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Genetic differentiation for nuclear, mitochondrial and chloroplast genomes in common wild rice (*Oryza rufipogon* Griff.) and cultivated rice (*Oryza sativa* L.)

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Abstract The genetic differentiation of nuclear, mitochondrial (mt) and chloroplast (cp) genomes was investigated by Southern and PCR analysis using 75 varieties of cultivated rice (*Oryza sativa* L.) and 118 strains of common wild rice (CWR, *Oryza rufipogon* Griff.) from ten countries of Asia. The distinguishing differences between the Indica and Japonica cultivars were detected both in the nuclear genome and the cytoplasmic genome, confirming that the Indica–Japonica differentiation is of major importance for the three different classes of genome in cultivated rice. This differentiation was also detected in common wild rice with some differences among the genome compartments and the various regions. For nuclear DNA variation, both Indica-like and Japonica-like types were observed in the Chinese CWR, with the latter more-frequent than the former. No Japonica-like type was found in South Asia, and only two strains of the Japonica-like type were detected in Southeast Asia, thus the Indica-like type is the major type among South and Southeast Asian CWR. For mtDNA, only a few strains of the Japonica-like type were detected in CWR. For cpDNA, the Japonica type was predominant among the CWR strains from China, Bangladesh and Burma, while the Indica type was predominant among the CWR strains from Thailand, Malaysia, Cambodia and Sri Lanka, and both types were found in similar frequencies among the Indian CWR. Altogether, however, the degree of Indica–Japonica differentiation in common wild rice was much-less important than that in cultivated rice. Cluster analyses for nuclear and mitochondrial DNA variation revealed that some CWR strains showed large genetic distances from cultivated rice and formed clusters distinct from cultivated rice. Coincidence in the genetic differentiation between the three different classes of genome was much higher in cultivated rice than in CWR. Among the 75 cultivars, about 3/4 entries were “homoeotype” showing congruent results for nuclear, mt and cpDNA regarding the Indica–Japonica differentiation. In CWR, the proportions of homoeotypes were 5.7%, 15% and 48.8% in China, South Asia and Southeast Asia, respectively. Based on the average genetic distance among all the strains of CWR and cultivated rice for nuclear and mitochondrial genomes, the variability of the nuclear genome was found to be higher than that of the mitochondrial genome. The global pattern based on all genomes shows much-more diversification in CWR than that in cultivated rice.

Keywords Rice · Nuclear genome · Mitochondrial genome · Chloroplast genome · Genetic differentiation

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Introduction

Rice is a very important food crop in both China and the rest of the whole world. Knowledge concerning the genetic differentiation of the genome of rice and its wild relatives, enables us to clarify the biosystematic relationship and provide basic information for rice breeding. The development of molecular markers has provided powerful tools in assessing genetic differentiation and detecting the phylogenetic relationship between cultivated rice and wild rice (Wang and Tanksley 1989; Wang et al. 1992; Nakano et al. 1992; Zhang et al. 1992; Doi et al. 1995; Sun et al. 1995).

The chloroplast and mitochondrial genomes of rice are both circular molecules of double-stranded DNA and have the size of 134,525 bp for chloroplasts and an approximate size of 495 kbp for mitochondria (Hiratsuka et al. 1989; Iwahashi et al. 1992). Restriction endonuclease analyses of mitochondria DNA (mtDNA) and chloro-

plast DNA (cpDNA) were used in the study of phylogenetic relationship in rice and other higher plants (Timothy et al. 1979; Kemble and Shepard 1984; Ichikawa et al. 1986; Ishii et al. 1986, 1988, 1993; Breiman 1987; Dally and Second 1990; Laurent et al. 1993; Luo et al. 1995).

It has been generally accepted that Asian cultivated rice is divided into *indica* and *japonica* subspecies. Indica–Japonica differentiation is of major importance in cultivated rice as proved by a wealth of data from morphological traits, isozyme and nuclear DNA markers (Morishima and Oka 1981; Second 1982, 1985b; Glaszmann 1987; Oka 1988; Wang and Tanksley 1989; Sano and Morishima 1992; Zhang et al. 1992; Cai et al. 1993).

The results of Ishii et al. (1986) and Dally and Second (1990), showed that there were two major types of cpDNA in Asian cultivated rice. Type1 and Type 3 identified by Ishii et al. (1986), and plastotype e1 and a1 named by Dally and Second (1990), corresponded to Japonica and Indica cultivars respectively. A deletion of the 69-bp sequence in ORF100 (Open Reading Frames 100) region was identified in the cpDNA (Type 3) of the Indica varieties compared to the Japonica varieties (Ishii et al. 1986, 1988; Kanno et al. 1993). The deletion can be detected by PCR amplification (Chen et al. 1993, 1994; Kanno et al. 1993).

The Indica–Japonica differentiation was also detected in common wild rice, ancestral species of cultivated rice, by isozyme and DNA markers (Second 1985; Morishima and Gadrinab 1987; Ishii et al. 1988; Dally and Second 1990; Sano and Sano 1990; Wang et al. 1992, 1994; Sun et al. 1995). Based on the Indica–Japonica differentiation of common wild rice (CWR), a “Diphyletic hypothesis” for the origin of cultivated rice was proposed (Chou 1948; Second 1985; Ishii et al. 1988; Dally and Second 1990).

However, information on the genetic variation in wild rice at the DNA level is still limited. To clarify the diversity and domestication process of this important food crop, analysis of the polymorphism of nuclear, mitochondria and chloroplast DNA is required using a larger number of strains of cultivated rice and common wild rice from different countries or regions. In the present study, we investigated the variation of nuclear, mitochondrial and chloroplast DNA among 75 cultivars and 118 strains of common wild rice from ten Asian countries by Southern and PCR analysis. The objective of our study is to analyze and integrate the genetic diversity of the three different classes of genome to infer the phylogenetic relationship of cultivated rice and its wild ancestor.

