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Nuclear genome differentiation in Asian cultivated rice as revealed by RFLP analysis

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ABSTRACT

RFLP analysis was carried out to clarify the nuclear genome differentiation in Asian rice varieties of *Oryza sativa*. Based on the restriction fragment patterns with two endonucleases, *Eco*RI and *Hin*dIII, using 12 single-copy rice DNA probes, 93 types of nuclear genome were found among 112 local varieties from 17 Asian countries. In a dendrogram showing genetic relationships among nuclear genome types, they were mainly divided into eight groups, A, B1, B2, C1, C2, D1, D2 and E. These results were compared with previous isozyme analysis and RFLP analysis on chloroplast genome using the same varieties. Classification on isozyme analysis matches well with that on nuclear genome, indicating synchronous differentiation of isozyme constitutions and nuclear genomes in Asian varieties. Considering the correspondence between them, nuclear genomes were grouped into Indica (A, B1 and B2), intermediate (C1, C2 and D1) and Japonica (D2 and E) types. From the comparison of chloroplast with nucleus for genome differentiation, two major chloroplast genomes (types 1 and 3) were found in the varieties with several nuclear genome types. However, Japonica group with D2 and E nuclear genomes has only type 1 chloroplast genome, whereas Indica and intermediate groups contain both two major chloroplast genomes. Especially, type 3 chloroplast genome which was not found in Japonica group is dominant type in Indica varieties. The results indicate the differentiation of nuclear genome has partially synchronized with that of chloroplast genome.

1. INTRODUCTION

Rice (*Oryza sativa*) is an important crop and a major source of food for more than half of the world population. Since *O. sativa* is widely distributed under variable environmental condition, a broad genetic differentiation is observed in

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this species. In order to provide basic information for rice breeding, intraspecific variation has been studied by many researchers.

Kato et al. (1928) were the first to divide *O. sativa* into two types, "Japonica" and "Indica", based on morphology and sexual affinity. Matsuo (1952) investigated 22 external morphological characters of several thousand varieties and recognized three main plant types, A, B and C. From the main location of distribution, they were referred to as "Japonica", "Javanica" and "Indica" types. Oka (1958) examined 12 morphological and physiological traits of 120 varieties, and classified them into three groups, Continental, Temperate-Insular and Tropical-Insular groups, which correspond to "Indica", "Japonica" and "Javanica" types, respectively. These studies on morphological and physiological traits suggest *O. sativa* can be classified into three main groups, Japonica, Javanica and Indica.

Biochemical studies using isozyme polymorphism have provided further useful information on classification in *O. sativa*. By examining 40 presumed isozyme loci, Second (1982) found that *O. sativa* varieties could be mainly divided into two groups, corresponding to Japonica and Indica. Large-scale survey on isozyme polymorphism in *O. sativa* was carried out by Glaszmann (1987). He examined more than 1600 Asian varieties and classified them into six varietal groups, i.e., two major (corresponding to Japonica and Indica), two minor, and two satellite groups. These isozyme studies indicate that *O. sativa* can be divided into two major groups, Japonica and Indica.

As for variation at molecular level in *O. sativa*, Wang and Tanksley (1989) surveyed RFLP patterns of 70 varieties using rice single-copy probes, and measured genetic variation of nuclear DNA. Chloroplast (ct) DNA was studied by Ishii et al. (1988) and Dally and Second (1990). They found two major chloroplast genome types which existed in Japonica and Indica varieties, respectively. Ishii et al. (1993) examined mitochondrial DNA variation in *O. sativa*, and found mitochondrial genome had differentiated between Japonica and Indica varieties. Since variation at molecular level in *O. sativa* has been observed in several studies, we intended to determine the correspondence between these analyses. In this study, we carried out RFLP analysis with rice single-copy probes using the same materials which were already characterized by isozyme analysis (Glaszmann, 1985) and RFLP analysis of ctDNA (Ishii and Tsunewaki, 1991). Based on these results, the differentiation in *O. sativa* is discussed.

