Theor Appl Genet (2002) 105:608–613
DOI 10.1007/s00122-002-0898-0

R. Ishikawa · I. Nakamura · T. Nishihara · M. Kikuchi
M. Senda · S. Akada · T. Harada · M. Niizeki

Origin of cytoplasm substituted rice cultivars found in Japan

Received: 1 October 2001 / Accepted: 12 December 2001 / Published online: 23 May 2002

Abstract Genetic variation of Japanese rice cultivars were examined. Five of 450 lowland cultivars and another five of 200 upland cultivars were determined as the *indica* type by using isozyme genotypes and the remainder were of the *japonica* type. The major characteristics of these *indica* cultivars, revealed a slender shape of grains, a short apiculus hair length, a positive allele for *Ph* reaction, and allele-3 for the *Pgd1* locus. Three of these *indica* cultivars showed a non-deletion ORF100, which is essential to the *japonica*-type plastid. The plastid subtype identity (PS-ID) sequences of these plastids is 6C7A, which is also a *japonica*-specific repeat unit. Thus, these cultivars were concluded to be naturally generated cytoplasm substituted lines. These plastids were introduced into a *indica* genetic background from *japonica* cultivars grown elsewhere. The rest of the *indica* cultivars revealed a deletion-type ORF100 and plastid subtype 8C8A, both of which are *indica*-specific. These cultivars carried *indica*-type allelic constitutions for diagnostic isozyme loci. However, other characters were identical to the cytoplasm-substituted cultivars in Japan. In East and Southeast Asia, cultivars carrying a *indica*-type nuclear genotype with a *japonica*-type plastid are restricted to Aus cultivars in the Bengal region. Genetic and historical records suggest that Japanese *indica* cultivars and the Aus cultivars are closely related. The Aus cultivars acquire necessary genetic constitutions from both *indica* and *japonica* cultivars through naturally occurring out-crossing to adapt to a particular cultivation condition in the region. The wide adaptability enabled them to be introduced into a northern region like Japan.

Keywords Nuclear genotype · Non-coding cpDNA · *Oryza sativa* L. · Adaptability

Communicated by R. Hagemann

R. Ishikawa (✉) · T. Nishihara · M. Kikuchi · T. Harada
M. Niizeki
Laboratory of Plant Breeding and Genetics, Hirosaki University,
Hirosaki 036-8561, Japan
e-mail: ishikawa@cc.hirosaki-u.ac.jp
Fax: +81-172-39-3750

M. Senda · S. Akada
Gene research Center, Hirosaki University,
Hirosaki 036-8561, Japan

I. Nakamura
Laboratory of Plant Cell Technology, Department of Horticulture,
Graduate School of Science and Technology, Chiba University,
Matudo 271-0092, Japan

Introduction

Asian cultivated rice plants (*Oryza sativa* L.) were mainly grouped into two types, subspecies *indica* and *japonica*, by hybrid fertility and other characters (Kato et al. 1928). Terao and Mizushima (1939), however, could only distinguish the major two groups by using hybrid sterility. The sterility relationships were too complicated to recognize the two types, because there is no tight reproductive barrier and no geographical barrier between these two subspecies. Oka (1953) combined several characters to classify Asian cultivars into these two categories. Then, he recognized the two varietal groups and found that the *japonica* type could be sub-grouped into temperate and tropical types. Several characters were found to be concerned with genetic differentiation. But none of them was a unique character distinguishing them completely. Thus, several characters were statistically formulated to recognize the differentiation between these varietal types (Morishima and Oka 1981). As these differentiations were also detected with enzyme variation by electrophoresis (Chu 1967; Shahi et al. 1969; Pai et al. 1975), such biochemical markers were preferred to recognize the *indica* and *japonica* types because the markers accurately reflected their nuclear compositions (Sano and Morishima 1992). This method was also applied to evaluate the genetic resources of Japanese lowland and upland cultivars (Ishikawa et al. 1991, 1992). In our former report (Ishikawa et al. 1992), a few *indica* cultivars could be recognized, based on their isozyme genotype. Additional *indica* cultivars were found in this report and further analyzed their uniqueness not

only at the nuclear level, but also at the cytoplasmic level. We also discussed their wide adaptability.