Materials and methods

Plant materials

A total of 193 strains including 118 strains of CWR and 75 cultivars were used. These CWR strains were from China (35), India (27), Sri Lanka (7), Bangladesh (6), Thailand (17), Burma (12), Cambodia (5), Malaysia (6), Indonesia (2) and the Philippines (1). All strains of the South and Southeast Asia CWR, and a part of the strains of Chinese CWR, were kindly provided By Prof. Morishima, National Institute of Genetics (NIG), Japan. The 75 strains of cultivated rice included landraces, primitive cultivars, historically important cultivars and modern elite varieties. These materials represent a broad spectrum of the gene pool of common wild rice and cultivated rice.

DNA extraction

Fresh leaves of a single plant in each strain were collected and ground in liquid nitrogen. Total DNA was extracted from ground tissues by the CTAB (Cetyltrimethyl ammonium bromide) method (Rogers and Bendich 1988).

RFLP markers of nuclear and mitochondrial genomes

Forty eight probes of the nuclear genome mapped on RFLP maps (Saito et al. 1991; Kurata et al. 1994; Tsunematsu et al. 1996), and seven probes of mitochondrial DNA (Iwahashi et al. 1992), were used in the study. A part of the probes of the nuclear genome were kindly provided by the MAFF DNA Bank of Japan. Among seven clones of the mitochondrial genome, *cox* I (the subunit I gene of rice cytochrome oxidase), *cox*II (the subunit II gene of rice cytochrome oxidase), *atp*9 (the subunit 9 gene of rice ATPase), *atpA* (the alpha subunit gene of rice ATPase) (Morikami and Nakamura 1987), and *nad*3 (the subunit 3 gene of rice NADH dehydrogenase) were provided kindly by Prof. Hirai, the University of Tokyo, Japan, and *ATPA* (the alpha subunit gene of pea ATPase) and *rrn*18 + 5 (18S + 5S genes of wheat), were kindly provided by Dr. Ishii, Kobe University, Japan.

Southern hybridization

Three micrograms of genomic DNA digested with one kind of restriction endonuclease was electrophoresed on a 0.8% agarose gel. The gels were blotted onto a positively charged Nylon membrane (Boehringer Mannheim) by capillary transfer in 0.4 N NaOH for 12 h and the membrane was washed in 2 × SSC, dried and then baked at 120 °C for 20 min. Probes were labeled with HRP (horseradish peroxidase) according to the protocol of the ECL direct nucleic acid labeling and detection system (Amersham). Hybridized filters were detected by enhanced chemiluminescence on Fuji X-ray film for 1–3 h. For Southern analysis of the nuclear genome, only *Dra*I was used for DNA digestion, while *Hin*dIII, *Eco*RV, *Pst*I, *Sal*I besides *Dra*I were used for mitochondrial analysis.

Data analysis

Each fragment detected by Southern analysis was treated as a unit character and the character was quantified by "1” for presence and "0” for absence of the fragment. The genetic distance between strains was calculated by the following equation (Nei 1987):

$$D = -\ln[2Mxy/(Mx + My)],$$

where Mxy is the total number of bands common to lines X and Y, and Mx and My are the total number of bands present in lines X and Y, respectively. A dendrogram was then constructed by the unweighted pair-group method with an arithmetic mean (UPGMA) (Sokal and Michener 1958).

PCR amplification of chloroplast DNA

Two oligonucleotides (5′-GGCCATCATTTTCTTCTTTAG-3′, 5′-AGTCCACTCAGCCATCTC-TC-3′) (Chen et al. 1993) were used

as a pair of primers for the PCR reaction. A total volume of 25 μl of reaction mixture was composed of 10 ng of total DNA, 10 mM of Tris-HCl (pH 9.0), 50 mM of KCl, 1.5 mM of $MgCl_2$, 0.1% Triton X-100, 1 μM of each primer and 1 unit of *Taq* DNA polymerase (Promega), and mixture was covered by 25 μl of mineral oil. Amplification was performed on a Iwaki Thermal Sequencer (TSR-300) programmed for 40 cycles of 1 min at 94 °C, 1 min at 51 °C, 2 min at 72 °C, followed by 7 min at 72 °C. Reaction products were resolved by electrophoresis at 80 V for 5 h in 1.4% agarose gels containing 0.5 μg/μl of ethidium bromide.

Results

Genetic differentiation of nuclear DNA

A total of 201 RFLP bands were detected when considering the 193 strains with 48 probe-enzyme (*Dra*I) combinations. The number of RFLP variants per probe-enzyme combination ranged from 2 to 9, with an average of 4.18. This average was less than that detected by Wang et al. (1992) in the genus *Oryza*, but more than that detected in *Oryza sativa* (Wang and Tanksley 1989).

The simplified dendrogram produced by the cluster analysis is shown in Fig. 1. The complexes of 75 varieties of cultivated rice and 118 accessions of CWR were classified into four distinct groups (Group n I, n II, n III and n IV in Fig. 1).

In Group n I, there were 74 strains of CWR and 37 cultivars. These 74 strains of CWR were from China (11), India (19), Burma (7), Thailand (17), Sri Lanka (3), Bangladesh (7), Indonesia (2), Cambodia (4) and Malaysia (4). All of 37 cultivars belong to the Indica type, classified by morphysiological traits. So this group was called the Indica Group. The nuclear genomes of all strains in this group were of the Indica type (for cultivars) and the Indica-like type (for CWR).

Group n II consisted of 19 strains of CWR and 38 cultivars. The strains of CWR in the group were from China (17), Cambodia (1) and Burma (1). All 38 cultivars were recognized as Japonica varieties by traditional classification. Nipponbare, Akihikari, Taichuang 65 etc., well known Japonica varieties, were included in this group. Hence, we called Group n II the Japonica Group. The nuclear genomes in this group were identified as Japonica type (for cultivars) and Japonica-like (for CWR strains).