2. MATERIALS AND METHODS

Plant materials

One-hundred-twelve local varieties of *O. sativa* from 17 Asian countries were used for nuclear DNA analysis (Table 1). Isozyme constitution of these varieties was already determined by Glaszmann (1985). Chloroplast genomes from 66 out

Table 1. Materials used for RFLP analysis of nuclear DNA

No.	Name	Origin	Enzymatic group[1)]	Chloroplast genome type[2)]	Nuclear genome type[3)]
1	JC 92	India	I	—	A-1
2	DA 9	Bangladesh	I	—	A-2
3	Chhote Dhan	Nepal	[V]	1	A-3
4	Guan-Yin-Tsan	China	I	3	B1-1
5	Madael	Sri Lanka	[I]	3	B1-2
6	Suduwee	Sri Lanka	*	3	B1-2
7	Sinna Sithina Kali	Sri Lanka	I	3	B1-3
8	Champa Tong 54	Thailand	I	3	B1-4
9	Niaw Tew	Thailand	I	—	B1-5
10	Kaw Luyoeng	Thailand	I	—	B1-6
11	Kenanga	Indonesia	I	—	B1-7
12	Pelita Janggut	Indonesia	I	3	B1-8
13	Salumpikit	Unknown	I	—	B1-9
14	Birain 360	Bangladesh	I	3	B1-10
15	IR 36	—	I	3	B1-11
16	CO 12	India	I	—	B1-12
17	Chiem Chanh	Vietnam	I	—	B1-13
18	Nep Cai Chiem 1	Vietnam	I	3	B1-14
19	Rathuwee	Sri Lanka	I	3	B2-1
20	CO 18	India	I	3	B2-2
21	llis Air	Indonesia	I	3	B2-3
22	Pa-Tou-Hung	China	I	3	B2-4
23	Pin Tawng	Thailand	I	3	B2-5
24	Chau	Vietnam	I	3	B2-6
25	S 624	India	I	1	B2-7
26	Kalukantha	Sri Lanka	*	3	B2-8
27	ASD 1	India	I	—	B2-9
28	Ai-Chiao-Hong	China	I	—	B2-9
29	Lu-Lu-Tsan	China	I	3	B2-10
30	Patik	Indonesia	I	11	B2-11
31	Cere Air	Indonesia	I	3	B2-12
32	Arang	Indonesia	I	—	B2-12
33	JC 120	India	I	—	B2-13
34	ADT 12	India	II	—	B2-14
35	PTB 25	India	I	1	B2-15
36	PTB 9	India	I	1	B2-16
37	Popot	Indonesia	I	—	B2-17
38	Leuang Pratew	Thailand	[I]	3	B2-18
39	Chaing Roneal	Kampuchea	I	—	B2-18
40	Kaukkyisaw	Myanmar	V	1	C1-1
41	Kaukkyi	Myanmar	V	1	C1-2
42	Yelaik Meedon	Myanmar	V	1	C1-2
43	Kaukkyi Ani	Myanmar	*	1	C1-3
44	Tchampa	Iran	[V]	—	C1-4
45	Dom-Zard	Iran	[V]	12	C1-5
46	Basmati 1	Pakistan	[V]	1	C1-5
47	Domsiah	Iran	V	12	C1-5
48	Dom-Sofid	Iran	V	—	C1-5
49	Mehr	Iran	V	—	C1-5
50	Basmati 217	India	V	—	C1-5
51	Rayada 16-05	Bangladesh	IV	—	C1-6
52	Rayada 16-04	Bangladesh	[IV]	10	C1-7
53	Rathal	Sri Lanka	I	1	C1-8
54	T 26	India	[V]	—	C1-9
55	Basmati Lamo	Nepal	V	1	C1-10
56	Bikyat	Philippines	VI	1	C1-11
57	Chahora 144	Pakistan	V	—	C2-1
58	Basmati 370	Bangladesh	V	1	C2-2
59	Rayada 16-02	Bangladesh	IV	—	C2-3

