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Restriction fragments homologous to mitochondrial plasmid-like DNAs are located within limited chromosomal regions on the rice nuclear genome

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Abstract. The chromosomal locations of restriction fragments of nuclear DNA that were homologous to four mitochondrial plasmid-like DNAs, namely, B1, B2, B3 and B4, were analyzed by restriction fragment length polymorphism (RFLP) analysis in cultivated rice. Nine kinds of fragments homologous to plasmid-like DNAs were analyzed for their segregation in three different F_2 populations derived from intercrosses between rice subspecies; these were found to be localized in three chromosomal regions: three, one and five kinds of nuclear homologues were situated on chromosomes 1, 3 and 8, respectively. Nuclear homologues on a given chromosome were tightly linked even though they were homologous to different plasmid-like DNAs. The loci of nuclear homologues found commonly in two or three cultivars were found to be highly conserved, a result that is consistent with their stable transmission. These results and those of the Southern analysis suggest the independent integration of these sequences during the varietal differentiation of rice. The concentration of loci for nuclear homologues on chromosomes 1 and 8 suggests that these sequences were integrated non-randomly into these chromosomal regions.

Key words: Mitochondrial DNA – Plasmid-like DNA – Nuclear homologue – Inter-organellar sequence transfer – Rice

Introduction

The mitochondria of higher plants often contain small circular and linear DNA molecules, referred to as plasmid-like DNAs, in addition to the main mitochondrial genome (Pring and Lonsdale 1985). Four kinds of circular plasmid-like DNA, namely, B1, B2, B3 and B4, have been detected in rice mitochondria (Yamaguchi and Kakiuchi 1983; Shikanai et al. 1987, 1989; Shikanai and Yamada 1988). Sequence analysis has revealed that these are related to 1.9-kb and 1.4-kb circular plasmid-like DNAs found in maize (Shikanai et al. 1989). Their presence or absence is well correlated with the varietal differentiation of cultivated rice (*Oryza sativa* L.; Kanazawa et al. 1992). These molecules do not exhibit extensive homology to either the main mitochondrial genome or the chloroplast genome, but they do exhibit extensive homology to the nuclear genome (Shikanai et al. 1987, 1989; Shikanai and Yamada 1988). Fukuchi et al. (1991) determined the sequences in the nuclear genome that were homologous to B4, and they also suggested the inter-organellar transmission of these sequences from the mitochondrion to the nucleus.

The nuclear homologues of these four plasmid-like DNAs revealed restriction fragment length polymorphisms (RFLPs) among varietal groups of *O. sativa*, and also between *O. sativa* and *O. glaberrima* (Sakamoto et al. 1991). Using these RFLPs, we recently carried out genetic studies on the nuclear homologues in F_2 populations derived from crosses between two subspecies of rice, namely, *Indica* and *Japonica*. Our results indicated that the nuclear homologues were stably located on chromosomes and transmitted according to the principles of Mendelian inheritance (Sakamoto et al. 1991; Kanazawa et al. 1991).

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Although nuclear homologues of mitochondrial plasmid-like DNAs have been detected in several plant species other than rice, namely, faba bean (Flamand et al. 1992), maize (Abbot et al. 1985; Kemble et al. 1983; Smith and Pring 1987) and sunflower (Crouzillat et al. 1989; de la Canal et al. 1991), no information about their locations on the respective nuclear genomes has been published. In this report, we describe the chromosomal locations of nuclear homologues of mitochondrial plasmid-like DNAs on the RFLP linkage map that was recently constructed by Saito et al. (1991) for rice. Furthermore, we report on our investigation of the conservative nature of the loci of the nuclear homologues using two different F_2 populations in combination with the linkage analysis of the nuclear homologues that were specifically found in the parental cultivars of these F_2 populations. Most of the nuclear homologues that we analyzed were tightly linked to one another, and three chromosomal regions were identified.

Materials and methods

Plant materials

Six rice cultivars were used in this study: 'Kasalath', 'Liuzhou baoya zao', 'Hong xie nuo', 'FL134', '74F4-9' (GS1211) and 'FL27'. The first three belong to the subspecies *Indica* and the latter three to the subspecies *Japonica*. F_2 individuals derived from crosses between these *Indica* and *Japonica* cultivars were used for genetic analysis.

Preparation of DNA

Total DNA was extracted from the leaves of each F_2 individual and the respective parental cultivars by the method of Murray and Thompson (1980). Mitochondrial plasmid-like DNAs, namely, B1, B2, B3 and B4, that had previously been cloned (Sakamoto et al. 1989; Kanazawa et al. 1991), and rice genomic RFLP markers (Saito et al. 1991) were used as probes for Southern hybridization.

