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用 RAPD 和 ISSR 检测的橡胶树野生种质和栽培品种的遗传多样性

安泽伟，孙爱花，程 汉，黄华孙*，方家林
(中国热带农业科学院橡胶研究所，国家橡胶树育种中心，农业部热带作物栽培生理学重点实验室，海南 儋州 571737)

摘要：用 19 个 RAPD 引物和 12 个 ISSR 引物对 14 份野生橡胶树种质和我国的 37 份栽培品种进行了遗传多样性分析。RAPD 引物共产生 132 条带，多态性带占 88.6%，相似系数变化范围在 0.432-0.947。ISSR 引物共产生 101 条带，多态性带占 87.1%，相似系数为 0.505-0.941。平均基因杂合度分析表明野生种质比栽培品种具有较高的遗传多样性。根据 UPGMA 法对 51 份材料进行聚类分析，结果表明，ISSR 分析中所有材料可分为 2 类：第一类为野生种质，第二类为栽培品种；而 RAPD 分析中野生种质和栽培品种不能被分为明显的两大类。虽然 ISSR 和 RAPD 的聚类分析结果存在差异，但对两种方法进行的相关分析表明，他们之间仍存在极显著相关性，相关系数为 0.574。品种 PR107、热研 217 等一些栽培品种可以通过特异带在 51 份供试材料中被区分开。这些结果可以对橡胶树的育种工作起到一定的指导作用，同时 RAPD 和 ISSR 技术也是进行橡胶树品种鉴定和遗传多样性研究的有效手段。

关键词：橡胶树；种质资源；遗传多样性；RAPD；ISSR
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Genetic Diversity among Wild and Cultivated Accessions of *Hevea brasiliensis* (Rubber Tree) Detected by RAPDs and ISSRs

AN Ze-wei, SUN Ai-hua, CHENG Han, HUANG Hua-sun*, FANG Jia-lin
(Rubber Research Institute, Chinese Academy of Tropical Agricultural Sciences; State Center for Rubber Breeding; Key Laboratory of Cultivation Physiology for Tropical Crops, Ministry of Agricultural, Danzhou 571737, China)

Abstract: In order to select suitable *Hevea* accessions for extending genetic base of Chinese rubber tree in future breeding schemes, the genetic diversity of 14 wild accessions and 37 cultivated clones were detected by RAPD and ISSR. Thirty-one reliable primers (19 RAPD primers and 12 ISSR primers) were chosen. Based on RAPD, a total of 132 bands were generated, polymorphic bands accounted for 88.6%, and similarity coefficient ranged from 0.432 to 0.947 among all accessions. Otherwise based on ISSR, 101 bands were produced, polymorphic bands accounted for 87.1%, and similarity coefficient was 0.505 to 0.941. The wild accessions showed higher polymorphism than cultivated clones according to the average heterozygosity. Based on ISSR, the 51 accessions were divided into 2 clusters according to unweighted pair-group method with arithmetic averages (UPGMA) cluster analysis: Cluster I contained 9 wild accessions and Cluster II consisted of 37 cultivated clones and 5 wild accessions. But all of the accessions can not be divided into wild cluster and cultivated cluster according to RAPD. Some cultivated clones, i.e. PR107, Re yan 217, could be screened from 51 accessions. The significantly high correlation between RAPDs and ISSRs among 51 accessions was observed ($r = 0.574$), although the differences between the RAPD and ISSR dendrograms were observed. It is proven that all of the results can be used in *Hevea* breeding programs, and RAPD and ISSR can be used in clonal identification and diversity study of *Hevea*.

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*Corresponding author

Key words: *Hevea brasiliensis*; Germplasm; Genetic diversity; RAPD; ISSR

Rubber tree (*Hevea brasiliensis* Muell. Arg.), the main source of natural rubber, is indigenous to the Amazon basin of South American. The English man, Wickham H. K., collected a lot of *Hevea* seeds from Amazon basin in 1876. He grew the *Hevea* seedlings in England, and transplanted them in Indonesia, Malaysia, Singapore and Sri Lanka. Today rubber tree is extensively cultivated in Southeast Asia, in which the yield of nature rubber is more than 90% of the total all over the world. The cultivated clones (known as Wickham clones) of Southeast Asia were all derived from 22 seedlings surviving from the Wickham collection in 1876, and showed a narrow genetic base [1]. Therefore, the International Rubber Research and Development Board (IRRDB) organized a survey of *Hevea* wild germplasm in the Amazon forest in 1981. Exploitation of these genetic resources requires the knowledge of their genetic diversity. Now molecular markers have been used to detect the genetic variation of rubber tree, because they are more precise, efficient than morphological markers.