Six strains of CWR from the Jiangxi (3), Hunan (1) and Yunnan Province (2) of China, were clustered into Group n III. The habitats of these CWR strains have been well-isolated from cultivars up to now. It is recognized that few genes of cultivated rice have been introgressed into their natural populations. Various characteristics found in these CWR strains, such as long anthers (more than 5 mm), purple stigmas, red long awns, black hulls, red pericarps and prostrate growth habit, were considered to be primitive and distinguishable from cultivated rice. These morphological and ecological characteristics accord with that of the primitive ancestral CWR strain proposed by Pang et al. (1995) based on the morphological classification of a larger number of CWR accessions. So we considered this group as the Chinese specific CWR group, and the nuclear genomes of these strains were identified as Chinese Wild-specific type.

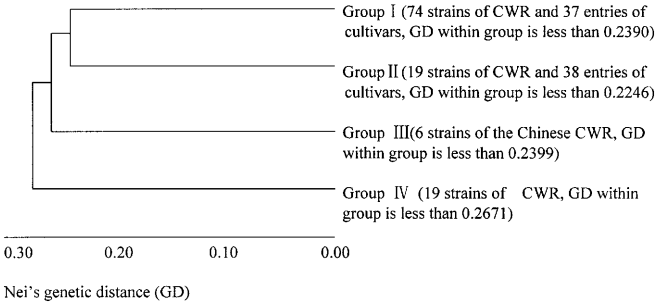

Fig. 1 Simplified UPGMA dendrogram of 118 accessions of CWR and 75 varieties of *O.sativa* based RFLP data of nuclear DNA

Group n IV included 19 strains of CWR from India (4), Sri Lanka (4), Burma (4), Philippines (1) and Malaysia (2). Although these CWR strains were not so typical or primitive as strains of Group n III in morphological characters, they show primitiveness in some of the traits; for example, a longer anther and a purple stigma etc. Consequently, we named this group as the South and Southeast Asia specific CWR group, and the nuclear genomes of these CWR strains were identified as the South and Southeast Asian Wild-specific type.

The above results demonstrated that the Chinese CWR could be classified into three types, Indica-like, Japonica-like and Chinese Wild-specific type, while the South Asian CWR could be only clustered into Wild-specific and Indica-like type. For the 43 strains of the Southeast Asian CWR, the Indica-like type is a major type in the nuclear genome, although they included two strains of Japonica-like type and seven strains of Wild-specific type CWR. Interestingly, the large genetic distance between Group n III and Group n IV indicated that there were distinguishing differences between the Chinese Wild-specific type CWR and the South, Southeast Asian Wild-specific type CWR.

Genetic differentiation of mitochondrial DNA

A total of 99 polymorphic fragments were detected with 17 enzyme/probe combinations in the strains of CWR examined and 75 cultivars. A dendrogram constructed based on mtDNA RFLP data by using the 193 accessions is shown in Fig. 2. All the materials were clustered into five groups.

Group m I consisted of 86 strains of CWR and 35 varieties of cultivated rice. The 86 strains of CWR were from nine countries of Asia. Among the 35 cultivars in this group, 30 varieties are Indica by traditional classification. So the genotypes of the mitochondrial genome of strains in this group were designated as Indica type (for cultivars) or Indica-like type (for CWR).

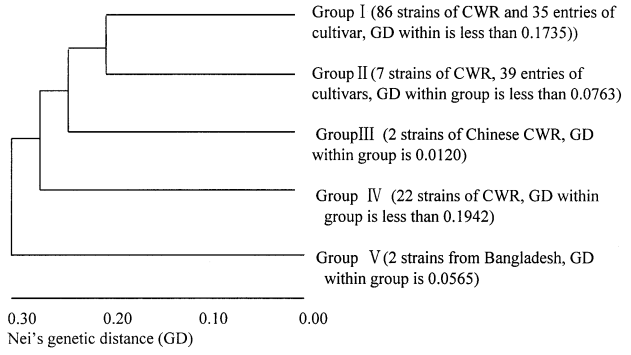

Fig. 2 Simplified UPGMA dendrogram of 118 strains of *O.rufipogon* and 75 varieties of *O.sativa* based on RFLP data of mitochondrial DNA

Seven strains of CWR and 39 cultivars were clustered into Group m II. The seven strains of CWR were from China (3), India (2), Cambodia (1) and Philippines (1) respectively. Among the 39 cultivars, 32 are Japonica and seven cultivars are Indica varieties by conventional classification. Milyang 23, an Indica variety from Korea, was clustered in this group. However, we identified the mitochondria of all the strains in this group as Japonica type (for cultivars) or Japonica-like type (for CWR). The genetic distances between strains within this group were less than 0.076, indicating that the variation within this group was relatively small.

In Group m III, there were only two strains of CWR, WA113 and WA114, from Dongxiang, Jiangxi Province, China. These two strains showed eight specific fragments in four enzyme-probe combinations.

Group m IV consisted of 22 accessions of CWR. These strains were from eight countries including India (8), Thailand (3), Burma (3), Bangladesh (1), Indonesia (1), Cambodia (1), Sri Lanka (1) and Yuanjiang, Yunnan Province, China (four strains). This group could be further clustered into five subgroups. One of the subgroups consists of four accessions of CWR from Yuanjiang, Yunnan Province of China. But these four strains showed a close relationship in their nuclear genome with the CWR of Jiangxi and Hunan Province, the middle valley of Yangtze river, China.

Considering the large differences of Group m III and Group m IV, consisting of CWR strains from Group m I and Group m II which included almost all cultivars, we identified the mitochondrial genome of Group m III and Group m IV as the Wild-specific type.

Group m V had two strains, Bonsaj and W1818, both from Bangladesh. The difference of mtDNA between the two strains was small, but there were large distances between Group m V and all other groups. It is difficult to identify the type of mitochondrial genome of the two strains. However, we called them the Bangladesh specific-type temporarily.

The above results showed that the mitochondrial genome of 75 cultivars could be clustered into Indica and Japonica types except for "Bonsaj", the ratio of Indica and Japonica types of mitochondrial genomes is near 1:1. This suggests that the Indica–Japonica differentiation is also the main trend in the mtDNA of cultivated rice. In CWR, two other kinds of Wild type mitochondral genomes specific to CWR were detected besides Indica-like and Japonica-like types which are shared with cultivars. The average genetic distance within the CWR (0.2608) was larger than that within cultivated rice (0.1460). This indicates that mtDNA is more variable in CWR than in cultivated rice.