RFLP analysis

For Southern hybridization, total DNA from F_2 individuals was probed with cloned mitochondrial plasmid-like DNAs and RFLP markers as previously described (Kanazawa et al. 1991). The locations of the nuclear homologues on a rice RFLP map, based on their segregation in F_2 populations, were identified using 144 individual F_2 plants derived from the the cross of 'Kasalath' × 'FL134' (Saito et al. 1991). Segregation and linkage analyses were also carried out using 70 individual F_2 plants derived from crosses between 'Liuzhou baoya zao' and '74F4-9', and between 'Hong xie nuo' and 'FL27'. Recombination values were estimated by the maximum likelihood method (Allard 1956). The putative order of loci within a linkage group was estimated by use of the MAPL program (Ukai et al. 1990). Genetic map distances were estimated from recombination values by application of the Kosambi function (Kosambi 1944).

Results

Chromosomal location of the nuclear homologues of mitochondrial plasmid-like DNAs on the RFLP linkage map

Our previous studies showed that five restriction fragments homologous to mitochondrial plasmid-like DNAs could be examined for their segregation in F_2 populations derived from intercrosses between *Indica* and *Japonica* subspecies of rice (Kanazawa et al. 1991). These fragments were a B1-homologous 2.5-kb *Eco*RV fragment, a B1-homologous 7.1-kb *Eco*RV fragment, a B2-homologous 15-kb *Eco*RV fragment, a B3-homologous 11-kb *Eco*RV fragment and a B4-homologous 35-kb *Bgl*II fragment, which were found specifically in *Indica*, *Japonica*, *Indica*, *Indica* and *Indica*, respectively (Table 1). To locate these nuclear homologues of mitochondrial plasmid-like DNAs on rice chromosomes, we performed linkage analysis using the segregation data of both nuclear homologues (Kanazawa et al. 1991) and RFLP markers (Saito et al. 1991) in the F_2 population derived from the cross of

Table 1. List of the restriction fragments homologous to mitochondrial plasmid-like DNAs analyzed in this study together with their presence or absence in the parental cultivars of F_2 plants

Fragment	FL134[a]	Kasalath	Liuzhou baoya zao	Hong xie nuo
B1-homologous 2.5-kb *Eco*RV	−	+	+	+
5.7-kb *Eco*RV	−	−	+	+
7.1-kb *Eco*RV	+	−	−	−
13-kb *Eco*RV	−	−	−	+
B2-homologous 3.3-kb *Eco*RV	−	−	−	+
15-kb *Eco*RV	−	+	+	+
B3-homologous 11-kb *Eco*RV	−	+	−	−
B4-homologous 2.2-kb *Bgl*II	−	−	+	+
5.5-kb *Bgl*II	−	−	−	+
7.6-kb *Bgl*II	−	−	−	+
35-kb *Bgl*II	−	+	+	+

+, Present; −, absent

[a] 74 F4-9 and FL27 gave the same pattern as FL134

'Kasalath' (*Indica*) x 'FL134' (*Japonica*). As a result, the five nuclear homologues of mitochondrial plasmid-like DNAs were mapped to three chromosomes. Linkage maps around the loci of these nuclear homologues are shown in Figs. 1A, 2A, 3A. The B1-homologous 7.1-kb *Eco*RV fragment, the B1-homologous 2.5-kb *Eco*RV fragment and the B2-homologous 15-kb *Eco*RV fragment correspond to B1-1, B1-2 and B2, respectively, on the linkage map of Saito et al. (1991). Two linked pairs, the 7.1-kb B1-nuclear homologue of 'FL134' and the 35-kb B4-nuclear homologue of 'Kasalath', and the 15-kb B2- and 11-kb B3-nuclear homologues of 'Kasalath' were mapped to chromosomes 1 (Fig. 1A) and 8 (Fig. 3A), respectively. The 2.5-kb B1-nuclear homologue of 'Kasalath' was located on chromosome 3 (Fig. 2A). The nuclear homologues that showed RFLPs and that could be mapped using 'Kasalath' and 'FL134' were of limited use even though we tested two or more restriction enzymes for our analyses. Accordingly, we tried using other materials in the next phase of the study.

Polymorphism of nuclear homologues among parental cultivars

To determine whether the locations of nuclear homologues were conserved during the varietal differentiation of rice and whether other nuclear homologues that could not be mapped by use of 'Kasalath' and 'FL134' were present in other cultivars, we analyzed the segregation of nuclear homologues using F_2 populations derived from other *Indica* and *Japonica* hybrids. Prior to F_2 analysis, we compared the hybridization profiles of the parental cultivars used in this experiment. Two *Indica* cultivars that were used as maternal parents, namely, 'Liuzhou baoya zao' and 'Hong xie nuo', are different from 'Kasalath' in terms of their geographical origin. The former two originated in China, whereas 'Kasalath' originated in India (Nakagahra 1977). The *Japonica* cultivars used as paternal parents, namely, '74 F4-9' and 'FL27', are mutant marker lines, as is 'FL134'.