Linker sequences between rice plastid genes *rpl16* and *rpl14* are used as cytoplasmic markers with the later-described polymorphic region of ORF100. The linker sequences are moderately variable in comparison with stable plastid sequences in higher plants, but could still be amplified by using a single pair of primers (Nakamura et al. 1997). The sequence identities were compared and used to distinguish plastid subtype-identity (PS-ID) sequences. The PS-ID sequences corresponded to differences between *indica* and *japonica* cultivars, and also between tropical and temperate *japonica* cultivars at the cytoplasmic level. CA repeats existing in the linker sequences are polymorphic in *sativa* species. Polymorhic PS-ID sequences were described as mCnA (m and n are the number of the repeats in the sequence). But plastid subtypes 6C7A and 7C6A were only detected in *japonica* cultivars. Three types, revealed as 7C7A, 8C8A and 9C7A, were known as *indica*-specific plastid subtypes and are consistent with a deletion in ORF100 region of each plastid genome; but *japonica*-specific plastid subtypes do not have the deletion (Nakamura et al. 1998). Both markers enabled us to analyze maternal lines of rice plants. In addition to the plastid sequences, data from the nuclear genotypes were combined with data of the cytoplasmic genotypes. The combined data show us how particular populations were formed and introduced into other countries. Also, this information show us how rice acquired wide adaptability to be dispersed from tropical areas to subarctic zones.

Materials and methods

Plant materials

Four hundred and fifty lowland cultivars and 200 upland cultivars in Japan were examined in this report. Two hundred and sixty eight lowland and 66 upland cultivars were examined for their isozyme genotypes in our previous reports (Ishikawa et al. 1991, 1992), which described genotypic variation in Japanese lowland and upland populations. In the present report, additional *indica* cultivars were screened in order to compare them to Asian *indica* cultivars obtained from the National Institute of Genetics, Japan. Thirty six Aus cultivars were also examined in this report. Aus cultivars are known as *indica* type and are cultivated in the summer season in Bangladesh and the west Bengal region of India, which is sympatric with the Aman cultivars. Aus cultivars are grown from spring and harvested before the flood season at the end of summer because of their photoperiodic insensitivity. The isozyme genotypes of Aus cultivars are different from those of Aman cultivars, which are photoperiod sensitive (Glaszmann 1987). The two groups of Aus and Aman cultivars are also known as ecospecies based on their different biological habits (Morinaga 1968).

Morphological and physiological traits

Apiculus hair length was measured by using a microscope. Ten apiculus hairs on three hulls were averaged. The length and width of ten hulls were measured for each cultivar. Each length was averaged and a ratio of hull length to width was calculated as the L/W ratio. Hulls were soaked in 1.5% phenol solution for 3 h and dried. Cultivars of hulls which turned to black were scored as positive for the phenol reaction (Ph^+). Cultivars of the alternative type showed an unchanged color of the hulls which were scored as negative for the phenol reaction (Ph^-). This character is regulated by a*Ph* gene on chromosome 4.

Isozymes

Genotypes of cultivars were isolated for 17 genes, *Acp1*, *Adh1*, *Amp1*, *Amp2*, *Amp3*, *Cat1*, *Est2*, *Est5*, *Est9*, *Mal1*, *Pgd1*, *Pgd2*, *Pgi1*, *Pgi2*, *Pgi3*, *Pox2* and *Sdh1* from ten isozyme species, to examine genotypic variation in Japanese lowland and upland cultivars. Seeds were germinated at 30 °C in the dark. Crude extracts of 3-day old seedlings were used for isozyme analysis as described by Ishikawa et al. (1991). Seven isozyme loci, *Acp1*, *Amp2*, *Cat1*, *Est2*, *Pgi1*, *Pgi2* and *Pox2*, were examined to determine the genotypes of each cultivar. Discriminant (D) scores given to alleles for these loci, which are used to classify each cultivar into *indica* or *japonica* types, were averaged, as described in our previous report (Ishikawa et al. 1991). Cultivars carrying scores from 0.0 to 0.4 were regarded as *japonica* and those carrying scores from 0.6 to 1.0 as *indica*. Some of these genotypes had already been determined by our previous reports (Ishikawa et al. 1991, 1992). The others were done in this report. In total, 450 lowland and 200 upland cultivars were examined.