The above results also demonstrated that the proportion of Japonica type mtDNA was very low in CWR. It is well known that CWR is essentially distributed in the regions of Indica cultivation. So we inferred that the high proportion of Indica type mtDNA in CWR might be related to their distribution.

Indica–Japonica differentiation of chloroplast DNA

By PCR amplification, the Indica type (with deletion of the 69-bp sequence in ORF100) and Japonica type (with no-deletion of the 69-bp sequence in ORF100) of the chloroplast genome was determined for all strains. As shown in Table 1, among 75 cultivars, the Japonica type was more frequent than the Indica type. Most of the Indica cultivars, such as IR24, IR36, Kasalath etc., showed the Indica type cpDNA. Most of the Japonica varieties,

Table 1 Distribution of cpDNA types in common wild rice and cultivated rice

Species	Origin	Number of accessions	Indica type	Japonica type	Proportion of Indica type
O.rufipogon	China	35	9	26	26
	India	27	13	14	48
	Sri Lanka	7	7	0	100
	Bangladesh	6	1	5	17
	Thailand	17	16	1	94
	Burma	12	3	9	25
	Malaysia	6	4	2	67
	Cambodia	5	4	1	80
	Indonesia	2	2	0	100
	Philippines	1	0	1	0
	Total	118	59	59	50
O.sativa		75	26	49	35

Table 2 Indica–Japonica type of nuclear, mitochondrial and chloroplast genomes of the cultivars used in this study. Note: mt: mitochondria, cp: chloroplast, I: Indica type; J: Japonica type; B: specific type of Banladesh

Varietal name	Origin	Indica–Japonica type			Varietal name	Origin	Indica–Japonica type		
		Nuclear	mt	cp			Nuclear	mt	cp
Rayada 7	Bangladesh	J	I	J	IR36	IRRI	I	I	I
Bomota	Bangladesh	I	I	J	Balilla	Italy	J	J	J
Kalimekri	Bangladesh	I	I	I	Shinriki	Japan	J	J	J
Awasina	Bangladesh	I	I	J	Kameji	Japan	J	J	J
Dhala Aman	Bangladesh	J	I	I	Nekken2	Japan	J	J	J
Muja Shail	Bangladesh	I	I	J	Norin 8	Japan	J	J	J
Bonsaj	Bangladesh	J	B	J	Nipponbare	Japan	J	J	J
Qingkeng	China	J	J	J	Akihikari	Japan	J	J	J
Yidu	China	J	J	J	Asahi	Japan	J	J	J
Kengdao	China	J	J	J	Kinmaze	Japan	J	J	J
Jiaodongqing	China	J	J	J	Milyang 23	Korea	I	J	J
Hangmangkeng	China	J	J	J	Mack Kham	Laos	I	I	I
Changgangbaidao	China	I	J	J	Khao Eo-1	Laos	J	J	J
Hunanxian	China	I	I	I	Khao Eo-2	Laos	J	J	J
Xiaobaigu	China	J	J	J	Khao Eo-3	Laos	J	J	J
Liuzhoubaoyazao	China	I	I	I	Pulat Balachan	Malaysia	I	I	I
Hongxienuo	China	I	I	I	Bongor	Malaysia	I	I	I
Dakanalo	China	I	I	I	Pulat Beludu	Malaysia	I	I	I
Daorenqiao	China	I	I	I	Menalam	Malaysia	J	J	J
Hongmang 1	China	J	J	J	Gawhtun	Myanmar	I	I	J
Guangluxuan	China	I	I	I	Ngasein	Myanmar	I	I	I
Guangluai 4	China	I	I	I	Masho	Myanmar	J	J	J
Luzhenzao 1	China	I	I	I	Dange Maruwa	Nepal	I	I	I
Mangge 136	China	J	J	J	Marsi	Nepal	J	J	J
Taichuang65	Taiwan,China	J	J	J	Dhan	Nepal	J	I	J
Herosi Bola	India	I	I	I	Red Basmati	Nepal	J	I	J
Nona Bokra	India	I	I	J	Col/Mk/Palistan	Pakistan	J	J	J
Juma	India	I	J	J	Canabongbong	Philippines	J	J	J
TKM6	India	I	J	J	Dinalaga	Philippines	J	J	J
Kasalath	India	I	I	I	Shinaba	Philippines	J	J	J
Pusur	India	I	J	J	Seenaddi	Sri Lanka	I	I	J
Surjamkhi	India	J	I	I	Niaw Dam	Thailand	I	J	J
Pangkai Kepal	Indonesia	J	J	J	Geraldine	U.S.A	J	J	J
Ketan Pitik	Indonesia	J	J	J	CPSLO	U.S.A	J	J	J
IR26	IRRI	I	I	I	Nang Dum To	Vietnam	I	I	I
IR24	IRRI	I	I	I	Ngoc Chum	Vietnam	I	J	J
IR28	IRRI	I	I	I	Nang Toi	Vietnam	I	I	I
IR29	IRRI	I	I	I					

such as Nipponbare, Akihikali, Taichuang65 etc., showed the Japonica type cpDNA. However, a few of Indica cultivars showed the Japonica type cpDNA, and some Japonica cultivars are of the Indica type cpDNA. For example, Milyang 23, an elite Indica variety of Korea derived from Japonica × Indica breeding, has Japonica type cpDNA. Dally et al. (1990) also reported that some Indica cultivars have a Japonica type cpDNA.

The relative frequecies of the Indica and Japonica type cpDNAs in the CWR strains were different among regions or countries. In the Chinese CWR the Japonica type cpDNA was more predominant. The same trend was also observed in the CWR from Bangladesh and Burma. In the Indian CWR, the frequencies of Indica and Japonica types were nearly equal. However, the Indica type cpDNA was found more frequently than the Japonica type in the CWR from other countries, such as Thailand, Malaysia, Cambodia, Sri Lanka. The above tendency is generally consistent with the results detected by Chen et al. (1993), though they used few CWR strains from China and India.

