum* appears to be highly diverged from all the other taxa.

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Key words: *Hibiscus cannabinus*; Related species; RAPD; Phylogenetic relationship

The genus *Hibiscus* is a large polymorphic group comprising approximately 400 annual and perennial species, and they are classified into 6 sections (Hochreutiner 1900), namely, *Furcaria*, *Alyogne*, *Abelmcschus*, *Ketmia*, *Calyphylliand* and *Axanza*. Tropical zone and subtropical zone are their distribution areas[1].

In section *Furcaria*, there are more than 40 species, most of them are tetraploid, some species are diploid, octoploid or even decaploid, and their somatic chromosome number ranges from 36 to 188. Among *Hibiscus* sect. *Furcaria* species, *H. cannabinus* (kenaf) and *H. sabdariffa* (roselle) are cultivated as fiber crops[1]. Kenaf is one of the main bast fiber crops in China.

There is some valuable gene source in wild species of *H. cannabinus*, for which the cultivated species lack, such as some resistance genes (resistance to nematode, insect pest, and harsh condition, etc.) and superior fiber quality gene[1]. Because the genome relationships among the species are not very clear, we lack the effective pathways to transfer the valuable genes into the cultivars of *H. cannabinus*. Morphological characters for most species of sect. *Furcaria* have been studied, they were preliminarily classified according to their morphological feature. The chromosome number and ploidy for some species in this section were investigated [2-5]. Menzel and Wilson[3] studied the affinity of the species hybridization among species of sect. *Furcaria*, showing that a few species, such as *H. radiatus*, *H. sabdariffa*, were the most closely related to *H. cannabinus* species. Xiao et al[6] had studied the compatibility of 5 species of *Hibiscus*, and selected a interspecific hybrid from the subsequent generations of *H. cannabinus* × *H. radiatus* hybridization, and the chromosome behavior from F_1 to F_8 were investigated. Although these studies have shed some light on the species relationships among *Hibiscus* sect. *Furcaria*, there is no any evidence for the species relationship on DNA molecule level at present. It is evident that further studies are necessary to ascertain the genetic relationships among various species.

Williams and Kubelik[7] and Welsh and McClelland[8] have described simple methods for assessing genetic variability and for construction of gene maps, based on the amplification of genetic DNA with single primers of arbitrary nucleotide sequence. These primers have been shown to detect polymorphisms in the absence of specific nucleotide sequence information in DNA. In RAPD (random amplification of polymorphic DNA), fingerprinting technique, the oligonucleotides are randomly produced and do not require any DNA target information. The observed fingerprints for a given DNA sample will depend on the length and sequence of the primer, as well as on the optimization of reaction conditions.

In the past a few years, many analysis have been performed using RAPD to solve genetic relationships among different species, such as *Brassica*[9, 10], rice[11], *Avena sterilis*[12], mahoganies[13], watermelon[14], *Pisum*[15], etc. The polymorphisms can provide unique information for

1)International Jute Organization. Proceedings of the Workshop on Application of Biotechnology in the Improvement of Jute, Kenaf and Allied Fibers—Phase I. Beijing, China, 23-25 November, 1998. 45-46.

classification. The aim of this work is to study genetic relationships among *H. cannabinus* and the 6 related species of *Hibiscus* sect. *Furcaria* using RAPD.

1 Materials and methods

Plant materials Seven species including 25 accessions in *Hibiscus* sect. *Furcaria* were used. All the materials were obtained from International Jute Organization (IJO) and Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences (IBFC, CAAS). A complete list of species, accession number and their chromosome number is presented in Table 1.

DNA isolation and characterization DNA was prepared from fresh tender leaf tissues, and the method described by Pich and Schubesrt[16] was adopted for DNA isolation. Using a Backmen DU-7 spectrophotometer to measure the value of $OD_{260\ nm}$ and $OD_{280\ nm}$ for DNA samples, the concentration and quality was tested. Using the λ DNA/Hind III as molecular standard, the size of DNA molecular was evaluated by electrophoresis on 0.7% agarose gel. Samples were diluted in TE (10 mmol/L Tris-HCl, pH 8.0, 1 mmol/L EDTA) to achieve a final concentration of 40 ng μl^{-1}.

