Theor Appl Genet (1995) 91:481–488

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Genetic variation detected by DNA fingerprinting with a rice minisatellite probe in *Oryza sativa* L.

Received: 12 August 1994 / Accepted: 24 October 1994

Abstract A rice minisatellite probe detecting DNA fingerprints was used to assess genetic variation in cultivated rice (*Oryza sativa* L.). Fifty-seven cultivars of rice, including 40 closely related cultivars released in the US, were studied. Rice DNA fingerprinting revealed high levels of polymorphism among distantly related cultivars. The variability of fingerprinting pattern was reduced in the closely related cultivars. A genetic similarity index (S) was computed based on shared fragments between each pair of cultivars, and genetic distance (D) was used to construct the dendrograms depicting genetic relationships among rice cultivars. Cluster analysis of genetic distance tended to group rice cultivars into different units corresponding with their varietal types and breeding pedigrees. However, by comparison with the coefficients of parentage, the criterion of relatedness based on DNA fingerprints appeared to overestimate the genetic relationships between some of the closely related US cultivars. Although this may reduce the power of fingerprints for genetic analysis, we were able to demonstrate that DNA fingerprinting with minisatellite sequences is simpler and more sensitive than most other types of marker systems in detecting genetic variation in rice.

Key words Rice · *Oryza sativa* · DNA fingerprinting · Minisatellites

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Communicated by G. S. Kush

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Introduction

The development of methods to obtain an accurate assessment of genetic variations among cultivated rice (*Oryza sativa* L.) has been of a great interest to scientists. Rice systematists are interested in the rapid classification of different taxonomic groups, while breeders are concerned with the determination of valuable variations in breeding programs. In the context of rice cultivar registration, plant patents, and breeder's right protection, a quick and reliable method for cultivar identification is particularly appealing. The traditional techniques, based on morphological and cytological characters, breeding behavior, and ecological distribution, have been used to assess the genetic variability and relationships among rice cultivars (Oka 1958; Sato 1986). Variations at the biochemical level, such as isozymes and seed proteins, have also been investigated in an attempt to classify taxonomic groups of rice cultivars (Ladizinsky and Hymowitz 1979; Second 1982; Glaszmann 1987). Both traditional and biochemical methods have proven themselves as valuable diagnostic tools in differentiating and identifying rice cultivars.

The detection of restriction fragment length polymorphisms (RFLPs) with DNA markers has allowed for the construction of linkage maps in many plant species (Beckmann and Soller 1986; Helentjaris et al. 1986; Tanksley et al. 1987; McCouch et al. 1988). It has also provided a new tool for measuring and monitoring genetic variation in plants (Saghai-Maroof et al. 1984; Song et al. 1988). Using RFLP analysis, genetic variation (i.e., polymorphism) can be evaluated independently from the stage of plant development and environmental effects. Therefore, RFLP markers, which can detect not only changes at DNA level in coding regions of the genome but in non-coding as well, are more sensitive than other traditional morphological and biochemical traits in assessing genetic variation. Several researchers have demonstrated that genetic variation among cultivated rice can be detected by RFLP analysis

(Wang and Tanksley 1989; Wang et al. 1992; Zhang et al. 1992). Results from these studies proved consistent with the observations from the other traditional and biochemical methods. However, in these studies, the probes detecting RFLPs were isolated from random collections of single-copy DNA or cDNA sequences of rice. In cases where the level of RFLPs detected by single-copy DNA sequences is low, a group of RFLP markers and several different restriction enzyme combinations were required to obtain results.

Recently, the discovery of hypervariable regions (HVRs) in many species of animals and plants has provided new opportunities for evaluating genetic variability. HVRs contain minisatellite sequences which consist of a series of tandem repeats of a core consensus sequence (Jeffreys et al. 1985b). Variability of such regions arises from differences in the copy number of tandem repeats. By using the minisatellite sequence as a probe, HVRs which share sufficient sequence similarity will be systematically detectable, thus yielding an individual-specific fingerprint (Jeffrey et al. 1985c). DNA fingerprinting with minisatellite sequences has been widely used in both humans and animals for individual identification and pedigree examination (Gill et al. 1985; Jeffreys et al. 1985a, 1986; Wetton et al. 1987; Hillel et al. 1989). In plants, this new technology has also been used for studying genetic variation and variety identification (Nybom 1990; Nybom and Schaal 1990; Lavi et al. 1991; Nybom and Hall 1991; Rogstad et al. 1991).