Comparison of the Indica–Japonica differentiation between nuclear, mitochondrial and chloroplast genomes in cultivated rice

The Indica–Japonica type of nuclear, mitochondrial and chloroplast genomes of 75 cultivars are presented in Table 2. Of the 75 cultivars, 24 showed the Indica type, and 32 showed the Japonica type consistently in the three different classes of genome. We defined such a type as a “homoeotype”. The strains showing the homoeotype were about 3/4 of the total cultivars, suggesting that most cultivars showed coincidence in Indica–Japonica differentiation among nuclear DNA, mtDNA and cpDNA. The proportion of homoeotypes in the Indica–Japonica type between nuclear and mitochondria, nuclear and chloroplast, and mitochondria and chloroplast, was 83%, 80% and 87% respectively.

However, Indica–Japonica differentiation was not always consistent among the three different classes of genome. For example, Rayada 7, a local cultivar from

Table 3 Genetic differentiation of nuclear mitochondrial and chloroplast genomes in common wild rice from China. Note: W: Wild-specific type; I: Indica-like type; J: Japonica-like type; NIG: National Institute of Genetics, Japan

Strain no	No. at NIG	Origin	Nuclear	mt	cp	Strain no.	Origin	Nuclear	mt	cp
WA11	W509	Unknown	J	I	J	WA115	Hunan	W	I	I
WA53	W1651	Guangxi	I	I	J	WA116	Guangxi	J	J	J
WA54	W1654	Guangxi	J	I	J	WA117	Yunnan	J	W	I
WA55	W1660	Guangxi	J	I	J	WA118	Yunnan	J	W	I
WA64	W1718	Guangdong	I	J	J	WA119	Guangxi	I	I	I
WA65	W1719	Guangdong	I	I	J	WA120	Guangxi	I	I	J
WA66	W1721	Guangdong	I	I	J	WA121	Guangxi	I	I	J
WA94	W1944	Guangxi	I	I	J	WA122	Guangxi	J	I	J
WA95	W1954	Guangxi	J	I	J	WA123	Guangxi	I	I	J
WA96	W1956	Guangxi	J	I	J	WA124	Guangxi	J	I	J
WA97	W1958	Guangxi	J	I	I	WA125	Guangdong	J	I	J
WA98	W1960	Guangxi	J	I	J	WA126	Guangdong	I	I	J
WA99	W1962	Guangxi	J	I	J	WA127	Guangxi	J	I	J
WA100	W1965	Guangxi	I	J	J	WA128	Yunnan	W	W	I
WA101	W1967	Guangxi	J	I	J	WA129	Yunnan	W	W	I
WA111		Jiangxi	W	I	J	WA130	Guangxi	J	I	J
WA113		Jiangxi	W	W	I	W38	Guangdong	J	I	J
WA114		Jiangxi	W	W	I					

Bangladesh, had the Japonica nuclear and chloroplast type, but an Indica type of mitochondria. We called such a type as a "heterotype". Almost all heterotype strains are landrace varieties. Some of them, e.g. Rayada 7, Bomota, Awasina, Dhala Aman, Muja Shail, Bonsaj and Changgang Baidao etc., were found to carry the same allele as that frequently found in common wild rice at the Est-10 locus (Wang et al. 1992). Rayada 7, Red Basmati and Surjamkhi belong to particular groups based on isozmyes (Glaszmann 1987).

Comparison of genetic differentiation between nuclear, mitochondrial and chloroplast genomes in common wild rice of China

The differentiation types of the three different classes of genome observed in Chinese CWR are presented in Table 3. It can be seen from this table that only two strains, WA116 and WA119 (both from Guangxi), showed a homoeotype in the three different classes of genome. The proportion of homoeotypes between nuclear and mitochondria, nuclear and chloroplast, and mitochondria and chloroplast, were 40%, 43% and 18% respectively, being much lower than that of cultivated rice.

It was also found that the Wild-specific type of nuclear or mtDNA was observed only in the three provinces, Jiangxi, Hunan and Yunnan, but not in the other two provinces of South China, Guangdong and Guangxi, where common wild rice is widely distributed. It seems that there are distinguishing differences between the CWR distributed in South China and other regions.

Comparison of genetic differentiation between nuclear, mitochondrial and chloroplast genomes in common wild rice of South and Southeast Asia

The genotypes of the three different classes of genome in the CWR of South and Southeast Asia are presented in Tables 4 and 5, respectively. It can be seen from Table 4 that among 40 accessions of South Asian CWR, six (15%) are homoeotypes in all three genomes, and are of the Indica type. The ratio of homoeotypes between nuclear and mitochondria, nuclear and chloroplast, mitochondria and chloroplast, were 42%, 39% and 37%, respectively.

Among 43 strains of Southeast Asian CWR, 21 (48.8%) showed homoeotypes in the three different classes of genome, with a higher proportion than those from China and South Asia. The proportion of homoeotypes between nuclear and mitochondria, nuclear and chloroplast, mitochondria and chloroplast were 63%, 70% and 65%, respectively, being also much higher than those of the Chinese and South Asian CWR. Coincidence of genetic differentiation in the three different classes of genome is higher in Southeast Asian CWR than in the Chinese and South Asian CWR. It was also found that the CWR strains of Thailand have a higher proportion (76%) of homoeotypes in the three genomes, being similar to that of cultivated rice.