Table 1 List of species studied, their accession number, chromosome number and source

Species	Number	Accession name	Chromosome number[1]	Source[1]
H. cannabinus	1	J-1-113	36	America
	2	Qingpi No.3	36	Vietnam
	3	722	36	China
	4	85-98	36	China
	5	Alian	36	Qatar
	6	74-1	36	China
	7	PI248901	36	America
	8	PI248895	36	America
	9	KB6	36	China
	10	84201	36	China
	11	KB5	36	China
	12	85-239	36	Kenya
	13	85-335	36	Kenya
	14	85-133	36	Kenya
	15	85-225	36	Kenya
H. sabdariffa	16	85-122	72	China
	17	H152	72	Thailand
	18	H153	72	Thailand
	19	H181	72	Thailand
H. radiatus	20	H158	72	Kenya
H. trionum	21	H003	72	Kenya
H. acetosella	22	H071	72	Kenya
H. penduriformis	23	H321	72	Kenya
	24	H322	72	Kenya
H. calyphyllus	25	H334	72	Kenya

RAPD analysis　RAPD analysis were performed using 16 primers obtained from Operon Technologies Corporation. These 16 primers were screened from 80 primers including OPE, OPG, OPH and OPN four series by using two DNA samples for amplification reaction. The nucleotide sequence of each random primer is shown in Table 2.

Table 2　List of primers and their respective oligonucleotide sequence

Primer	Sequence	Total bands	Polymorphic bands
OPE-13	5'CCCGATTCGG 3'	14	10
OPE-16	5'GGTGACTGTG 3'	13	11
OPG-03	5'GAGCCCTCCA 3'	8	8
OPG-06	5'GTGCCTGCGG 3'	14	9
OPG-07	5'GAACCTGCGC 3'	9	6
OPG-08	5'TCACGTCCAC 3'	10	10
OPG-15	5'ACTGGGACTC 3'	12	10
OPH-05	5'AGTCGTCCCC 3'	13	12
OPH-12	5'ACGCGCATGT 3'	14	11
OPH-13	5'GACGCCACAC3'	13	10
OPH-14	5'ACCAGGTTGC 3'	12	9
OPH-15	5'AATGGCGCAG 3'	15	12
OPN-02	5'ACCAGGGGCA 3'	13	10
OPN-03	5'GGTACTCCCC 3'	13	8
OPN-13	5'AGCGTCACTC 3'	9	7
OPN-15	5'CAGCGACTGT 3'	10	6
Total		192	149

Polymerase chain reactions (PCR) were performed in 25 μl volumes containing 10 mmol/L Tris-HCl, 1% Triton X-100, 1.5 mmol/L $MgCl_2$, 0.1 mmol/L each of dATP, dCTP, dGTP and dTTP, 40 ng DNA sample, 1.5 units of Taq polymerase and 5 pmol/L primer. A negative control containing water instead of template DNA was included in each reaction set. The amplifications were carried out in a PERK DNA Thermal Cycler and programmed for 5 min at 95℃ predenature, 5 cycles of 1 min at 94℃ to denature, 1 min at 36℃ for annealing primer, and 2 min at 72℃ for extension, then 40 cycles of 30 seconds at 94℃, 1 min at 36℃, 2 min (10 min for the last cycle) at 72℃, after the 45 cycles were completed, 10 μl of each DNA amplification products was analyzed by electrophoresis on 1.4% agarose gel containing ethidium bromide (0.5 μg ml^{-1}) in 1×TAE buffer at 5 V cm^{-1} and recorded by UV photography[16]. The molecular standard used was the PCR markers obtained from Sino-American Biotechnologies Corporation (SABC).

Data analyses　Only clear amplified DNA bands were scored. The occurrence of a specific band of amplified DNA was scored as 1 and absence as 0 within a fingerprint. Therefore, a sequence of 0's and 1's was generated for each primer/accession to form a data matrix. The data for all the primers were used to estimate the Nei's similarity based on the number of bands. A dendrogram based on similarity coefficients was generated by using the parsimony.