The genome of cultivated rice (*O. sativa* L.) was one of the first in plants where human-like HVR sequences were shown to exist (Dallas 1988). In a previous study, we reported on two human-like minisatellite sequences isolated from rice (Winberg et al. 1993). In this paper, a rice minisatellite sequence has been used to reveal DNA fingerprints for rice cultivar identification and genetic analysis. Most of the cultivars analyzed could be unambiguously distinguished by rice DNA fingerprinting. The applications of DNA fingerprinting to detect genetic variation and estimate relatedness among closely related rice cultivars have also been discussed.

Materials and methods

Plant materials and DNA extraction

The plant material used in this study included 57 accessions of cultivated rice (*O. sativa* L.) from the International Rice Research Institute (IRRI, Manila, Philippines), the China National Rice Research Institute (CNRRI, Hangzhou, P. R. China), and R. H. Dilday (USDA-ARS, Rice Research Center, University of Arkansas) (Table 1). Leaf materials were harvested from four plants of each cultivar grown in a Missouri greenhouse, and were freeze-dried and ground to a fine powder with a Tekmar sample mill. Total genomic DNA was extracted following the method of Saghai-Maroof et al. (1984).

Table 1 List of rice cultivars (*Oryza sativa* L.) included in study

No.	Name	Type[a]	Origin[a]
1	Taichung Native 1	indica	Taiwan
2	IR 8 (IR 8-288-3)	indica	Philippines
3	Ai-Zai-Zhan	indica	China
4	Ta-Hei	japonica	China
5	Dular	indica	India
6	Ai-Ma-Bai-Gu	indica	China
7	Yuan-Feng-Zhao	indica	China
8	Zhen-Zhu 19 (Nuo)	indica	China
9	IR 29	indica	Philippines
10	China 91	japonica	China
11	Suweon 283	indica	Korea
12	Pecos	indica	USA
13	La-Ai 64	indica	China
14	Qui-Guang	japonica	China
15	Zhen-Zhu 19	indica	China
16	Dourado Precoce	indica	Brazil
17	Bellemont	indica	USA (Texas)
18	Ketan Nangka	japonica	?
19	Lun-Hui 422	japonica	?
20	Fortuna	indica	Puerto Rico
21	Colusa	japonica	USA (Louisiana)
22	Carolina Gold	indica	USA
23	Rexoro	indica	USA (Louisiana)
24	Nira	indica	USA (Louisiana)
25	Lady Wright	?	Unknown
26	Calady	indica	USA (California)
27	Zenith	indica	USA (Arkansas)
28	Arkrose	japonica	USA (Arkansas)
29	Bluebonnet	indica	USA (Texas)
30	Rexark	indica	USA (Arkansas)
31	LaCrosse	indica	USA (Louisiana)
32	Calrose	japonica	USA (California)
33	TP-49	indica	USA (Texas)
34	Century Patna 231	indica	USA (Texas)
35	Hill Long Grain	indica	USA
36	HLSL/BBNT	indica	USA
37	Northrose	indica	USA (Arkansas)
38	Belle Patna	indica	USA (Texas)
39	Nova	indica	USA (Arkansas)
40	Nova 66	indica	USA (Arkansas)
41	Bluebelle	indica	USA (Texas)
42	Northrose/Zenith	indica	USA (Arkansas)
43	CS-M3	japonica	USA (California)
44	BBLE/BLPT/Dawn	indica	USA (Texas)
45	Mars	indica	USA (Arkansas)
46	Nova 76	indica	USA (Arkansas)
47	S6	japonica	USA (California)
48	Calrose 76	japonica	USA (California)
49	M7	japonica	USA (California)
50	S-201	japonica	USA (California)
51	Caloro	japonica	USA (California)
52	Tainan iku 487 (T487)	japonica	Taiwan
53	Dee Geo Woo Gen	indica	Taiwan
54	KPF 6 (AUS)-PAK	?	Pakistan
55	Bluebelle/6x TN-1	indica	Philippines
56	Shoemed	indica	Philippines
57	Lemont	indica	USA (Texas)

[a] ?, information unavailable

DNA probe preparation

The fingerprinting probe was prepared from the rice minisatellite clone pOs 6.2H using PCR as previously described (Winberg et al. 1993). The PCR products were passed through a Sepharose CL-6B spin column to remove unincorporated primers and dNTPs. The hybridization probe was then labelled with α-^{32}P-dCTP by the random primer method (Feinberg and Vogelstein 1983).

Restriction digests, electrophoresis, and Southern analysis

A DNA sample of 10 μg from each cultivar was digested with *Dra*I restriction endonuclease under conditions recommended by the supplier (Promega) and electrophoresed in 0.8% agarose gels (1V/cm, 24 h, 1 × TBE). The gels were Southern transferred onto a Hybond-N^+ charged nylon-membrane (Amersham International) according to the manufacturer's instruction and fixed at 80 °C for 2 h. Filters were prehybridized and hybridized at 65 °C for 16 h according to Westneat et al. (1988), and then were washed once in 2 × SSC, 0.5% SDS at room temperature for 30 min and twice in 0.2 × SSC, 0.1% SDS at 65 °C for 30 min. Autoradiographs were made at − 80 °C for 2–5 days with intensifying screens.