Clustering of the CWR and cultivated rice based on the genetic differentiation of the three different classes of genome

Classification of CWR and cultivars were considered separately for *O.sativa* and three geographical groups of CWR (Fig. 3). Of the three genomes, the genes in the nuclear genome might play a principal role in the growth and development of rice. Thus, we classified the CWR strains and cultivars into different main groups, firstly based on the genetic differentiation of the nuclear genome and then subdivided by cytotypes. According to the genetic differentiation of nuclear genomes, cultivated rice was classified into two major groups, the Indica and Japonica groups. Considering their cytoplasmic type they were further classified into three Indica and four Japonica sub-groups, respectively. Of the seven types, $I_nI_mI_c$ and

Table 4 Genetic differentiation of nuclear mitochondrial and chloroplast genomes in common wild rice from South Asia. Notes as in Fig. 3

Strain no.	No. at NIG	Origin	Nuclear	mt	cp	Strain no.	No. at NIG	Origin	Nuclear	mt	cp
WA1	W106	India	I	I	I	WA103	W1983	India	I	W	I
WA2	W107	India	I	W	I	WA104	W1987	India	I	J	J
WA3	W120	India	W	I	J	WA105	W2000	India	W	I	J
WA4	W130	India	I	I	J	WA106	W2001	India	I	I	J
WA5	W136	India	W	I	I	WA107	W2003	India	I	I	J
WA6	W139	India	W	I	I	WA108	W2004	India	W	I	J
WA35	W1084	India	I	I	I	WA109	W2011	India	W	I	J
WA36	W1090	India	I	I	I	WA7	W144	Sri Lanka	I	W	I
AW56	W1670	India	I	W	I	WA12	W555	Sri Lanka	I	I	I
WA57	W1677	India	I	W	I	WA37	W1161	Sri Lanka	W	W	I
WA58	W1680	India	I	W	I	WA79	W1803	Sri Lanka	I	I	I
WA59	W1681	India	I	W	I	WA80	W1807	Sri Lanka	W	W	I
WA68	W1737	India	I	W	I	WA81	W1810	Sri Lanka	I	I	I
WA69	W1741	India	I	I	J	WA82	W1811	Sri Lanka	W	I	I
WA70	W1750	India	I	W	I	W78	W1802	Bangladesh	I	I	J
WA71	W1753	India	I	W	I	WA83	W1818	Bangladesh	I	W	J
WA72	W1757	India	I	I	J	WA84	W1820	Bangladesh	I	I	J
WA73	W1764	India	I	I	J	WA85	W1821	Bangladesh	I	I	J
WA74	W1769	India	I	I	J	WA86	W1822	Bangladesh	I	I	J
WA75	W1781	India	W	I	I	WA87	W1823	Bangladesh	I	W	I

Table 5 Genetic differentiation of nuclear mitochondrial and chloroplast genomes in common wild rice from Southeast Asia. Notes as in Fig. 3

Strain no.	No. at NIG	Origin	Nuclear	mt	cp	Strain no.	No. at NIG	Origin	Nuclear	mt	cp
WA8	W145	Thailand	I	I	I	WA17	W574	Malaysia	I	I	I
WA9	W168	Thailand	I	W	I	WA18	W587	Malaysia	I	I	J
WA10	W234	Thailand	I	I	I	WA19	W589	Malaysia	I	I	I
WA13	W556	Thailand	I	I	I	WA20	W593	Malaysia	W	I	I
WA45	W1546	Thailand	I	I	I	WA21	W595	Malaysia	I	I	I
WA46	W1619	Thailand	I	I	J	WA22	W596	Malaysia	W	I	J
WA60	W1690	Thailand	I	W	I	WA24	W610	Burma	I	I	J
WA61	W1695	Thailand	I	W	I	WA25	W621	Burma	W	I	J
WA62	W1698	Thailand	I	I	I	WA26	W623	Burma	W	I	J
WA63	W1699	Thailand	I	I	I	WA27	W625	Burma	W	I	J
WA67	W1729	Thailand	I	I	I	WA28	W626	Burma	I	W	I
WA76	W1794	Thailand	I	I	I	WA29	W627	Burma	I	W	I
WA89	W1860	Thailand	I	I	I	WA30	W629	Burma	W	I	J
WA90	W1863	Thailand	I	I	I	WA31	W630	Burma	I	I	J
WA91	W1965	Thailand	I	I	I	WA32	W633	Burma	I	I	J
WA92	W1866	Thailand	I	I	I	WA33	W635	Burma	I	I	J
WA93	W1904	Thailand	I	I	I	WA34	W638	Burma	I	W	I
WA14	W557	Cambodia	I	I	I	WA110	W2036	Burma	J	I	J
WA15	W558	Cambodia	I	I	I	WA39	W1294	Philippines	W	J	J
WA16	W559	Cambodia	J	J	J	WA38	W1292	Indonesia	I	I	I
WA40	W1295	Cambodia	I	I	I	WA102	W1976	Indonesia	I	J	J
WA77	W1800	Cambodia	I	W	I						

$J_nJ_mJ_c$ are major types indicating that the Indica–Japonica differentiation is of major importance in cultivated rice.

For the Chinese CWR, the 35 strains were classified into three groups, Indica-like, Japonica-like and Wild-specific group. The Wild-specific group contains three types with different cytotypes, $W_nW_mI_c$, $W_nI_mI_c$ and $W_nI_mJ_c$. The Indica-like and Japonica-like groups could be further classified into three and four types, respectively. Among the ten types found in Chinese CWR, $J_nJ_mJ_c$ is a major type followed by the type of $I_nI_mJ_c$.

In the South Asian CWR, the 40 strains were classified firstly into the Wild-specific group and the Indica-like group. The Wild-specific group included two types, and the Indica-like group contains five types in which $I_nI_mJ_c$ and $I_nW_mI_c$ were the two major types.

The 43 strains of Southeast Asia CWR were divided into Wild-specific, Indica-like and Japonica-like Groups. Under these three groups, nine types were recognized where $I_nI_mI_c$ is the major type in Southeast CWR.

From the above results, it is obvious that the genomic type in common wild rice is much-more diversified than that of cultivated rice. All the types observed in the latter could be detected in the former. In contrast, many types detected in CWR could not be found in cultivated rice.