Genetic diversity of *H. brasiliensis* has been detected by several molecular markers, i.e., isozyme[2], RFLP[3,4], minisatellite[5], SSR[6], AFLP[7] and RAPD[8]. Inter-simple sequence repeats (ISSR) have not been reported in *Hevea* genetic study yet, but it has been used in other crops, e.g. rice[9], *Eucalyptus* [10], sweet potato[11], potato[12], sesame[13].

In China, the cultivated rubber clones have a very narrow genetic base, most of which were derived from several foreign clones, such as RRIM600, PR107. The narrow genetic base has hold back the development of Chinese rubber breeding. In order to extend the genetic base of Chinese rubber clones, more and more wild rubber germplasm should be exploited in breeding. In this study, we evaluated the *Hevea* diversity by RAPD and ISSR among 14 wild germplasm (1981 Amazonian accessions) and 37 cultivated clones. The objective of this study was to give more information to select suitable parents for extending rubber genetic base in the future breeding schemes of China.

1 Materials and Methods

Plant materials This investigation was based on 14 wild accessions and 37 cultivated clones (Table 1). The 14 wild accessions come from the 1981 Amazonian collections, which have some outstanding traits e.g. fast-growing, cold resistance. The 37 cultivated clones are elite rubber clones in China. All of the 51 accessions have been conserved in state germplasm garden in Rubber Research Institute, Chinese Academy of Tropical Agricultural Sciences.

DNA extraction Total genomic DNA were isolated from fresh young leaves using modified CTAB extraction buffer[8].

RAPD Nineteen primers were selected in this analysis. PCR was carried out in a 10 μl volume containing 50 mmol/L KCl, 10 mmol/L Tris-HCl, 0.1% Triton X-100, 2 mmol/L $MgCl_2$, 0.1 mmol/L dNTPs, 0.34 μmol/L primer, 0.5 U Taq Polymerase, template DNA 30 ng, ddH_2O 6.5 μl. The reaction was run for 45 cycles (denaturing at 94℃ for 4 min, 10 cycles each consisting of denaturing at 94℃ for 30 s, annealing at 36℃ for 30 s, extension at 72℃ for 70 s; 35 cycles each consisting of denaturing at 94℃ for 20 s, annealing at 36℃ for 20 s, extension at 72℃ for 60 s; a final extension at 72℃ for 7 min). The amplification products were separated on 1.5% agarose gels, stained with ethidium bromide, visualised with ultraviolet light and photographed.

ISSR Twelve primers were selected in this analysis. PCR was carried out in a 10 μl volume containing 50 mmol/L KCl, 10 mmol/L Tris-HCl, 0.1% Triton X-100, 2 mmol/L $MgCl_2$, 0.1 mmol/L dNTPs, 0.4 μmol/L primer, 0.5 U Taq Polymerase, template DNA 30 ng, ddH_2O 6.64 μl. The reaction was run for 45 cycles (an initial denaturing at 94℃ for 4 min, 35 cycles each consisting of denaturing at 94℃ for 30 s, annealing at 50℃ for 30 s, extension at 72℃ for 70 s; a single extension at 72℃ for 7 min). The amplification products were separated on 2% agarose gels, stained with ethidium bromide, visualised with ultraviolet light and photographed.

Data analysis Fragments amplified by

Table 1 The origins of wild accessions and cultivated clones of *H. brasiliensis* used in this study