Data analysis

A genetic similarity index (S) was calculated as the fraction of shared fragments between pairs of cultivars (Jeffreys et al. 1985c; Lynch 1990). For cultivars i and j, $S_{ij} = 2\ N_{ij}/(N_i + N_j)$, where N_{ij} is the number of common fragments in the two cultivars, while N_i and N_j are the number of fragments scored in cultivars i and j, respectively. Genetic distance (or genetic diversity) was expressed as $D = -\ln(S)$ and used to construct the dendrograms with the statistical program Systat 5.2 (SYSTAT, Inc. 1992) using the unweighted pair-group method with arithmetic mean (UPGMA, Sokal and Michener 1958) on a Macintosh computer. The fingerprint data for the first 19 cultivars (Table 1) was analyzed separately from the US cultivars.

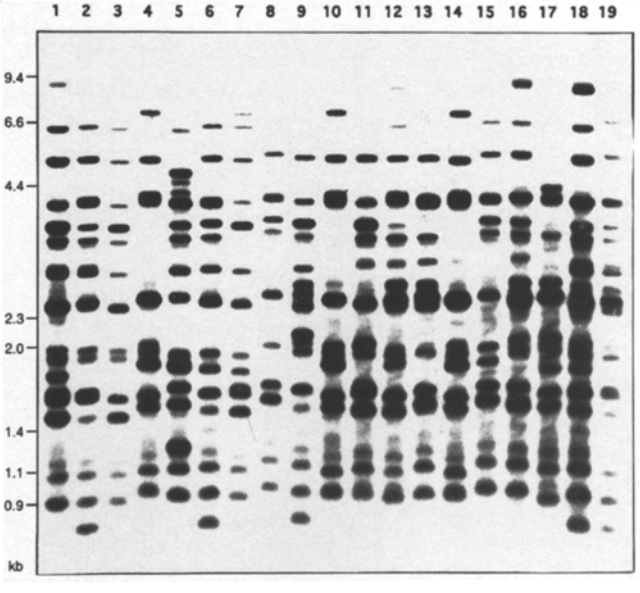
Results and discussions

DNA fingerprinting with the rice minisatellite probe

The hybridization patterns detected by the rice minisatellite probe pOs 6.2H in genomic DNA from 19 cultivars of *O. sativa* L. are shown in Fig. 1. On average, 13.5 discrete fragments ranging in size from 0.6 kb to 9.4 kb were visible on the autorads. The restriction fragment patterns differed between all of these cultivars. No within-cultivar variation was found with pOs 6.2H (data not shown). The wash stringency (0.2 × SSC, 0.1% SDS, 65 °C) used was relatively higher than that reported in other rice studies (Dallas 1988; Winberg et al. 1993). Under this condition, the hybridization patterns proved to be reproducible even after stripping and re-hybridization several times.

The inheritance of polymorphic fragments detected by pOs 6.2H was studied in the F_2 progeny of a cross between cultivars Bulu Dalam and IR34583 (data not shown). The individual fragments appeared to segregate in a Mendelian fashion and no linkage was detected for most of the polymorphic fragments. This suggested that the fragments detected by pOs 6.2H are dispersed throughout the rice genome. Such a property is important for obtaining a true individual-specific fingerprint. Our results demonstrated that the rice minisatellite probe pOs 6.2H can systematically detect RFLPs in the rice genome and provide informative DNA fingerprints for genetic analysis and cultivar identification. Since the rice fingerprints were highly variable, the polymorphic loci detected by the rice minisatellite sequence are probably hypervariable regions (HVRs) in the genome.

Fig. 1 DNA fingerprints detected by the PCR-amplified minisatellite probe pOs 6.2H in *Dra*I-digested genomic DNA from 19 rice cultivars (*O. sativa*). *Lanes 1–19*: TN-1, IR8, Ai-Zai-Zhan, Tai-Hei, Dular, Ai-Ma-Bai-Gu, Yuan-Feng-Zhao, Zhen-Zhu 19 (Nuo), IR29, China 91, Suweon 283, Pecos, La-Ai 64, Qiu-Guang, Zhen-Zhu 19, Dourado Precoce, Bellemont, Ketan NangKa, and Lun-Hui 422