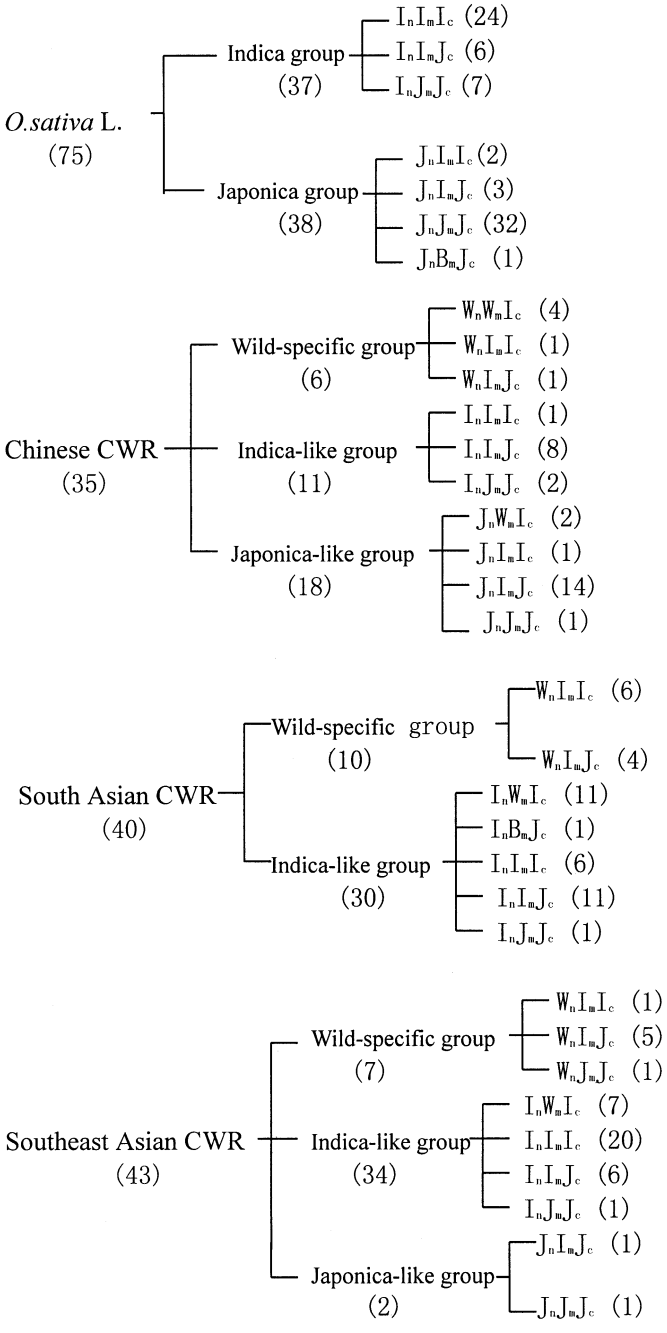

Fig. 3 Classification of the CWR and cultivated rice based on the Indica–Japonica differentiation of the three genomes

This suggests that the genetic diversity had been decreased during the course of evolution from wild rice to cultivated rice.

Comparison of the genomic types between the geographical groups of CWR, demonstrated that the Chinese CWR possesses more variable types than South and Southeast Asian CWR. This is consistent with our previous results that the Chinese CWR strains have a higher genetic diversity than those of other Asian countries (Sun et al. 2001).

Interestingly, among the 121 strains of Indica-type mtDNA, both Indica- and Japonica-type cpDNAs were detected. On the other hand, for the 46 strains of Japonica-type mtDNA, all possessed Japonica-type cpDNA. Namely, for the Indica–Japonica type of cytoplasmic organelles only three types were found, I_mI_c (Indica mtDNA and Indica cpDNA), I_mJ_c (Indica mtDNA and Japonica cpDNA) and J_mJ_c (Japonica mtDNA and Japonica cpDNA). The J_mI_c type (Japonica mtDNA and Indica cpDNA) could not be observed in the CWR strains and cultivars used in the study.

Discussion

Comparison of genetic diversity of the three different classes of genome between common wild rice and cultivated rice

The nucleus, mitochondria and chloroplast are the three different classes of genome found in the cells of plants. They contain their own genomes, and can be autonomously replicating and independent in inheritance. Genetic information in the nucleus is inherited from both parents, whereas those of mitochondria and chloroplasts are usually inherited maternally.

Contrary to the results of Wang et al. (1992), who classified the common wild rice into two groups based on nuclear-DNA RFLPs, the present study indicated that 118 strains of common wild rice were classified into four groups in the nuclear-genome type. Among the four groups, Group n I and Group n II corresponded to Indica and Japonica, and both accompanied CWR with the same nuclear DNA types, respectively, whereas Group n III and Group n IV consisted of only CWR. That discrepancy between their results and ours is probably because they examined only 17 strains of common wild rice with 25 RFLP markers.

The mitochondrial genomes of the strains examined were clustered into five groups. Group m I and Group m II corresponded to Indica and Japonica types, both including cultivars and CWR strains, respectively. Ishii et al. (1993) grouped ten strains, including two Japonica, two Javanica, four Indica and two *Oryza glaberrima*, into four main clusters. Although in their study the genetic distances between clusters were small, variation between the Indica and Japonica cultivars was also observed. In our study, however, two different types specially observed in common wild rice were identified.

The chloropast genomes of our materials were classified into two types, Indica type and Japonica type, which correspond to type 3 and type 1 of Ishii et al. 1986, 1988, 1993), and plastotype e1 and a1 of Dally et al. (1990).

In the combination of nuclear, mitochondrial and chloroplastic DNA types, seven types were detected in cultivated rice, with a total of 16 different types in CWR (Fig. 3). Morishima (1997) and the present authors (Sun et al. 2001) demonstrated that allele numbers in cultivated rice is about 60% of that in CWR in terms of isozyme

Table 6 Average genetic distances within and between species based on the nuclear and mitochondrial genomes differentiation

Genetic distance	Average genetic distance	
	Nuclear DNA	mtDNA
Within all strains	0.4057	0.2384
Within *O.rufipogon*	0.4242	0.2608
Within*O.sativa*	0.3376	0.1460
Between *O.rufipogon* and *O.sativa*	0.4104	0.2497

and DNA markers respectively. These results verified that the genetic diversity has decreased with domestication, and some alleles were lost through natural and human selection, not only in the nucleus but also in cytoplasmic organelles.