No.	Name	Type	Origin	No.	Name	Type	Origin
1	Re shi99-11	Cultivated	China	27	Da ling17-155	Cultivated	China
2	Re shi99-10	Cultivated	China	28	Wen chang217	Cultivated	China
3	Yun yan277-5	Cultivated	China	29	Tian ren31-45	Cultivated	China
4	Da feng318	Cultivated	China	30	RRIM600	Cultivated	Malaysia
5	Da feng95	Cultivated	China	31	PR107	Cultivated	Indonesia
6	Re yan7-33-97	Cultivated	China	32	Wen chang193	Cultivated	China
7	Wen chang11	Cultivated	China	33	Re yan88-13	Cultivated	China
8	Re yan7-20-59	Cultivated	China	34	Re yan 8-79	Cultivated	China
9	Yun yan77-2	Cultivated	China	35	Hai ken 1	Cultivated	China
10	Xu yu141-2	Cultivated	China	36	Re yan 78-3-5	Cultivated	China
11	Tian ren93-114	Cultivated	China	37	Wen chang 24-29	Cultivated	China
12	IAN873	Cultivated	Sri Lnaka	38	AC/S/10 37/93	Wild	Amazon
13	Guang xi6-68	Cultivated	China	39	AC/X/21 64/221	Wild	Amazon
14	He kou3-11	Cultivated	China	40	RO/OP/4 20/125	Wild	Amazon
15	Nan hua1	Cultivated	China	41	MT/IT/18 31/125	Wild	Amazon
16	Min lin71-22	Cultivated	China	42	AC/F/5 21/197	Wild	Amazon
17	Hai ken2	Cultivated	China	43	AC/S/12 42/494	Wild	Amazon
18	PB86	Malaysia	China	44	RO/C/8 24/272	Wild	Amazon
19	PB235	Malaysia	China	45	AC/X/21 64/177	Wild	Amazon
20	RRIM623	Malaysia	China	46	RO/C/9 23/238	Wild	Amazon
21	Da ling68-38	Cultivated	China	47	MT/IT/16 34/5	Wild	Amazon
22	Re yan217	Cultivated	China	48	AC/AB/15 54/418	Wild	Amazon
23	Re yan8-333	Cultivated	China	49	RO/CM/10 44/768	Wild	Amazon
24	PB260	Malaysia	China	50	RO/CM/10 44/683	Wild	Amazon
25	Wen chang162	Cultivated	China	51	RO/J/5 33/38	Wild	Amazon
26	Wen chang215	Cultivated	China				

RAPD and ISSR primers were scored as present (1) and absent (0). Genetic similarity (GS) were estimated according to Simple Matching Coefficient based on the probability that the amplified fragment from one genotype will be present in another genotype, and the fragment can't be amplified in both genotypes. $GS = (a+d)/(a+b+c+d)$, (a: number of shared fragments, b: number of fragments in line A, c: number of fragments in line B, and d: number of absent fragments in both line A and line B). The average heterozygosity was evaluated according to Nei M.[14]. Correlation between assays was calculated using the SPSS software package. Cluster analysis was performed with the NTSYS-pc software package based on UPGMA (unweighted pair-group method with arithmetic average).

2 Results

2.1 Level of polymorphism

In this study, 51 accessions were detected by means of 19 RAPD primers and 12 ISSR primers. A total of 132 bands were generated with 19 RAPD primers (Fig. 1), and 7 bands were detected per primer. Of them, 117 bands (88.6%) were polymorphic. 101 bands were scored for ISSRs (Fig. 2), and 87.1% of those were polymorphic. On average, 8 bands were detected per ISSR primer. The number of polymorphic bands based on RAPD was 95 (79.2%) in wild accessions, and it was 92 (80.0%) in cultivated accessions. The number of polymorphic bands based on ISSR was 84 (84%) in wild accessions, and it was 64 (68.8%) in cultivated accessions (Table 2).

2.2 Genetic relationship of 51 *Hevea* accessions

Similarity coefficient was calculated based on polymorphic data. Based on ISSR, the coefficient ranged from 0.505 to 0.941 among all accessions, and it was 0.432 to 0.947 according to RAPD (data not showed). Wild accessions showed lower similarity and higher polymorphism than cultivated clones. Based on ISSR, the coefficient ranged from 0.505 to 0.871 in