PCR and DNA sequencing

Total genomic DNA was isolated with the CTAB method from 100 mg of leaf tissues. PCR amplification was performed with *Taq* DNA polymerase (Promega Co.), the company's supplied buffer, 0.2 mM of dNTPs, 2 mM of $MgCl_2$ in a final concentration, and one pair of primers, 5Psk (AAAGATCTAGATTTCGTAAACAACATAGAGGAAGAA) and 3P(ATCTGCAGCATTTAAAAGGGTCTGAGGTTGATCAT) (Nakamura et al. 1997). These primers can amplify PS-ID sequences from dicot and monocot plants. Additional oligomers, whose sequence is complementary to the labeled primer for sequencing, were added to the 5P primer for direct sequencing. After 3-min heat treatment at 94 °C, 45 cycles (98 °C for 10 s, 55 °C for 30 s and 72 °C for 1 min) were used in the amplification, followed by 72 °C for 5 min as a post-treatment. From the PCR products, excess primers and free nucleotides were removed with a QIAGEN PCR purification kit according to the company's recommendations. Then, the purified PCR product was used as a template-DNA for sequencing. The LICOR 4200S sequencer was used with a SequiTherm Excel II (EPICETRE TECHNOLOGIES Co.) sequence kit. A IRD41 labeled-primer was used in the sequencing reaction.

The deletion in the ORF 100 region was checked by PCR reactions. The genotypes of plastid DNA were characterized by PCR amplification using the primer sets, #3 (AGTCCACTCAGCCATCT) and #4 (CTCGGCCATCATTTTCTTCTTTAG), which amplify the ORF100 region in rice plastid DNA (Kanno et al. 1993). Most of the *indica* cultivars possess a 69-bp deletion in this region, while most of the *japonica* cultivars do not. This region has been considered to be an effective marker for *indica-japonica* differentiation (Chen et al. 1993). After PCR amplification, the products were electrophoresed in a 1.5% agarose gel. *Takara Taq* DNA polymerase (Takara Co.) was used with the company's supplied reaction buffer, 0.2 mM of dNTPs and 5 mM of $MgCl_2$. High Mg concentration inhibited the amplification of additional bands for unknown reasons.

Results

Distinctive *indica* cultivars in Japan

Lowland and upland cultivars were examined for 17 isozyme loci. More than 80% lowland cultivars revealed

Table 1 Genotypic variation of 450 lowland and 200 upland cultivars in Japan

Locus	Genotypes																															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Acp1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	1	1	1	1	2	1	1	1
Adh1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Amp1	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Amp2	1	1	2	3	1	1	1	1	1	1	1	2	1	1	2	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	1	2
Amp3	1	1	1	1	3	1	1	1	1	1	1	1	2	1	2	1	1	1	1	1	2	2	2	1	1	1	3	1	3	1	2	1	1
Cat1	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2	1	1	1	2	2	2	1	1	1	1	1	2	1	1	1	1
Est2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	1	1	2	0	0	0	2	0	2	1	2	2
Est5	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Est9	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	2	1	2	1	2	1	1	1	1	1
Mal1	1	1	1	1	1	1	1	1	2	1	1	2	1	2	1	1	2	1	2	1	1	2	1	2	2	2	1	2	2	2	2	2	2
Pgd1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	1	3	3
Pgd2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Pgi1	2	2	2	2	2	2	2	2	2	0	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	0	1	1	1	1	1	1	1
Pgi2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	1	2	2
Pgi3	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1
Pox2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	0	0
Sdh1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Total no.																																	
Lowland	363	4	16	2	2	1	1	1	49	1	2	1	1	1	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0
Upland	23	0	0	0	0	2	0	0	0	0	0	0	0	0	1	77	41	34	4	1	8	3	1	0	0	0	0	0	1	1	1	1	1

Table 2 Frequency distribution of D-scored cultivars in Japan

Population	Total	No. of D-scored cultivars				
		0.0–0.2	0.2–0.4	0.4–0.6	0.6–0.8	0.8–1.0
Lowland	450	424	21	0	2	3
Upland	200	152	43	0	3	2