2 Results and discussion

2.1 Polymorphic bands

RAPD polymorphisms were evaluated using 16 primers for each species/accessions. Three replications were performed for each primer. There were 192 bands for 16 primers in total. Among all the bands, 149 markers were polymorphic while the other 43 markers were monomorphic. The polymorphic bands comprised 77.6% of the total bands. Several polymorphic

bands were observed for each primer. Most primers produced multibanded fingerprints. Figures 1 and 2 illustrated the results obtained for primer OPG-07 and OPN-15, respectively. The data of these bands were used in similarity evaluation. The similarity matrix was obtained after multivariate analysis using Nei's coefficient, then these similarity coefficients were used to produce dendrogram by parsimony.

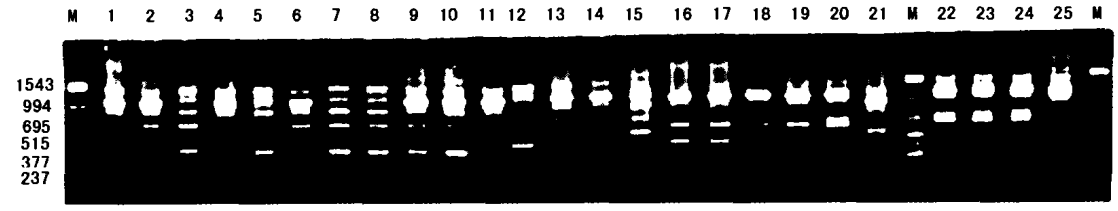

Fig. 1 Fingerprinting analysis for 25 accessions of *Hibiscus* sect. *Furcaria* using primer OPG-07. Lables for each lane were the accession numbers listed in Table 1, M: PCR markers

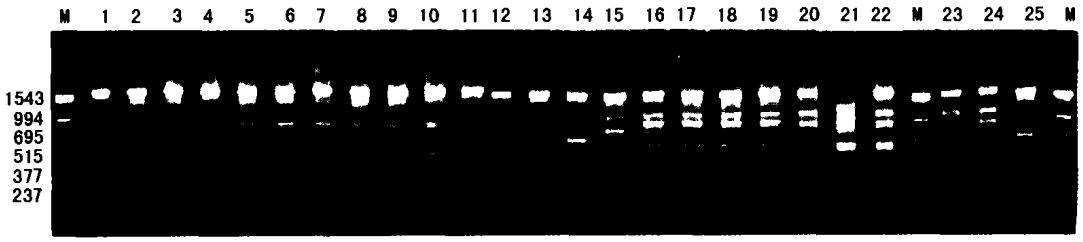

Fig. 2 Fingerprinting analysis for 25 accessions of *Hibiscus* sect. *Furcaria* using primer OPN-15. Lables for each lane were the accession numbers listed in Table 1, M: PCR markers

2.2 Genetic distance analysis

The most parsimonious dendrogram obtained for all primers combined is presented in Figure 3. From the dendrogram, 25 accessions are clustered in seven clades, namely, A, B, C, D, E, F, and G. Clade A is the accessions of *H. sabdariffa*. In this clade, four accessions can be divided into two subgroups at similarity of 0.93, and the first subgroup is three wild type accessions (H153, H181 and H152), the second subgroup is the cultivar of roselle 85-122. Clade B is the species *H. radiatus*. Fifteen accessions of *H. cannabinus* are clustered in two clades, one (C) is the 11 cultivars, the other (D) is the wild type accessions. It reveals that among these species, the cultivars are different from the wild materials in genome, in fact, the variation of morphological characteristics is great between the two types of materials. Clade E is *H. acetosella*. Clade F included two species, which can also be divided into two subgroups at the similarity of 0.87, one is the accessions of species *H. pendurimormis*, and the other, species *H. calyphyllus*, which indicate that there is close genetic relationships between the two species. *H. trionum* (Clade G) appears to be the most distant species from the other taxa.