Genetic variation with DNA fingerprint analysis

From the data in Fig. 1, a genetic similarity index (S) for each pair of cultivars was calculated (Table 2). Each hybridization fragment from the DNA fingerprints was scored as a piece of independent data. With this one probe/enzyme combination, an average of 44% similarity was found among the cultivars analyzed. The indica type cultivars evaluated showed a 49% similarity, which ranged from 90% between TN1 and IR8, to 0% between Yuan-Feng-Zhao and Zhen-Zhu 19 (Nuo). The cultivars within the japonica group showed an average 34% similarity. Zhang et al. (1992) have suggested that indica rice is genetically more diverse than the japonica type. In their study using RFLP analysis, an average 0.476 genetic diversity within the indica group was detected over three restriction enzymes using 43 RFLP probes. In our study with a single probe/enzyme combination, the indica type cultivars showed a 49% genetic similarity; in other words a 0.713 genetic diversity. However, genetic variation within the japonica group (1.079) appears larger than in the indica cultivars. This result, which seems to contradict Zhang et al. (1992), may due to our small sample size and/or the different cultivars analyzed.

Figure 2 depicts the genetic relationships among the cultivars based on the genetic distance (D) computed from the DNA fingerprint data (Table 2). With a few exceptions, the indica-type cultivars were well separated from the japonica cultivars. The indica cultivar Zhen-

Table 2 Genetic similarity index (S) between pairs of cultivars (data from Fig. 1)

Cv	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1 TN 1																		
2 IR8	0.90																	
3 Ai-Zai-Zhan	0.79	0.76																
4 Tai Hei	0.46	0.44	0.33															
5 Dular	0.52	0.50	0.48	0.44														
6 Ai-Ma-Bai-Gu	0.76	0.73	0.81	0.32	0.40													
7 Yuan-Feng-Zhao	0.50	0.48	0.69	0.33	0.21	0.52												
8 Zhen-Zhu 19 (Nuo)	0.23	0.22	0.08	0.55	0.30	0.08	0.00											
9 IR29	0.67	0.65	0.57	0.38	0.52	0.62	0.50	0.15										
10 China 91	0.23	0.37	0.50	0.45	0.37	0.40	0.50	0.18	0.31									
11 Suweon 283	0.67	0.64	0.80	0.43	0.57	0.62	0.56	0.09	0.67	0.61								
12 Pecos	0.34	0.33	0.59	0.32	0.47	0.36	0.52	0.16	0.48	0.40	0.62							
13 Lai-Ai 64	0.46	0.37	0.58	0.36	0.52	0.48	0.42	0.09	0.54	0.55	0.78	0.72						
14 Qiu-Guang	0.15	0.30	0.50	0.27	0.22	0.24	0.42	0.09	0.38	0.45	0.52	0.56	0.64					
15 Zhen-Zhu 19	0.37	0.50	0.48	0.35	0.29	0.46	0.32	0.26	0.30	0.61	0.42	0.62	0.61	0.26				
16 Dourado Precoce	0.67	0.71	0.64	0.38	0.52	0.69	0.43	0.23	0.47	0.38	0.74	0.76	0.46	0.31	0.67			
17 Bellemont	0.30	0.36	0.48	0.43	0.50	0.38	0.24	0.17	0.44	0.52	0.50	0.46	0.70	0.43	0.33	0.59		
18 Ketan Nangka	0.48	0.47	0.39	0.28	0.53	0.44	0.32	0.07	0.42	0.34	0.67	0.44	0.62	0.28	0.40	0.48	0.53	
19 Lun-Hui 422	0.52	0.56	0.34	0.22	0.31	0.40	0.21	0.22	0.32	0.22	0.36	0.40	0.52	0.22	0.50	0.52	0.43	0.65

Mean indica/indica value $= 0.49 \pm 0.20$
Mean japonica/japonica value $= 0.34 \pm 0.13$