Table 3 Morphological and physiological characteristics of Japanese cultivars

Population	I/J[a]	*Pgd1*[b]	Total of cultivars	APH[c]	L/W[d]	Ph[e]	
						+	–
Lowland	J	1	445	0.72 ± 0.19	2.09 ± 0.34	32	413
	I	3	5	0.37 ± 0.12	2.79 ± 0.16	4	1
Upland	J	1	26	0.65 ± 0.12	2.14 ± 0.16	5	21
	J	2	169	0.44 ± 0.14	2.38 ± 0.21	131	38
	I	1/3	5	0.34 ± 0.13	2.93 ± 0.09	4	1

[a] I/J indicates the classification of *indica* (I) or *japonica* (J) types based on nuclear markers
[b] Allelic type of phosphoguluconate dehydrogenase are represented; 1/3 means that one cultivar revealed allele 1 but others revealed allele 3
[c] Length of averaged apiculus hairs revealed in mm
[d] Ratio of hull length and width
[e] Reaction of phenol absorption. + and – are positive and negative respectively

single genotypes Table 1. Others showed different genotypes at a few loci. Five cultivars showed different genotypes at multiple loci. The predominant genotype in the lowland cultivars was also found in the 200 upland cultivars. However, most upland cultivars possess the *Pgd1*^{3} allele which has never been found in the lowland cultivars. We also found another five cultivars carrying a different genotype at multiple loci in the upland cultivars.

Seven diagnostic loci out of the 17 loci were used to classify these cultivars into *indica* or *japonica* types. Five of the lowland cultivars were identified as *indica* type (Table 2). Another five of the 200 Japanese upland cultivars were also classified as *indica* type. The genotypes of these *indica* cultivars were not identical and were extremely varied.

The Japanese rice cultivars were roughly divided into three groups with the genotype of *Pgd1*. *Pgd1*^{1} as the predominant type in the lowland cultivars and *Pgd1*^{2} in the upland cultivars. *Pgd1*^{3} is specific to the *indica* cultivars found in Japan. The ten *indica* cultivars carry the *Pgd1*^{3} allele except for one cultivar. This allele is rarely found in Asian *indica* cultivars based on the results of our previous report (Ishikawa et al. 1991). In addition to the difference of the isozyme genotype, the two *japonica* and *indica* groups can be distinguished by morphological and physiological characters. The *indica* cultivars were characterized by a short apiculus hair length, a slender hull, and a positive allele for the phenol reaction of the hulls (Table 3). All but one revealed a red pericarp (Table 4). Although upland cultivars carrying the *Pgd1*^{2} allele tend to show a positive phenol reaction, which is a specific character for the *indica* type, they were classified into the *japonica* type based on isozyme genotypes.

Plastid subtype-identity (PS-ID) sequences were examined for 50 of the randomly chosen lowland cultivars and ten cultivars were classified as *indica* type. Three of the ten *indica* cultivars revealed the plastid subtype 6C7A (Fig. 1). Others revealed the plastid subtype 8C8A which is a *indica*-specific PS-ID sequence (Table 4). To confirm the plastid subtype, the ORF100 region was amplified by the PCR reaction, a deletion of which is

Table 4 Morphological and physiological characteristics of ten cultivars classified into *indica* type based on their nuclear genotypes

Population	Cultivar name	ORF100[a]	PS-ID[b]	Color of pericarp	*Pgd1*	APH	L/W	Ph[c]
Lowland	Kagoshimazairai	ND	6C7A	Red	3	0.42	2.68	+
	Karahoushi	ND	6C7A	Red	3	0.23	2.62	+
	J278	D	8C8A	Red	3	0.55	2.73	–
	J328	D	8C8A	Red	3	0.34	2.93	+
	J393	D	8C8A	Red	3	0.33	3.00	+
Upland	Up9	ND	6C7A	Red	3	0.25	3.07	+
	Up10	D	8C8A	Red	3	0.34	3.00	+
	Up74	D	8C8A	Red	3	0.47	2.86	+
	Up77	D	8C8A	White	1	0.18	2.89	+
	Up170	D	8C8A	Red	3	0.48	2.86	+