H. radiatus and *H. sabdariffa* were the most closely related species with the cultivars of *H.*

cannabinus. The result is in agreement with the conclusion got from species hybridization by Menzel et al.[2], and is also in agreement with the chromosome behavior observed in F_1–F_8 from *H. cannabinus* × *H. radiatus* [5].

The value of genetic distance between *H. radiatus* and *H. sabdariffa* is small, and these two species are also quite similar in some morphological characters[5].

The accessions within the same species, such as *H. sabdariffa*, *H. cannabinus* and *H. penduriformis*, appear to be closely affiliated, which is good evidence for the caliber of experimental data.

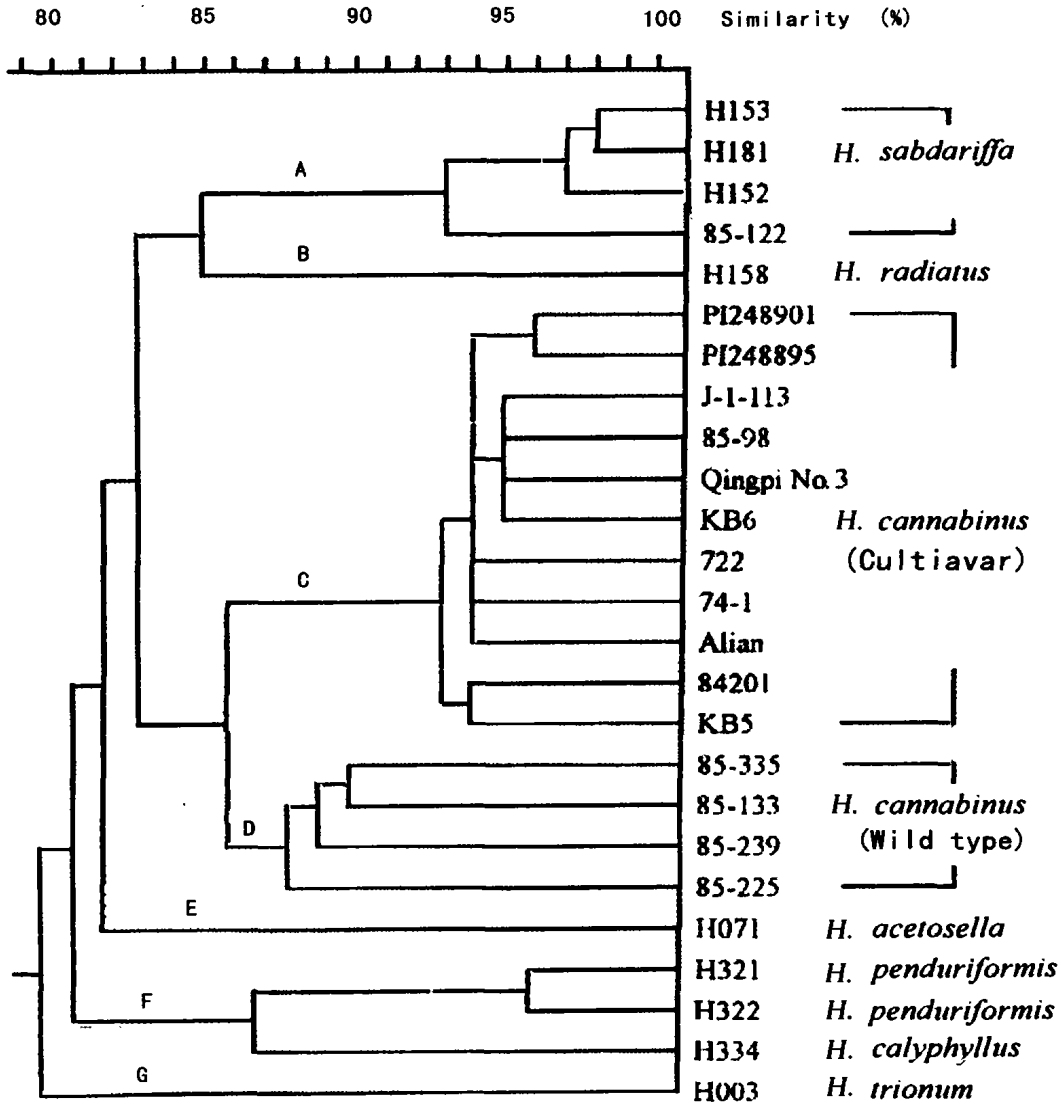

Fig. 3 The most parsimonious dendrogram showing the relationships among 25 accessions of *Hibiscus* sect. *Furcaria*