60	Bhadoia 233	Bangladesh	III	1	C2-4
61	Bamoia 341	Bangladesh	III	3	C2-5
62	JC 91	India	I	3	C2-6
63	Boteswar 2	Bangladesh	II	1	C2-7
64	T 1	India	II	—	C2-8
65	Ghati Kamma Nangarhar	Afghanistan	II	3	C2-8
66	Jhona 349	India	II	3	C2-9
67	Gerdeh	Iran	II	3	C2-9
68	Muthusamba	Sri Lanka	*	—	C2-10
69	DA 11	Bangladesh	I	1	C2-11
70	Dular (32561)	India	II	3	D1-1
71	Dular (3688)	India	II	3	D1-2
72	ARC 10372	India	II	—	D1-3
73	Jhum Begunbichi	Bangladesh	II	—	D1-4
74	Binulawan	Philippines	[VI]	—	D1-5
75	Kalamkati	India	II	3	D1-6
76	Aus 61	Bangladesh	II	—	D1-7
77	N 32	India	II	—	D1-8
78	DA 16	Bangladesh	II	—	D1-8
79	N 22	India	II	3	D1-9
80	Tepi Boro	Bangladesh	II	—	D1-10
81	Jagri Boro	Bangladesh	II	3	D1-11
82	Thahanala	Sri Lanka	*	—	D1-11
83	Baran Boro	Bangladesh	II	1	D1-12
84	Dholi Boro	Bangladesh	II	1	D1-12
85	PTB 30	India	II	—	D1-13
86	DA 8	Bangladesh	II	—	D1-14
87	DA 28	Bangladesh	II	1	D1-15
88	JC 117	India	I	—	D1-16
89	DA 1	India	I	3	D1-17
90	Dhola Aman	Bangladesh	I	—	D1-17
91	Chuan 3	Taiwan	VI	—	D2-1
92	Haifugoya	Taiwan	VI	1	D2-2
93	Beonjo	Korea	VI	1	D2-3
94	Y Chang Ju	China	VI	1	D2-4
95	Shan Kiu Ju	China	VI	—	D2-4
96	Ta Hung Ku	China	VI	1	D2-5
97	Darmani	Nepal	V	—	D2-5
98	Ken Chiao Ju Hsiao Li	China	VI	—	D2-6
99	Kap Nhay	Laos	VI	—	D2-7
100	NPE 844	Pakistan	*	1	D2-8
101	Chuan 4	Taiwan	VI	1	D2-9
102	Hei Chiao Chui Li	China	VI	1	D2-10
103	Trembese	Indonesia	VI	1	E-1
104	Azucena	Philippines	VI	1	E-2
105	Gogo Lempuk	Indonesia	[VI]	1	E-3
106	Sulig	Philippines	VI	1	E-4
107	Kinandang Patong	Philippines	VI	1	E-5
108	Gotak Gatik	Indonesia	VI	—	E-5
109	Cicih Beton	Indonesia	VI	1	E-6
110	Ma Hae	Thailand	VI	—	E-7
111	Hawm Om	Thailand	VI	—	E-8
112	Dam	Thailand	VI	—	E-9

[1] Enzymatic groups (I-VI) classified by Glaszmann (1985). *: unclassified varieties. Brackets indicate the enzymatic groups which were not confirmed with the varieties used in this study.

[2] Chloroplast genome types (1, 3, 10, 11 and 12) classified by Ishii and Tsunewaki (1991). —: not determined.

[3] Nuclear genome types identified in this study. They were mainly classified into eight groups, A, B1, B2, C1, C2, D1, D2 and E. In each group, nuclear genome types were numbered from the top branch of the dendrogram in Fig. 1.

of 112 varieties were also analyzed by Ishii and Tsunewaki (1991). Seeds of these varieties were obtained from the International Rice Germplasm Center, International Rice Research Institute, Philippines.

Southern hybridization analysis

Total DNAs from 112 varieties were isolated from leaves according to the method of Dellaporta et al. (1983). They were digested with two restriction endonucleases, *Eco*RI and *Hin*dIII (Boehringer Mannheim, Germany), and electrophoresed (5–10 μg per lane) in 1.0% agarose gel. After digested DNA was transferred to Nylon membrane (Hybond-N, Amersham), hybridization was carried out with 12 rice single-copy DNA probes. These probes were selected from 12 RFLP linkage groups corresponding to the chromosomes of rice (Table 2). They were kindly provided by Drs. S. R. McCouch and S. D. Tanksley, Cornell University, USA. Probe labeling with digoxigenin-dUTP and immunological detection were made after Ishii et al. (1990).