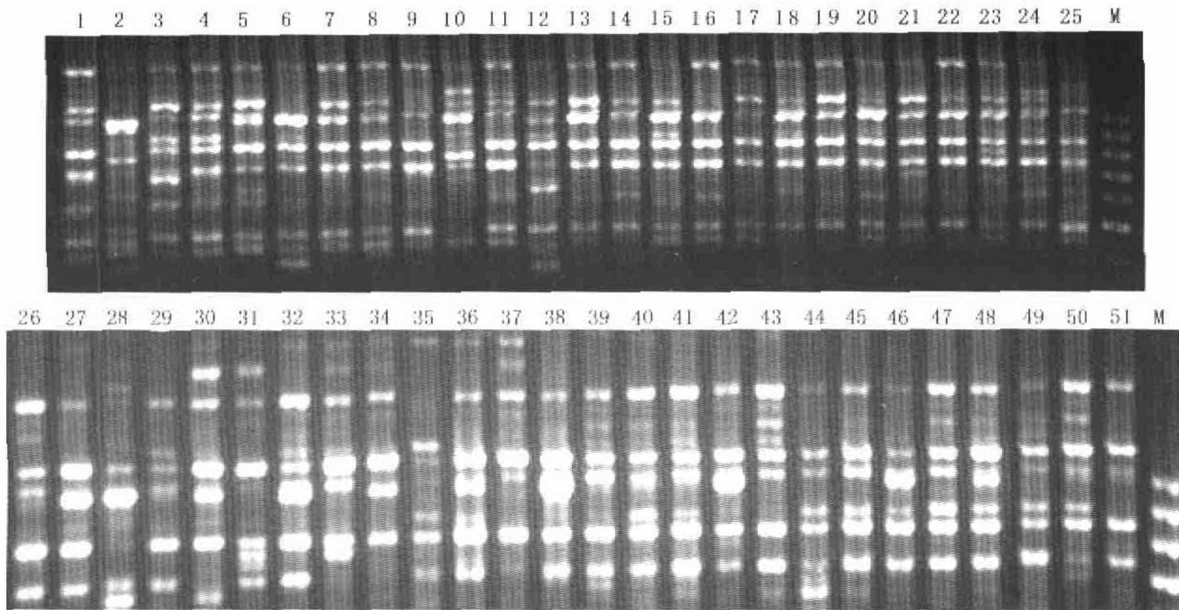

Fig. 1 RAPD pattern of all 51 accessions by primer OPA7
Marker is 100 bp ladder marker.

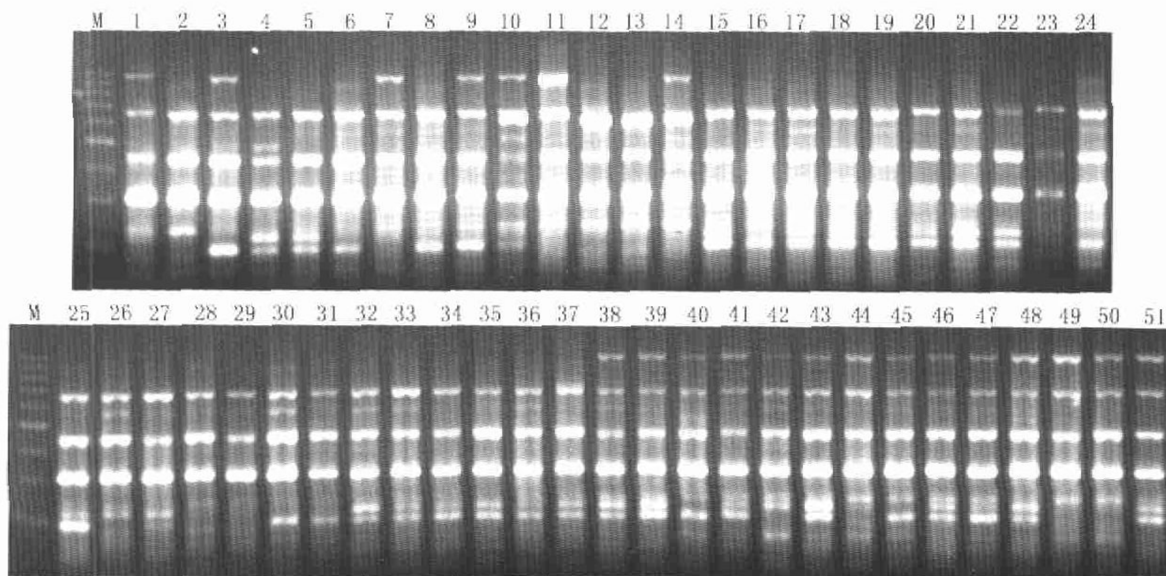

Fig. 2 ISSR pattern of all 51 accessions by primer 836
Marker is 200 bp ladder marker.

wild accessions, and it ranged from 0.663 to 0.941 in cultivated clones. Based on RAPD, the coefficient was 0.523 to 0.871 in wild accessions, and it was 0.530 to 0.947 in cultivated clones. The dendrogram revealed two distinct clusters according to ISSR (Fig. 3): Cluster I consisted of 9 wild accessions; Cluster II comprised of 37 cultivated clones and 5 wild accessions. Otherwise the 51 accessions can't be divided into wild cluster and cultivated cluster according to RAPD (Fig. 4).