[a] ND, D indicated non-deletion (ND) and deletion (D) types of ORF100. ND corresponds to the *japonica*-type plastid
[b] PS-ID indicated plastid subtype-identity sequence. Repeats units were indicated as 6C7A and 8C8A. These units represented six C and seven A repeats, or eight C and eight A repeats, respectively
[c] Reaction of phenol absorption. + and – are positive and negative reactions, respectively

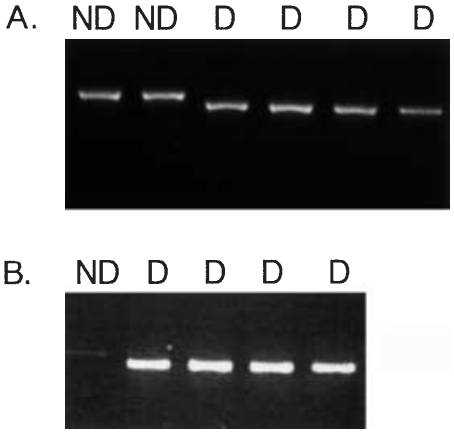

Fig. 1 Different plastid types detecting by size of ORF region. ND and D indicated non-deletion and deletion type plastids, respectively. Panel A showed lowland cultivars. In lanes from left ro right, PCR products from Kagoshimazairai, Karahoushi, J278, J328, J393, and L32 were loaded. Panel B showed upland cultivars. In lanes from left ro right, PCR products from Up9, Up10, Up74, Up77, and Up170 were loaded. ND type PCR products were larger than those of D type

Table 5 Nuclear-cytoplasmic combinations detected in Japanese rice cultivars

Population	I/J[a]	PS-ID	
		6C7A	8C8A
Lowland	J	49	1
Lowland	I	2	3
Upland	I	1	4

[a] I/J indicate the classification of *indica* (I) or *japonica* (J) types based on nuclear markers

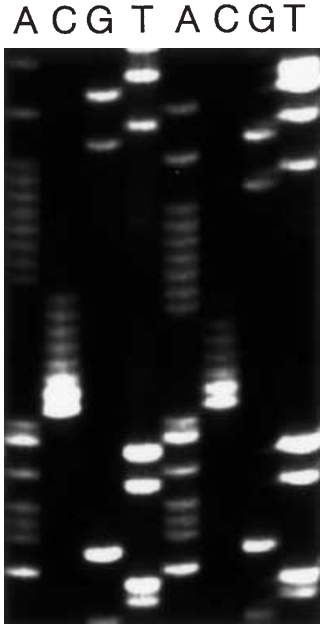

Fig. 2 Detection of different PS-ID sequences. First four lanes showed the sequence of a plastid subtype, 8C8A. Next four lanes showed the sequence of a plastid 6C7A. After C repeats, A repeats can be found

specific for the plastid subtype 8C8A. All cultivars carrying the plastid subtype 8C8A revealed smaller products (deletion type) than those (non-deletion type) amplified from the 6C7A-type cultivars. Thus, these polymorphic plastid subtypes are not due to mutation of the plastid sequence, but to chloroplast substitution that occurred in the *indica* cultivars. An additional *indica* cultivar from Japan, Toboshi, was added to the experimental material, which belongs to the Daitou-mai group with Karahoushi (Table 4). The Daitou-mai group was characterized by a red pericarp and slender-grain cultivars. Karahoushi is a typical one included in the group. Toboshi also carried the *indica* nuclear genotype with the plastid subtype 6C7A, i.e. the *japonica*-type cytoplasm (data not shown).