Table 2. Probes used for RFLP analysis of nuclear DNA[1)]

Probe	Size (kbp)	Chr. no.	Probe	Size (kbp)	Chr. no.
RG236	1.4	1	RG351	0.8	7
RG144	0.8	2	RG20	1.5	8
RG69	?	3	RG358	1.0	9
RG214	1.4	4	RG241	2.5	10
RG182	3.4	5	RG118	2.0	11
RG172	1.8	6	RG190	1.4	12

[1)] All probes were cloned using the *Pst*I site of pUC8 (McCouch et al. 1988).

Estimation of genetic distance and the construction of phylogenetic tree

All the hybridized fragments were scored, and the ratio of common fragments was calculated by the formula, $F_{ij}=2B_{ij}/A_{ij}$, where A_{ij} and B_{ij} are the numbers of total and common fragments observed between i^{th} and j^{th} varieties (Nei and Li, 1979). Using this value, a genetic distance between two varieties was estimated according to Nei (1987) (formula 5.53–5.55). Based on the genetic distances among the varieties, a phylogenetic tree was constructed by UPGMA method (Sneath and Sokal, 1973).

3. RESULTS AND DISCUSSION

Identification of the materials

In order to confirm whether the materials are the same as those analyzed by Glaszmann (1985) or not, part of their isozyme constitution was examined accord-

ing to the method of Glaszmann et al. (1988). The following five loci of two enzymes were surveyed; *Pgi-1* and *Pgi-2* of phosphoglucose isomerase, *Amp-1*, *Amp-2* and *Amp-3* of aminopeptidase. In most varieties (102 out of 112 varieties), the identical isozyme constitution was confirmed, however, ten showed different isozyme patterns (Table 1). Probably, they are not pure lines, or seed contamination may have given different results. Taking this fact into consideration, nuclear DNA from 112 varieties was analyzed.

RFLP analysis on nuclear genome in O. sativa

Total DNAs from 112 varieties were digested with two 6-bp cutters, *Eco*RI and *Hin*dIII. They were subjected to Southern hybridization with 12 single-copy DNA probes (Table 2). In total, 24 combinations of hybridization (2 endonucleases $\times$ 12 probes) were analyzed, and polymorphism was revealed in 17 combinations. Among 112 varieties, the total number of the fragments scored in