2.3 Comparison between assays

To compare the results obtained with the two techniques, we tested Pearson correlations. The result showed that 57.4% of the pairs of genotypes were ranked in the same order between RAPD and ISSR.

3 Discussion

The 51 accessions were distinctly separated into two clusters according to ISSR: cultivated clone cluster and wild accession cluster. This result is consistent

Table 2 Number of total and polymorphic bands generated by RAPD and ISSR among 51 accessions

Primer	Number of total bands (Number of polymorphic bands)			Primer	Number of total bands (Number of polymorphic bands)		
	Wild accessions and cultivated clones	Wild accessions	Cultivated clones		Wild accessions and cultivated clones	Wild accessions	Cultivated clones
OPA1	6(5)	6(3)	5(4)	OPAJ13	6(5)	5(4)	6(5)
OPA2	8(6)	8(5)	6(3)	OPAJ18	7(6)	7(6)	6(5)
OPA4	4(4)	3(1)	4(4)	OPAJ20	4(4)	4(4)	3(2)
OPA5	5(5)	4(4)	5(5)	807	6(5)	6(5)	6(5)
OPA6	4(4)	3(3)	3(3)	808	4(3)	4(3)	4(1)
OPA7	6(4)	4(2)	6(5)	809	6(4)	6(4)	6(4)
OPA9	7(6)	7(6)	7(5)	810	11(9)	10(8)	11(6)
OPB7	13(13)	11(9)	12(8)	811	13(12)	13(12)	9(5)
OPB8	15(15)	13(13)	11(11)	834	10(9)	10(8)	9(8)
OPB17	10(9)	9(7)	10(7)	835	1(0)	1(0)	1(0)
OPI9	2(1)	2(1)	2(1)	836	12(10)	12(10)	10(8)
OPQ1	8(7)	8(4)	8(5)	840	15(15)	15(14)	14(12)
OPX1	7(7)	7(6)	5(5)	841	8(8)	8(7)	8(7)
OPX9	8(8)	8(8)	7(7)	842	9(9)	9(9)	9(6)
OPAJ9	5(3)	4(2)	3(2)	864	6(4)	6(4)	6(2)
OPAJ11	7(7)	7(7)	6(5)				
Total	115(104)	104(81)	100(80)	Total	118(103)	116(98)	108(76)