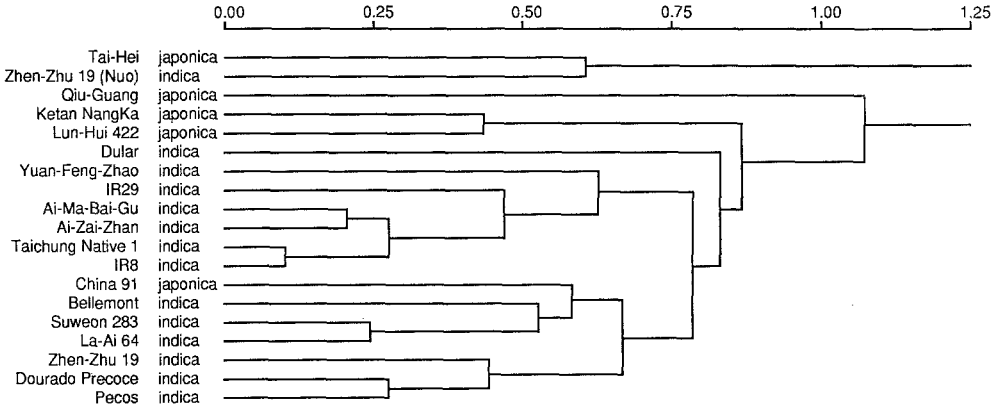


Fig. 2 Dendrogram of genetic relationships among 19 rice cultivars (data from Fig. 1). From left: first column, cultivar names; second column, varietal types. The scales indicate genetic distance. The branch of Tai-Hei and Zhen-Zhu 19 (Nuo) does not join with the rest because of the extreme case where the cultivars Yuan-Feng-Zhao and Zhen-Zhu 19 (Nuo) shared no fingerprint fragments (S = 0, D = ∞, see text for detail)

Zhu 19 (Nuo), which possesses some javanica characteristics, appeared to be closer to the japonica than to the indica type. The japonica cultivar China 91 tended towards the indica group (Fig. 2). Cultivars Yuan-Feng-Zhao and Zhen-Zhu 19 (Nuo) were found having no bands in common from the total of 24 bands detected (Fig. 1, lanes 7 and 8). Therefore, the genetic distance (D) between these two cultivars approached ∞ and a substitute value 999.9 was used in the cluster analysis. Due to this extreme case, the branch of Tai-Hei and Zhen-Zhu 19 (Nuo) could not be joined with the rest of the group (Fig. 2). We have found that cluster analysis with a substituted value did not affect the overall appearance of the dendrogram. Since both Tai-Hei and Zhen-Zhu 19 (Nuo) had a relatively low S with the other cultivars, the subsititution analysis, with either $D = \infty$ or $D =$ mean distance between Zhen-Zhu 19 (Nuo) and other cultivars, always clustered these two into a single group and separated them from the rest. By using isozyme and RFLP analysis, Glaszmann (1987) and Wang and Tanksley (1989) have suggested that there are some indica cultivars which may have been originally misclassified. Although there is no information to suggest that this would be the case for Zhen-Zhu 19 (Nuo), we found it is much closer to the japonica cultivar Tai-Hei (55% similarity). The pedigree information for most of these cultivars is unavailable.

DNA fingerprinting among closely related cultivars

Modern rice cultivars which are closely related usually have very narrow genetic backgrounds. In order to test the applicability of DNA fingerprinting for cultivar identification, 40 cultivars released in the United States were studied (Table 1). Figure 3 shows the DNA fingerprints of some these cultivars as detected by the rice probe pOs 6.2H. On average, 12.6 fragments were visible following hybridization autoradiography and 75% of similarity was found among these cultivars (Table 3). Based on these data, the probability of two US cultivars having the same DNA fingerprint pattern is

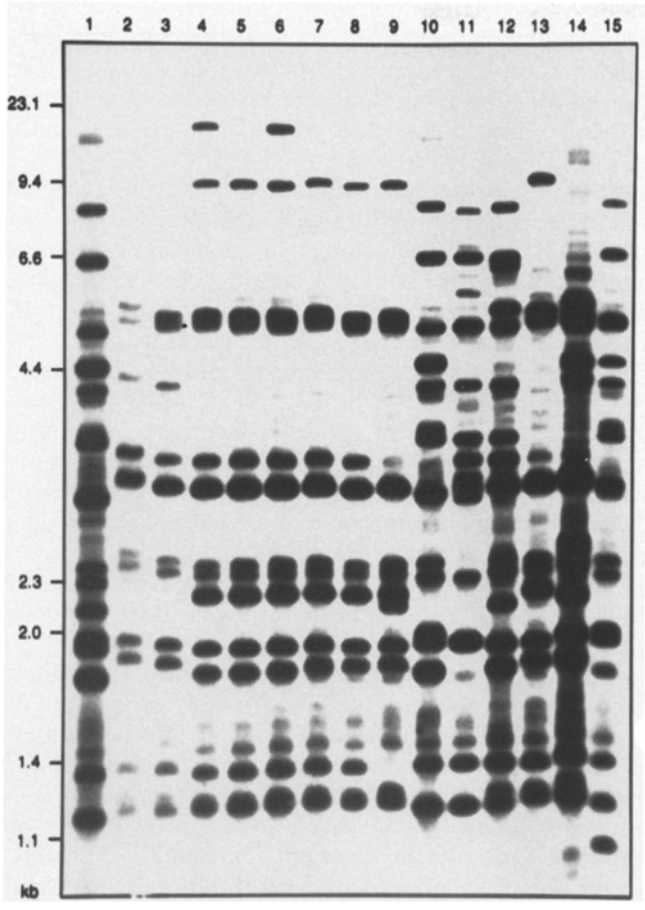


Fig. 3 DNA fingerprints detected by the PCR-amplified minisatellite probe pOs 6.2H in *Dra*I-digested genomic DNA from US rice cultivars. *Lanes 1–15*: TN-1, Mars, Nova 76, S6, Calrose 76, M7, S-201, Caloro, T-487, Dee Geo Woo Gen, KPF 6, BBLE/6 × TN-1, Shoemed, Lemont, and IR8