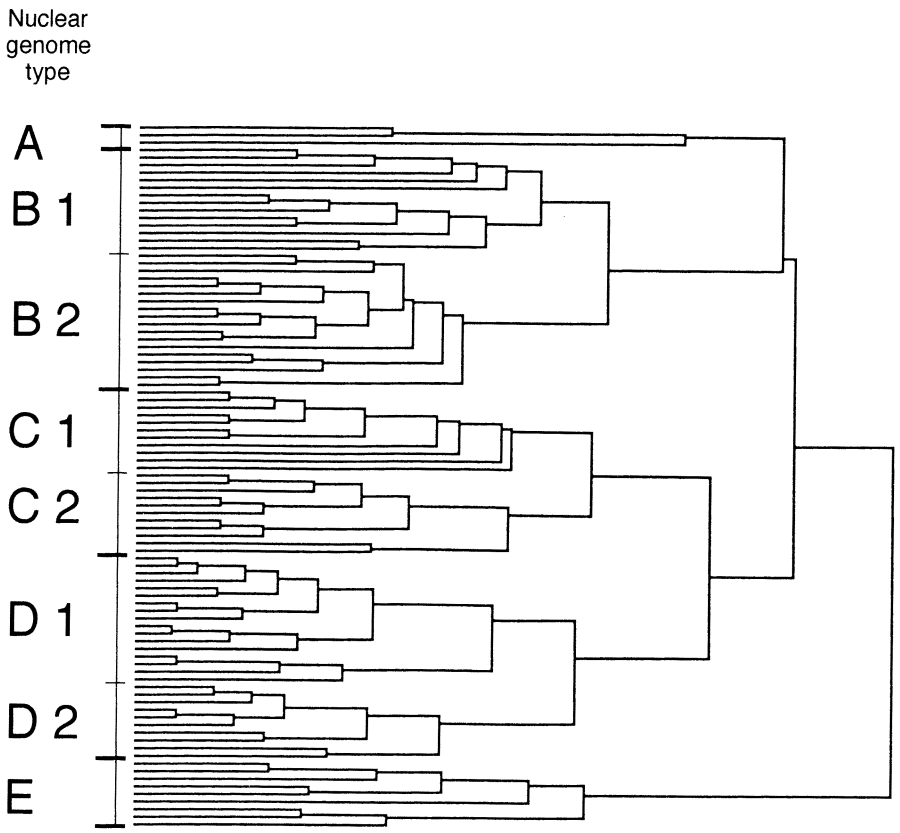

Fig. 1. Dendrogram showing genetic relationships of 93 nuclear genome types found among 112 varieties of *O. sativa*. They were divided into eight groups, A, B1, B2, C1, C2, D1, D2 and E. The varieties belonging to each branch were listed in Table 1.

all combinations ranged from 25 to 31. Based on the fragment patterns, 93 types of nuclear genomes were found among 112 varieties (Table 1). After calculating the proportion of common restriction fragments between 93 types, genetic distances were estimated, and a dendrogram showing genetic relationships was constructed (Fig. 1). Ninety-three nuclear genome types from 112 varieties were mainly divided into five groups, A, B, C, D and E. Further, groups B, C and D could be divided into two subgroups, respectively.

Uniqueness of RFLP patterns of individual varieties in O. sativa

On the basis of the results of RFLP analysis, nuclear DNAs from 112 varieties were classified into 93 types. Among them, 15 types were shared in at least two varieties. Therefore, in total, 78 out of 112 varieties (69.6%) could be individually identified. Wang and Tanksley (1989) reported that 58 out of 70 varieties (82.9%) in *O. sativa* were differentiated from one another. The percentages of uniqueness of the nuclear DNA of individual varieties differed in the two studies, because the materials were prepared in different ways as follows. Wang and Tanksley (1989) used five individuals as representative of each variety, and examined 50 hybridization patterns (10 probes$\times$5 endonucleases) for 70 varieties. They scored, regardless of frequency, all hybridized fragments detected in a single variety from five plants. In the present study, a single plant which had identical isozyme constitution to previous results (Glaszmann, 1985) was used as a representative of the variety in order to eliminate variation within variety which might be caused by contaminated or outcrossed seeds. Therefore, smaller number of the plants examined as representative of each variety seems to result in lower uniqueness of the nuclear DNA among varieties.

In scoring the fragments, only the fragments with strong signal were selected from each hybridization pattern, because it was difficult to judge whether fragments with weak intensity were from the same locus that the probe originated or from different loci of high homology with the probe. In the present study, number of the fragments scored in each hybridization pattern ranged from zero to three. In most cases, only one fragment was scored, the average fragment number being 1.04–1.24 among 112 varieties. This may also be one of the reasons why the percentage of uniqueness of individual varieties became lower than that of Wang and Tanksley (1989).

Comparison between enzymatic groups and nuclear genome types in Asian varieties

Nuclear DNAs of 112 varieties were classified into 93 types. They were divided into eight groups as shown in Fig. 1. Previously, Glaszmann (1985, 1987) analyzed the isozyme constitution of the same varieties, and classified them into six enzymatic groups, i.e., two major (groups I and VI), two minor (groups II and V), and two satellite (groups III and IV) groups. From the result of the cluster

Table 3. Number of the varieties classified into different nuclear genome type on the basis of enzymatic group or chloroplast genome type