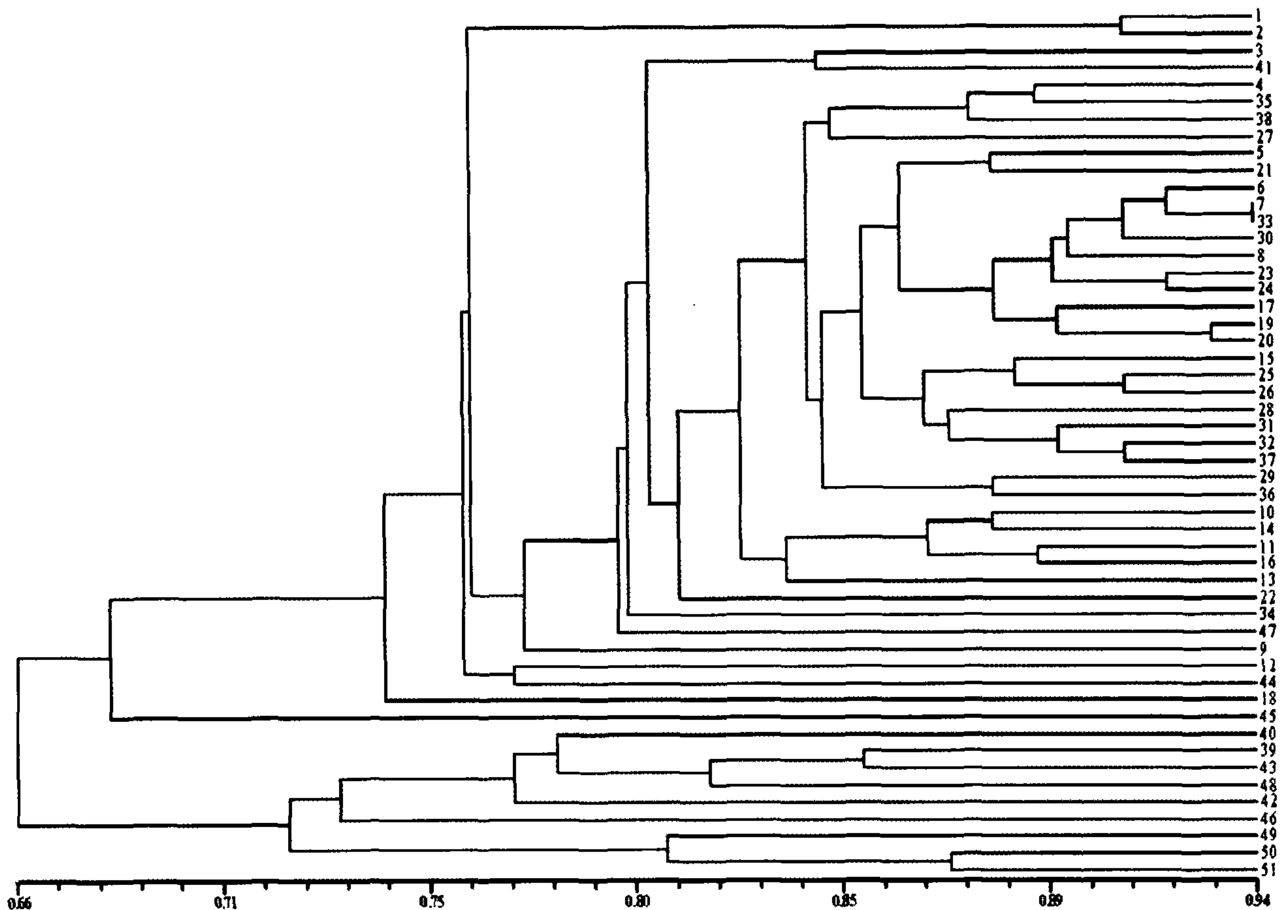

Fig. 3 Dendrogram of 51 *Hevea* accessions based on ISSR markers

with the pedigree information of 51 accessions. Wild accessions, i.e. MT/IT/18 31/125, MT/IT/16 34/15, AC/S10 37/93, AC/X/21 64/177, RO/C/8 24/272, are closely related to cultivated clones, and similar result has been detected by RFLP[15]. Based on RAPD, the 51 accessions can't be clearly separated into cultivated