The homoeotype and heterotype of the genetic differentiation of the three different classes of genome were observed both in CWR and cultivated rice. The proportion of homoeotypes was higher in cultivated rice than in CWR. This suggests that the coincidence of genetic differentiation among the three different classes of genome has increased with domestication. The proportion of homoeotypes of CWR strains from China, South Asia and Southeast Asia was 5.7%, 15% and 48.8%, respectively. This seems to indicate that the CWR from Southeast Asia is nearer to cultivation than the CWR from China and South Asia.

Comparison between the mitochondrial and nuclear genome differentiation

Genetic distance is independent of genome size, and gives an estimation of the rate of base change per site. The average genetic distances (Table 6) within all strains for nuclear genomes is much larger than that for mitochondral genomes. This suggests that the evolutionary variability of the nuclear genomes is higher than that of the mitochondrial genomes. Wolf et al. (1987) also reported that the mutation rate of the mitochondrial genomes is lower than that of nuclear genomes. However, Ishii et al. (1993) concluded that the evolutionary variabilities of the mitochondrial and nuclear genomes were almost the same, based on the genetic distances among the ten cultivars of *O.sativa* and *O.glaberrima*. The discrepancy between our findings and the results of Ishii et al. (1993) might be attributable to a small number of cultivars and a small number of loci used by them.

Whether or not the common wild rice has differentiated into the Indica and Japonica type like cultivars?

It is important to confirm whether or not the wild progenitors of cultivated rice, the common wild rice, have differentiated into the Indica and Japonica type like cultivars. As early as 1948, Chou proposed that the Keng (Japonica) varieties originated in China from the Keng-type wild rice found in Tsaohu Lake, Anhui Province, and the Hsien (Indica) varieties originated in some place outside China, probably in India. Later Second (1982, 1985a) advocated that CWR has differentiated into Indica and Japonica types through the study of isozyme variations, and proposed a diphyletic hypothesis for the origin of Indica–Japonica. More recently the hypothesis was supported by the genetic variation of chloroplast DNA and ribosomal DNA in the CWR and cultivated rice (Ishii et al. 1988; Dally and Second 1990; Sano and Sano 1990). On the other hand, Oka and Chang (1962) argued in their earlier study that the Asian CWR has not differentiated into the Indica and Japonica types. Their later results (Morishima and Gadrinab 1987), by a survey of many strains of CWR with regard to potassium chlorate resistance, low-temperature tolerance and isozymes, demonstrated that in some character combinations CWR tends to differentiate into Indica and Japonica types, especially in Chinese wild rice. Indeed, both the Indica type and the Japonica type of nuclear, mitochondria, and chloroplast genomes were found in the present study. But among 118 CWR strains, those in which nuclear DNA, mtDNA and cpDNA showed consistent differentiation, into either Indica-like or Japonica-like types, involved only 40 strains. The proportion of homoeotypes (essentially $I_nI_mI_c$) of CWR was only 34%, while that in cultivated rice was about 75%. These results showed that the degree of Indica–Japonica differentiation in CWR is much less than that in cultivated rice.

The difference in the Indica–Japonica differentiation between common wild rice from China and other countries in Asia has been discussed by Second (1985a) and Wang et al. (1992). They reported that Chinese CWR was similar to the Japonica-type, while the CWR from South and Southeast Asia were similar to the Indica type judging from isozyme and RFLP markers. Sano and Sano (1990) examined the variation of the intergenic spacer region of ribosomal DNA and demonstrated that CWR from Jiangxi Province, China, was Japonica-inclined. The results of the present study showed that Chinese CWR contained the Indica-like type besides the Japonica-like type, not only in the nuclear genome but also in the mitochondrial and chloroplast genomes. Undoubtedly, the Chinese CWR used in present study is more representative than that used by the above researchers. Consequently, we conclude that the CWR of South and Southeast Asia differentiated into mainly the Indica-like type, while the Chinese CWR differentiated into both the Indica-like and the Japonica-like type.

Inference on the origin and domestication of cultivated rice viewed from the Indica–Japonica differentiation of common wild rice

Regarding the origin of the Indica–Japonica differentiation, three main hypotheses were proposed in China as reviewed by Oka (1988). First, the hypothesis of Ting (1957, 1961) assumes that Hsien rice (Indica) was devel-

oped from the common wild rice in Southern China and that the Keng rice (Japonica) differentiated from Hsien later. The second hypothesis is the view presented by Wang et al. (1984). It assumes that the wild rice, domesticated in marshy lowlands, has become Hsien (Indica) and that in upland fields has become Keng (Japonica). The third hypothesis, a diphyletic one, was proposed by Chou (1948), as described above.

Oka (1988) thought that the three hypotheses were all intuitive speculations and were not supported by enough scientific evidence. The results of the recent studies revealed that Indica–Japonica differentiation occurred not only in the nuclear genome but also in the cytoplasmic genomes of common wild rice. This seems to support the hypothesis of a diphyletic origin. In the present study, however, among the 118 strains of CWR, 86 showed the Indica-like type mitochondria, whereas only seven strains showed the Japonica-like type. In cultivated rice, the frequency of the Indica and Japonica type of the mitochondrial genome is nearly equal. Moreover, among the strains of Indica mtDNA, both the Indica and Japonica type of cpDNA were detected, whereas in the stains of Japonica mtDNA only the Japonica type of cpDNA was observed. These results suggest that mitochondria of the Indica type might be more primitive than those of the Japonica type, or else that the mitochondria of the ancestral species domesticated into the Indica type earlier than the Japonica type. This evidence supports the first hypothesis proposed by Ting (1957, 1961).

To have a deeper understanding of the origin of the Indica–Japonica differentiation in Asian rice, an integration of the bio-archaeology and the DNA study is required. We are currently investigating the variation of DNA of the carbonized rice from different ages and neolithic sites of China.

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