Nuclear genome type	Enzymatic group[1]							Chloroplast genome type[2]				
	I	II	III	IV	V	VI	*[3]	1	3	10	11	12
A	2	0	0	0	0	0	0	1	0	0	0	0
B1	13	0	0	0	0	0	1	0	9	0	0	0
B2	18	1	0	0	0	0	1	3	10	0	1	0
C1	1	0	0	1	8	1	1	8	0	1	0	2
C2	2	5	2	1	2	0	1	4	5	0	0	0
D1	3	16	0	0	0	0	1	3	6	0	0	0
D2	0	0	0	0	1	10	1	7	0	0	0	0
E	0	0	0	0	0	9	0	6	0	0	0	0
Subtotal	39	22	2	2	11	20	6	32	30	1	1	2
Total	102[4]							66				

[1] Classified by Glaszmann (1985).
[2] Classified by Ishii and Tsunewaki (1991).
[3] Enzymatic group unclassified.
[4] Ten varieties showing different isozyme constitution from Glaszmann's results are excluded.

analysis, these enzymatic groups were divided into two main clusters, one consisting of groups I, II and III, and the other containing groups IV, V and VI. Especially, two major groups I and VI correspond to Indica and Japonica varieties, respectively. Table 3 gives the comparison between Glaszmann's enzymatic groups and the present nuclear genome types in Asian varieties. Most of the varieties belonging to two major enzymatic groups, I and VI, have three (A, B1 and B2) and two (D2 and E) nuclear genome types, respectively. This indicates typical Indica and Japonica varieties can also be distinguished on the basis of these nuclear genome types. Moreover, the varieties of two minor enzymatic groups, II and V, mainly made intermediate clusters of nuclear genome types D1 and C1, respectively. However, two satellite enzymatic groups, III and IV, could not be compared to the nuclear genome types, since the number of the varieties analyzed was still small. On the other hand, nuclear genome types were mainly found in the varieties belonging to a single enzymatic group except for C2 nuclear genome type which the varieties of five enzymatic groups possessed. These results suggest nuclear genome types and enzymatic groups have synchronously differentiated. Based on the correspondence with enzymatic groups, nuclear genomes were divided into Indica (A, B1 and B2), intermediate (C1, C2 and D1) and Japonica (D2 and E) types. In most of study, the extent of the differentiation in Japonica is much smaller than that in Indica (Second, 1982; Oka, 1988; Wang and Tanksley, 1989; Dally and Second, 1990; Ishii et al., 1993).

However, two Japonica nuclear genomes, D2 and E, were not so close to each other in the present RFLP analysis. Especially, nuclear genome type E was the most differentiated one from others in *O. sativa* (Fig. 1). The reason why different results appeared in this study might be due to the probes; one probe (RG182) showed polymorphisms in *Eco*RI and *Hin*dIII restriction patterns between accessions having type E nuclear genome and others. Therefore, in order to know the extent of nuclear genome differentiation in Japonica, it would be better to add more probes for the analysis.

Comparison between chloroplast and nuclear genomes in Asian varieties

Chloroplast DNA from 66 out of 112 varieties used in this study was previously examined by RFLP analysis (Ishii and Tsunewaki, 1991). In total, five chloroplast genome types (1, 3, 10, 11 and 12) were found among them. Especially, types 1 and 3 are major ones which were present in 62 out of 66 varieties. The rest three are minor types. Table 3 shows the correspondence between chloroplast and nuclear genome types. Both major chloroplast genome types 1 and 3 were widely found in the varieties with several nuclear genome types. Japonica group with D2 and E nuclear genomes has only type 1 chloroplast genome. On the other hand, Indica group contains both chloroplast genome types 1 and 3. Dally and Second (1990) reported chloroplast genome is well-associated with isozyme-characterized nuclear genome, since Indica varieties which have Japonica chloroplast genome are exceptional and intermediate varieties have generally Japonica chloroplast genome. In the present study, type 3 chloroplast genome was found dominantly in Indica group, however, Indica varieties having type 1 chloroplast genome (Japonica chloroplast genome type) can not be neglected. In intermediate group, both chloroplast genomes were observed almost equally; 15 and 11 varieties have type 1 and 3 chloroplast genomes, respectively. These facts indicate the differentiation of nuclear genome has partially synchronized with that of chloroplast genome.

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