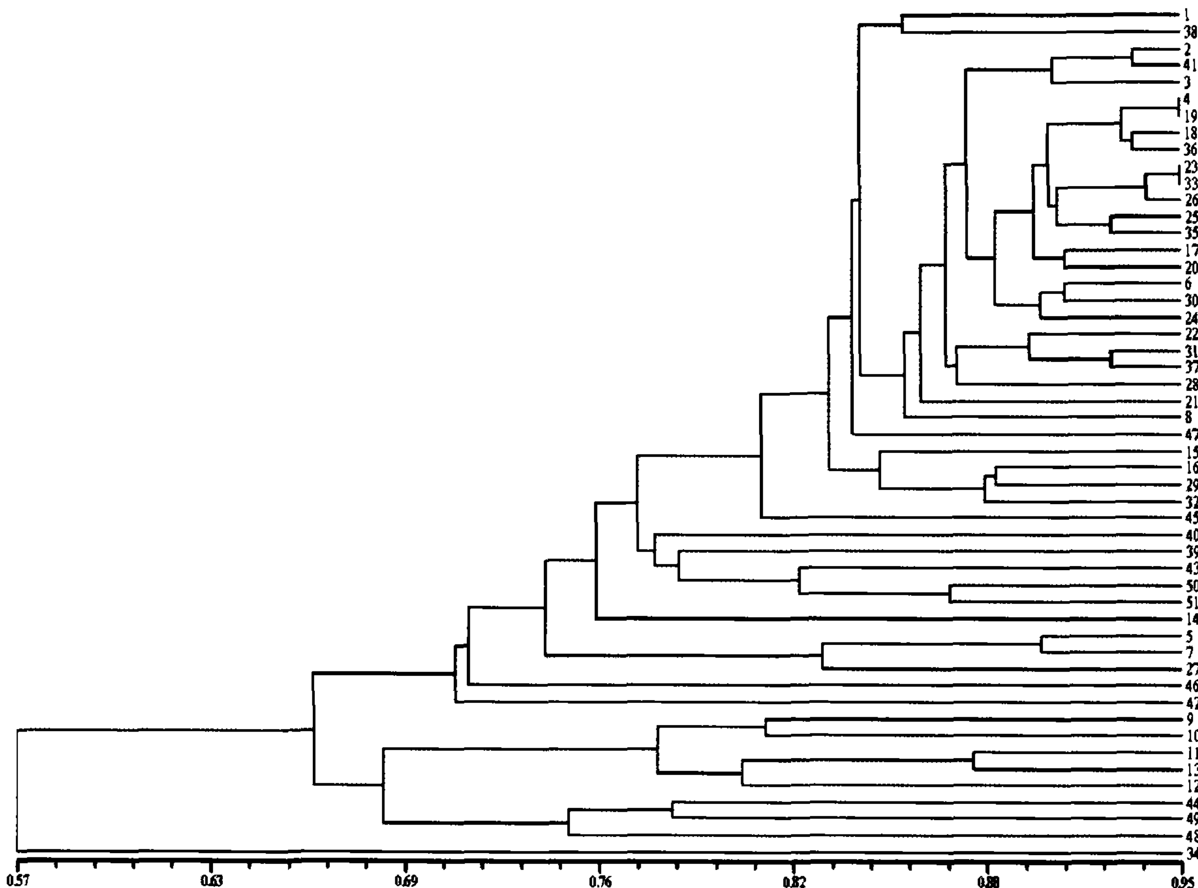

Fig. 4 Dendrogram of 51 *Hevea* accessions based on RAPD markers

cluster and wild cluster. The reason may well be that the two markers are generated by different target fragments. Increasing the large number of polymorphic primers to detect all accessions might give better solutions.

The polymorphic data unambiguously showed that cultivated clones had less polymorphism than wild accessions. Based on RAPD, the average heterozygosity was 0.406 in wild accessions, and was 0.383 in cultivated accessions. It was 0.425 in wild accessions according to ISSR, but was 0.400 in cultivated accessions. The similar results have already been reported by Bease P. et al.[3,4] and Lekawipat N. et al.[16]. Despite their narrow genetic base and high level of inbreeding, some cultivated clones could be screened from 51 accessions, i.e. PR107, Re yan 217. This result indicated that RAPD and ISSR markers could be used to identify cultivated clones. However, to increase the precision of cultivated clones identification, much more polymorphic primers should be needed.

Knowledge of genetic variation and the genetic relationship between genotypes is an important consideration for efficient utilization of germplasm resources. Furthermore, it is important for the optimal design of plant breeding programs, influencing the choice of genotypes to cross for the development of new populations. The genetic analysis of 51 accessions revealed that wild accessions had more variability than cultivated clones. Since all of the 51 accessions belong to *H. brasiliensis*, we should select the parents, which have distant genetic relationship, from cluster I and cluster II for cross breeding. Thus we can exploit the wild germplasm to broaden the genetic base of cultivated rubber tree. Genus *Hevea* has nine species. All of them, *H. brasiliensis* can produce the most nature rubber. The other species has some prospective traits, for example, *H. benhamiana* can resist *Microcyclus ulei* and *Phytophthora palmivora*. In future breeding schemes, we can exploit not only *H. brasiliensis* but also other useful species.

Molecular-based estimates of GS will allow plant breeders to make informed decisions regarding the

choice of genotype to cross, but we must choose a suitable assay. According to Vos et al.[17], the ideal fingerprinting assay should require no prior sequence knowledge of research objects. While RAPD and ISSR primers are random primers, and meet these requirements. In this study, we got the reliable results by RAPD and ISSR, and the significantly high correlation between RAPDs and ISSRs among 51 accessions was observed (r = 0.574), although differences were observed between the RAPD and ISSR dendrograms. It was proven that RAPD and ISSR markers could be used in *Hevea* clonal identification and diversity study.

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