Table 3 Summary of the data analysis of DNA fingerprints in 40 US cultivars[a]

No. of fragments per cultivar	
Average N ± SD	12.6 ± 1.5
Range in N	11–17
Proportion of sharing fragments (F)	
Average F ± SD	0.75 ± 0.13
Range in F	0.37–1.00
Probability of two cultivars matching	
$P = F^N$	2.7×10^{-2}

[a] Proportion of sharing fragments (F) between a pair of cultivars was computed as same as genetic similarity index (S). The probability of two cultivars having the same DNA fingerprints (P) was calculated as described by Jeffreys et al. (1985c)

2.7×10^{-2}. Some cultivars with common ancestries, such as S6 and M7, were found to have identical fingerprints, (Fig. 3). Rice probe pOs 6.2H was not able to detect any polymorphism between S6 and M7, even with 12 different AT-cutting restriction enzymes (data not shown).

The probability of two cultivars matching (2.7×10^{-2}) with this probe appeared much higher when compared to other minisatellite sequences for DNA fingerprinting in rice and other plant species (Dallas 1988; Nybom et al. 1990; Lavi et al. 1991; Nybom and Hall 1991). In the present study of 780 pair comparisons between a total of 40 accessions, 22 pairs involving 20 cultivars showed identical fingerprints. The percentage of common pairs (2.8%) observed is within the range of the predicted (2.7×10^{-2}) chance of matching (Table 3).

The results suggest that DNA fingerprinting may not be as powerful in rice as in humans and other animals for individual identification. This notion may be arguable since there are vastly different reproduction systems and selection pressures between the two situations. The outcrossing nature in humans and animals permits a large amount of genetic heterozygosity to be maintained in both populations and individuals. In contrast, through constant breeder selection and genetic manipulation, genetic variability in self-pollinated rice has been greatly reduced. Genetic uniformity in the US rice cultivars is a major concern to rice breeders (Dilday 1990). Cultivars with such a narrow genetic background present a great challenge to any technology of variety identification. In addition, an expectation of using one probe/enzyme combination to distinguish all existing rice cultivars would be too high to be realistic.

A number of approaches, based on cultivar-specific biochemical and chemical markers including the use of electrophoresis and high-performance liquid chromatography (HPLC), have been undertaken in an attempt to discriminate rice cultivars (Aliaga-Morell et al. 1987; Lookhart et al. 1987; Hussain et al. 1989). Although varying degrees of success have been reported in identifying closely related cultivars, these methods are generally environment-sensitive and sometimes require expensive and highly delicate equipment. The feasibility of using RFLP for cultivar identification has also been previously discussed in rice (Wang and Tanksley 1989) and several other plant species (Helentjaris et al. 1985; Nagamine et al. 1989; Smith and Smith 1991). However, the success rate of using the RFLP approach with closely related individuals has been limited, because of low polymorphism in such populations. Beckmann and Soller (1986) have estimated that 20–30 polymorphic markers would be needed to differentiate the existing inbred strains of maize. Smith and Smith (1991) used 38 DNA probes revealing RFLPs in order to discriminate 74 of 78 maize hybrids. The present approach clearly demonstrated a simple method of using DNA fingerprinting for cultivar identification. It was quite obvious that DNA fingerprinting with a minisatellite sequence could quickly and unambiguously identify different cultivars when they were unrelated or only distantly related (Fig. 1; the probability of a match was 1.5×10^{-5}). However, when dealing with closely related individuals having a narrow genetic base, such as the US cultivars, a high chance of matching is to be expected. Despite its obvious drawback, DNA fingerprinting with minisatellite sequences is still more sensitive and informative than other types of markers so far tested in detecting genetic

variation. By comparison, 58 of the 70 varieties representing a wide range of cultivated rice were distinguishable by RFLP analysis with ten probes and five enzyme combinations (Wang and Tanksley 1989). In our study with the rice minisatellite sequence, one probe and one enzyme was capable of unambiguously identifying 50% of the closely related US cultivars. This did not take into account the 20 unresolved cultivars which were clearly differentiated into seven groups (see Fig. 4).