Among 50 lowland cultivars, 49 revealed *japonica*-type nuclear genotypes with the *japonica*-specific plastid subtype 6C7A (Table 5). The remaining one, lowland *japonica* cultivar L32, revealed an *indica*-specific plastid subtype, 8C8A, and the deletion type ORF100 (Fig. 2). L32 possessed *japonica*-type nuclear genotypes for the isozyme loci examined. Such cytoplasm-substituted cultivars were rare in the lowland cultivars, because 49

Table 6 Nuclear-cytoplasmic combination among *indica* cultivars in southeast Asian countries

Country	Total	PS-ID – *Pgd1* genotype					
		6C7A-allele 1	6C7A-allele 2	6C7A-allele 3	8C8A-allele 1	8C8A-allele 2	8C8A-allele 3
Chinese	7	0	0	0	3	0	4
Taiwan	4	0	0	0	0	0	4
India	14	5	1	1	3	3	1

Table 7 Combination of phenol reaction and ORF100 types in Aus cultivars

Phenol reaction[a]	Total no. cultivars	ORF100[b]	
		D	ND
Ph^+	17	6	11
Ph^-	19	14	5

[a] Reaction of phenol absorption; + and – are the positive and negative reactions
[b] ND, D indicate the non-deletion (ND) and deletion (D) types of ORF100. ND corresponds to the *japonica*-type plastid

out of the 50 examined for PS-ID sequences revealed the plastid subtype 6C7A. And there were none in the 80 Japanese upland cultivars examined in our previous report (data not shown).

Comparison among Asian cultivars

Asian *indica* cultivars were also examined for their nuclear isozyme genotype at the *Pgd1* locus and their plastid sequence. Twenty five *indica* cultivars were selected from three countries, some of which carry $Pgd1^3$ which is the common allele to Japanese *indica* cultivars. All cultivars in China and Taiwan carried the plastid subtype 8C8A (Table 6). Seven of 14 Indian cultivars carried plastid subtype 6C7A. One Aus cultivar was included into the cytoplasm-substituted cultivars. Thirty six other Aus cultivars were examined for their morphological and physiological characteristics, and also their plastid type. The data showed extremely wide diversity. L/W ratios ranged from 1.92 to 3.37 (average±SD = 2.52±0.29) and APH ranged from 0.19 to 0.49 mm (0.32±0.07 mm). The most-prominent characteristic was observed for the phenol reaction. Out of 36 cultivars examined, 19 showed Ph^-. The remaining 17 cultivars showed Ph^+, although all Aus cultivars were classified into the *indica* type based on their isozyme genotypes. Although Ph^+ is usually consistent with the deletion type of ORF100 in the usual *indica* cultivars, Aus cultivars revealed various combinations for the phenol reaction and the ORF100 type. Five of 19 Ph^- cultivars and 11 of 17Ph^+ cultivars carried the non-deletion type ORF100, which is a unique characteristic of *japonica*-type plastids (Table 7). This would result from out-crossing between the *indica* and *japonica* cultivars which happened in the past.

Discussion

Japanese cultivars classified into the *indica* type belong to the Daitou-mai group, based on their cultivar name (Arashi 1974; Ishikawa et al. 1991). Usually, *indica* cultivars are adapted to tropical and sub-tropical zones with high photoperiod sensitivity. However, the Japanese *indica* cultivars showed weak photoperiod sensitivity with other distinctive characters, in contrast to other *indica* cultivars in Asian countries. Arashi (1974) suggested that historical records indicated that these Daitou-mai cultivars had originated from an ancient nation; Champa existed in the middle part of current Vietnam. Karahoushi and Toboshi both belonging to the Daitou-mai group, and was found to carry the *indica*-type nuclear genotypes with *japonica*-type plastids in this report. The PS-ID sequences of these cultivars were 6C7A. The Daitou-mai group consists of particular cytoplasm-substituted cultivars (6C7A non-Del type ORF100) in high frequency. Such cytoplasm-substituted cultivars are unusual. Our previous report (Ishikawa et al. 2001) showed only one out of 50 lowland and 80 upland cultivars in Japan revealed such a substitution. As to the nuclear genotypes of the Japanese *indica* cultivars, all but one were characterized as $Pgd1^3$, with a red pericarp, a slender hull, and a short apiculus hair length. Japanese cytolpasm-substituted cultivars seem to have originated from the same population, because cultivars carrying $Pgd1^3$ are rarely found in Asian countries (Ishikawa et al. 1991). As far as we examined there is no cultivar in Vietnam. Probably, ancestral cultivars belonging to the Daitou-mai group were cultivated in restricted areas or at a restricted time. We presume that cytoplasm-substituted cultivars would happen once in particular areas and anyhow were dispersed into other areas including Vietnam with the help of weak or non-photoperiod sensitivity, which would probably have originated from *japonica* parents. As cytoplasm-substituted cultivars were also seldom detected (Nakamura et al. 1998), we surveyed genetic resources which carried same genotypes with Daitou-mai. We found that such cultivars are frequent in India.