In this study, the power of RAPDs to reveal polymorphisms among 7 species of *Hibiscus* sect. *Furcaria* is clearly revealed. We attempt to use RAPD markers as a tool to implement studies of molecular systematics in *Hibiscus*. More accessions from *Hibiscus* species and more species which are not considered in present experiment will be included in our future studies, and the use of more primers will provide greater resolution of the relationships of these taxa.

References:

[1] Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences. Cultivation Sciences of Bast Fiber Crops in China [M]. Beijing: Publishing Company of Agriculture, 1993. 261-265. (in Chinese)

[2] Menzel M Y, Wilson F D. Cytotaxonomy of twelve species of *Hibiscus* sect. *Furcaria* [J]. Amer J Bot, 1963, 50(3):262-271.

[3] Menzel M Y, Wilson F D. Genetic relation in *Hibiscus* sect. *Furcaria* [J]. Brittonia, 1969, 21(2):91-125.

[4] Menzel M Y, Martrin D W. Chromosome homology in some intercontinental hybrids in *Hibiscus* sect. *Furcaria* [J]. Amer J Bot, 1971, 58:191-202.

[5] Menzel M Y. Some species of the African puzzle in *Hibiscus* sect. *Furcaria* [J]. Amer J Bot, 1983, 70:285-297.

[6] Xiao R Z, Guo A P, Cheng X Q, et al. Studies on karyotype of "Furong Hongma 369" — A interspecific hybrid of *H. cannabinus* × *H. radiatus* [J]. China's Fiber Crops, 1994, 3:1-5. (in Chinese)

[7] Williams J G K, Kubelik A. Polymorphism amplified by arbitrary primers are useful as genetic markers [J]. Nucl Acids Res, 1990, 18:6531-6535.

[8] Welsh J, McClelland M. Fingerprinting genomes using PCR with arbitrary primers [J]. Nucl Acids Res, 1990, 18:7213-7218.

[9] Quiros C F, This J H P. Development and chromosomal localization of genome-specific markers by polymerase chain reaction in *Brassica* [J]. Thero Appl Genet, 1991, 88:627-632.

[10] Thormann C F, Ferreira M E. Comparison of RFLP and RAPD markers to estimating genetic relationship within and among cruciferous species [J]. Thero Appl Genet, 1994, 88:973-980.

[11] Xial J. Genetic diversity and its relationship to hybrid performance and heterosis in rice as revealed by PCR-base markers [J]. Thero Appl Genet, 1992, 84(7):835-838.

[12] Heun M, Murphy J P, Phillips T D. A comparison of RFLP and RAPD markers to estimating genetic relationships among *Avena sterilis* L. accessions [J]. Thero Appl Genet, 1994, 87:689-696.

[13] Chalmers K J, Newton A C. Evaluation of the extent of genetic variation in mahoganies (Meliaceae) using RAPD markers [J]. Thero Appl Genet, 1994, 89:504-508.

[14] Lee S J, Shin J S. Detection of genetic diversity using RAPD-PCR and sugar analysis in watermelon [*Citrullus lanantus* (Thunb.) Mansf.] germplasm [J]. Thero Appl Genet, 1996, 92:719-725.

[15] Hoey B K, Crowe K R. A phylogenetic analysis of *Pisum* based on morphological characters, and allozyme and RAPD markers [J]. Thero Appl Genet, 1996, 92:92-100.

[16] Guo A P, Huang M, Cheng X Q, et al. Isolation and characterization of total DNA from several bast fiber crops and their allies [J]. China's Fiber Crops, 1997, 4: 9-14. (in Chinese)