It is not surprising that the rice probe pOs 6.2H failed to detect polymorphism between some closely related cultivars. This probe was derived from the human minisatellite sequence 33.6 and is probably just one of many minisatellites existing in the rice genome. Daly et al. (1992) reported on a rice sequence similar to the human minisatellite 33.15 which is polymorphic in cultivated rice. Additionaly, we have found that the minisatellite sequence from bacteriophage M13 (Vassart et al. 1987) can detect polymorphisms between cultivars S6 and M7 with the *Dra*I restriction enzyme (data not shown). Analyzing the same cultivars with additional minisatellite probes with different consensus sequences and different enzymes will certainly help to detect more polymorphisms in narrow gene-based rice germplasm.

Estimation of genetic relatedness with DNA fingerprinting

The genetic similarity index (S) calculated from the DNA fingerprint data was used to estimate the genetic relatedness between cultivars. On average, a 75% genetic similarity was found between the cultivars developed in the US, compared to 44% for the other cultivars in Fig. 1. The genetic similarity ranged from 100% (no polymorphism detected by pOs 6.2H) between S6 and M7, to 37% between IR8 and T-487. The high level of similarity among US cultivars was not unexpected. Recently, based on well-documented pedigree information, Dilday (1990) calculated the coefficients of parentage for 140 genotypes identified in US rice breeding programs. He traced the genetic base of these programs to 22 plant introductions in the southern Rice Belt (Arkansas, Louisiana, and Texas), and 23 plant introductions in the California Rice Belt. Of 23 accessions introduced in California, seven of them are common to the ancestry of Arkansas cultivars. This has therefore led to high coefficients of parentage for the US cultivars (Dilday 1990). The present results clearly demonstrated a narrow genetic background of the current rice cultivars released in the US, in that most of the DNA fingerprints were common among these cultivars (Fig. 3).

Based on the DNA fingerprint data, the genetic distances (D) were used to construct a dendrogram for the US cultivars analyzed (Fig. 4). It is interesting to note that, with a few exceptions, most of the cultivars were clustered into different groups corresponding to their pedigrees or origins. Cultivars from Texas were grouped into one unit, except for Lemont for which cannot be clustered into any group. The cultivars developed in California, mostly japonica, were clustered into

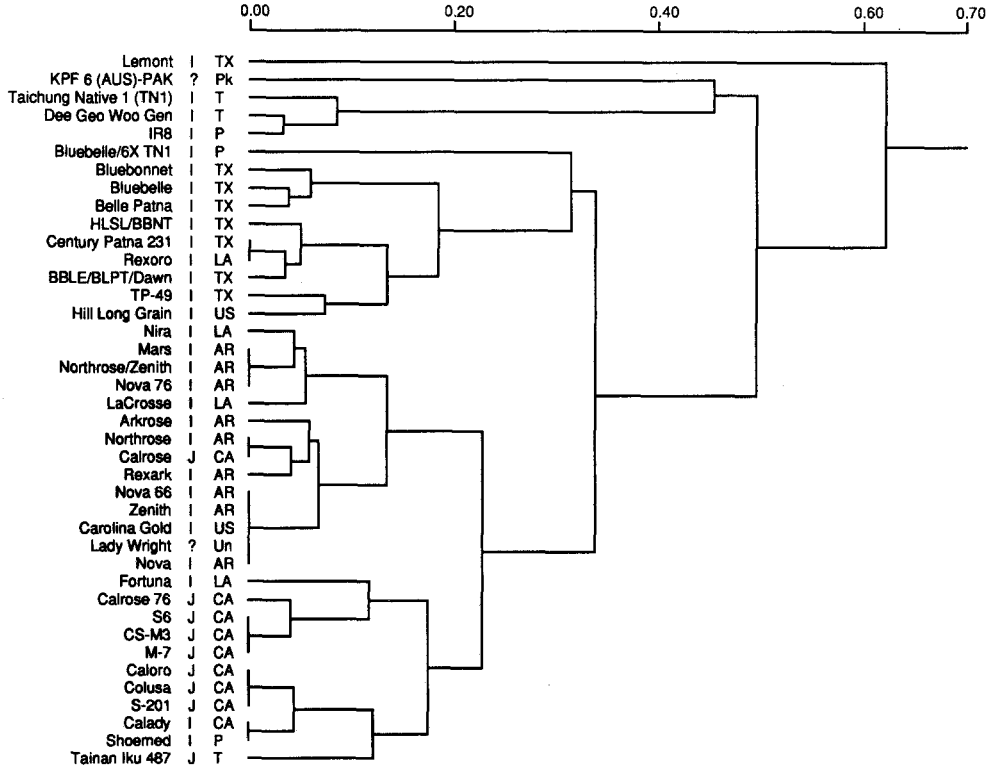


Fig. 4 Dendrogram of genetic relationships among 40 cultivars from US rice-breeding programs. From left: first column, cultivar names; second column, varietal types (*I* indica, *J* japonica, ? information unavailable); third column, origins (*P* Philippines, *Pk* Pakistan, *T* Taiwan, *AR* Arkansas, *CA* California, *LA* Louisiana, *TX* Texas, *US* USA, *Un* unknown). The scales indicate genetic distance