How are such cytoplasm-substituted cultivars created in nature? In upland fields, we can still see heterozygotes and extremely wide variation for morphological and physiological markers in single fields of Laos (Ishikawa et al. 2001). The cultivation system adopted in primitive upland fields enables rice plants to outcross. These out-crossed seeds are planted again in the fields. This system allows the hybrids and progeny to retain their seeds to the next generation without human selection. In this way, mixed cultures of both types would easily generate such out-crossed plants. Through repeated self-pollination of the heterozygotes, the nuclear condition would become homozygous. Some of them would also become cytoplasm-substituted cultivars.

Cytoplasm-substituted cultivars were surveyed among *indica* cultivars carrying $Pgd1^3$ Indian cultivars, especially Aus cultivars, revealed *indica* nuclear genotypes with *japonica*-cytoplasm representing plastid subtype 6C7A in a high frequency. Further, one of them carried $Pgd1^3$ which is a unique allele found in Japanese *indica*

cultivars. Probably Aus cultivars had been introduced into ancient Champa with the Hindu religion which was a national religion in the nation from India; then they were introduced into Japan.

The origin of the *japonica*-type cytoplasm of the substituted cultivars is still unknown. The plastid subtype 6C7A is common in tropical and temperate *japonica* types (Ishikawa et al. 2001). The alternative plastid subtype of *japonica* 7C6A is specific to the tropical *japonica* type. The latter plastid subtype was not found in the cytoplasm-substituted cultivars. This would not directly indicate that the temperate *japonica* cultivar is the maternal origin of the cytoplasm-substituted cultivars. However, the plastid subtype 6C7A and the short basic vegetative phase of Aus cultivars strongly suggested that their genetic components would originate from the temperate *japonica* type. Additionally, tropical *japonica* usually carry a long basic vegetative phase which is not found in Aus cultivars (Sato and Morishima 1987). Thus, we prefer the idea that the temperate *japonica* type would be the maternal progenitor. However, we need more information on maternally inherited genetic markers to confirm this assumption.

Out-crossing would introduce extremely wide diversity of nuclear genotypes into the Aus cultivars. Isozyme genotypes of the Aus cultivars are unique (Glaszmann 1987) and also various (Wan and Ikehashi 1997). Morphological and physiological analysis carried out in this report also suggested that Aus cultivars represented high polymorphism for several characters. Although they can be classified into *indica* by nuclear genotypes based on isozymes, some of them showed *japonica*-specific characteristics in particular traits, like the phenol reaction. Morinaga and Kuriyama (1955, 1958) also considered the Aus cultivars to be intermediate between the *indica* and *japonica* types. This would result from the out-crossing which naturally occurred between these two types of rice plants. The introduction of *japonica*-specific characteristics can be also explained by the high frequency of cytoplasm-substituted cultivars in Aus cultivars. More-detailed plastid sequence information will tell us about the maternal origin of these Aus cultivars and that of *indica* cultivars in Japan.

Interestingly, Aus cultivars are seen with other *indica* cultivars, Aman cultivars. Aus and Aman cultivars are known to be sympatric in the Bengal region of India. Aus cultivars are harvested in the summer season before the flood season in the Bengal region. In the flood season, Aman cultivars can elongate the length of the internodes quickly in order to survive in deep water and to be harvested after the flood season. In the Aus group, a short basic vegetative phase and a weak photoperiod sensitive phase are essential to adapt to the summer cultivation style in the Bengal region. In contrast, Aman cultivars carry a strong photoperiod sensitive phase in order to flower after the flood season in late summer. The different flowering habit allows the Aus cultivars to be grown in Japan experimentally (data not shown). In addition to the difference in flowering habits, isozyme genotypes were slightly different between these ecospecies (Glaszmann 1987). A comparison of the nuclear composition between these two groups, Aus and Aman, will tell us which genetic components were altered to gain wide adaptability by the result of the out-crossing which happened to ancestral strains of Aus cultivars in the past. This phenomenon is interesting not only in the genetic respect but also in the evolutionary aspect, which will explain how rice acquired wide adaptability to be cultivated from tropical to sub-arctic zones.