another group. The cultivars developed in Arkansas tended to be grouped in yet another separate cluster (Fig. 4). However, the Arkansas group showed a closer relationship to the group from California than to the Texas group. This notion is supported by the fact that some Arkansas cultivars have common ancestors with Californian cultivars (Dilday 1990).

In order to assess the accuracy of estimation of genetic relatedness *via* DNA fingerprinting, we compared Dilday's coefficients of parentage (1990) with our genetic similarity index between cultivars. The genetic similarity of 83% between the cultivars Calrose and Caloro compared very well with Dilday's results where these two cultivars showed that almost 90% of the genes were in common. However, in some of the cases, DNA fingerprinting appeared to overestimate the genetic relatedness between cultivars. Cultivars 'M7' *vs* 'Calrose 76', 'Nova' *vs* 'Nova 66', and 'Nova 66' *vs* 'Nova 76' were believed to have more than 70%, 70%, and 60% of their genes in common, respectively (Dilday 1990). But, based on the fingerprint data, we found that, the relatedness of M7 and Calrose 76, Nova and Nova 66, and Nova 66 and Nova 76 was 96%, 100%, and 91%, respectively. On the other hand, even though cultivar Shoemed showed no pedigree relationship with cultivar Calady, probe pOs 6.2H did not detect any polymorphism. Because we do not have information on the coefficients of parentage for some of the 40 cultivars, exactly how many pairs of comparison tend to be overestimated was unknown.

The overestimation of genetic relatedness between cultivars *via* DNA fingerprinting may be due to several reasons. First, we scored each fragment of the fingerprint as a piece of independent data, but these fragments may not be in linkage equilibrium. Previous studies have suggested that high levels of linkage disequilibrium can and do exist in self-pollinating plants (Clegg et al. 1972). However, it is not clear how much linkage disequilibrium exists in cultivated rice, or how this would affect the similarity index. Second, the probe pOs 6.2H detects multiple bands simultaneously to form a complex DNA fingerprint. Therefore, any fragments that are allelic cannot be identified. This posts another obstacle to the interpretation of DNA fingerprint data' (Hill 1987; Lynch 1988; Lewin 1989). Isolation of hypervariable single-locus probes might help to solve this problem (Lynch 1988). We have isolated several locus-specific minisatellite sequences from rice and further studies are in progress. Third, as described earlier, the rice probe pOs 6.2H might only detect one group of minisatellites and cover a small proportion of the rice genome. Genetic variance existing in other regions may well have been neglected. Analysis with additional minisatellites and enzymes may shed more light on this issue.

We realize that the data presented here involved only one fingerprinting probe and a small number of cultivars. Although its accuracy in estimating genetic relatedness remains to be determined, the genetic similarity indices obtained from DNA fingerprints do appear to reflect the genetic pedigrees of these rice cultivars. Thus, we believe that the dendrogram depicted in Fig. 2 delineates, in many aspects, a general genetic relationship for these cultivars. DNA fingerprinting should be considered a worthwhile approach for studying the genetic variability of rice cultivars, especially when pedigree information is unavailable.

The results of this study demonstrated a new novel technique of DNA fingerprinting in plants which can be useful to address questions relevant to rice systematics and breeding. Minisatellite loci will provide additional molecular markers for constructing a saturated RFLP map of the rice genome. This would be especially valuable if such sequences were located in regions which were not covered by the single-copy RFLP markers. The minisatellite probes could also be useful for gene-tagging and marker-assisted selection, if the agronomically important traits could be found closely linked to fingerprinting fragments. Although the accuracy of this method in estimating genetic relationship between cultivars remains to be determined, it is much simpler and more sensitive for detecting genetic variation than other types of genetic marker systems. Like other molecular methods, such as isozyme and RFLPs, DNA fingerprinting has great potential of practical use in plant breeding.

Acknowledgements This research was supported in part by a grant from the Rockefeller Foundation to J. P. G. The authors thank Drs. G. S. Khush, R. H. Dilday, and K-L. Zheng for providing rice seeds. We are grateful to Dr. J. F. Dallas for his help and guidance during the early part of this project.

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