References

Arashi K (1974) Some thoughts on red rice in Japan. Nihon akamai Ko, Yuzankaku, Tokyo

Chen WB, Nakamura I, Sato YI, Nakai H (1993) Distribution of deletion types in the cpDNA of cultivated and wild rice. Jpn J Genet 68:597–603

Chu Y (1967) Variations in peroxidase isozymes of *Oryza perennis* and *O. sativa*. Jpn J Genet 42:233–244

Glaszmann JC (1987) Isozymes and classification of Asian rice varieties. Theor Appl Genet 74:21–30

Ishikawa R, Maeda K, Harada T, Niizeki M, Saito K (1991) Classification of Japanese rice varieties into Indica and Japonica types by using isozyme genotypes. Jpn J Breed 41:605–622

Ishikawa R, Maeda K, Harada T, Niizeki M, Saito K (1992) Genotypic variation for 17 isozyme genes among Japanese upland varieties in rice. Jpn J Breed 42:737–746

Ishikawa R, Sato Y-I, Tang L, Nakamura I (2001) Different maternal origins of Japanese lowland and upland rice populations. Theor Appl Genet (in press)

Kanno A, Watanabe N, Nakamura I, Hirai A (1993) Variations in chloroplast DNA from rice (*Oryza sativa*): differences between deletions mediated by short direct-repeat sequences within a single species. Theor Appl Genet 86:579–584

Kato S, Kosaka H, Hara S (1928) On the affinity of rice varieties as shown by the fertility of rice plants. Central Agric Institute Kyushu Imp Univ 2:241–276

Morinaga T (1968) Origin and geographical distribution of Japanese rice. Jpn Agric Res Quart 3:1–5

Morinaga T, Kuriyama H (1955) Japonica type rice in the subcontinent of Indica and Java. Jpn J Breed 5:149–153

Morinaga T, Kuriyama H (1958) Intermediate-type rice in the subcontinent of Indica and Java. Jpn J Breed 7:253–259

Morishima H, Oka H (1981) Phylogenetic differentiation of cultivated rice. XXII. Numerical evaluation of the Indica–Japonica differentiation. Jpn J Breed 31:402–413

Nakamura I, Kameya N, Kato K, Yamanaka SI, Jomori H, Sato YI (1997) A proposal for identifying the short ID sequence which addresses the plastid subtype of higher plants. Breed Sci 47: 385–388

Nakamura I, Urairong, Kameya N, Fukuta Y, Chitrkon S, Sato YI (1998) Six different plastid subtypes were found in the *O. sativa*-*O. rufipogon* complex. Rice Genet Newslett 15:80–82

Oka HI (1953) Variations in various characters and character combination among rice varieties (Japanese/English). Jpn J Breed 3:33–43

Pai C, Endo T, Oka H (1975) Genic analysis for acid phosphatase in *Oryza perennis* and *O. sativa*. Can J Genet Cytol 17:637–6508

Sano R, Morishima H (1992) Indica–Japonica differentiation of rice cultivars viewed from the Himalayan hilly areas. Theor Appl Genet 84:266–274

Sato YI, Morishima H (1987) Distribution of the complementary genes causing F_1 weakness in the common rice and its wild relatives. 2. Distribution of *Hwc-1* and *Hwc-2* genes in the native cultivars and their relatives of tropical Asia. Euphytica 36:425–431

Shahi BB, Morishima H, Oka H (1969) A survey of variations in peroxidase, acid phosphatase and esterase isozymes of wild and cultivated *Oryza* species. Jpn J Genet 44:303–319

Terao H, Mizushima U (1939) Some considerations in the classification of *Oryza sativa* L. Jap J Bot 10:213–258

Wan J, Ikehashi H (1997) Identification of two types of differentiation in cultivated rice (*Oryza sativa* L.) detected by polymorphism of isozymes and hybrid sterility. Euphytica 94:151–161