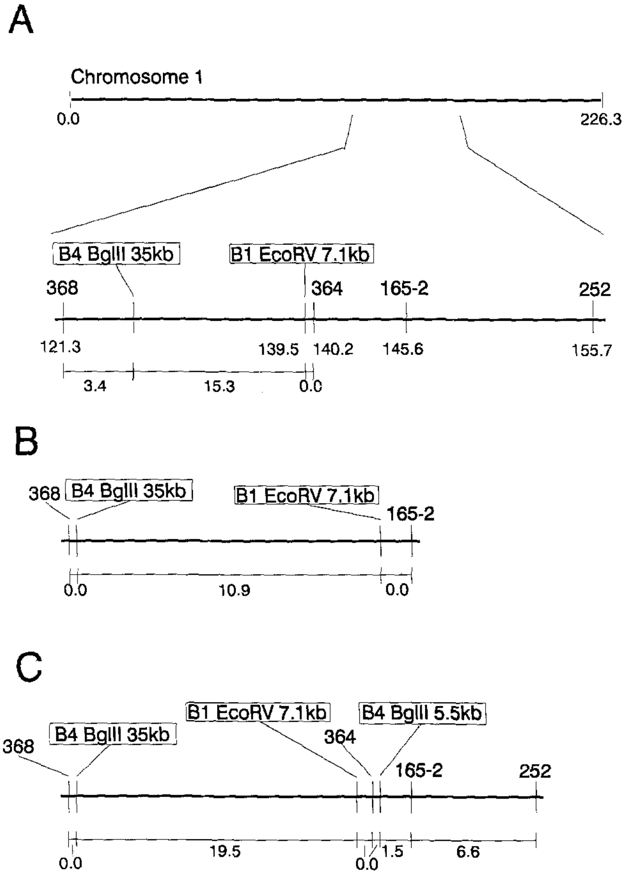


Fig. 1A–C. Linkage map of the nuclear homologues of mitochondrial plasmid-like DNAs and their neighboring RFLP markers on chromosome 1, constructed using the data from F_2 populations derived from 'Kasalath' x 'FL134' (**A**), from 'Liuzhou baoya zao' x '74 F4-9' (**B**) and from 'Hong xie nuo' x 'FL27' (**C**). Map distances are shown in cM *below a thin line*. Map distances on the RFLP linkage map, shown in cM *below a thick line* in panel A, are taken from Saito et al. (1991)

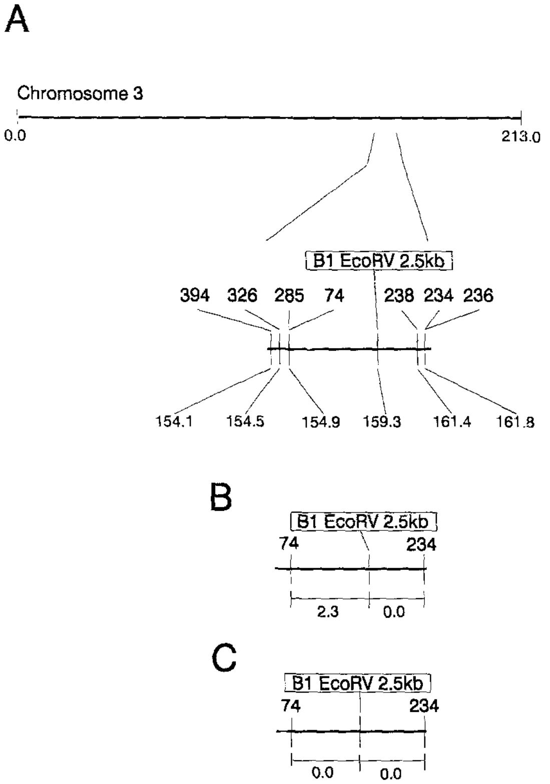


Fig. 2A–C. Linkage map of the nuclear homologues of mitochondrial plasmid-like DNAs and their neighboring RFLP markers on chromosome 3, constructed using the data from F_2 populations derived from 'Kasalath' x 'FL134' (**A**), from 'Liuzhou baoya zao' x '74 F4-9' (**B**) and from 'Hong xie nuo' x 'FL27' (**C**). For map distances, see legend to Fig. 1

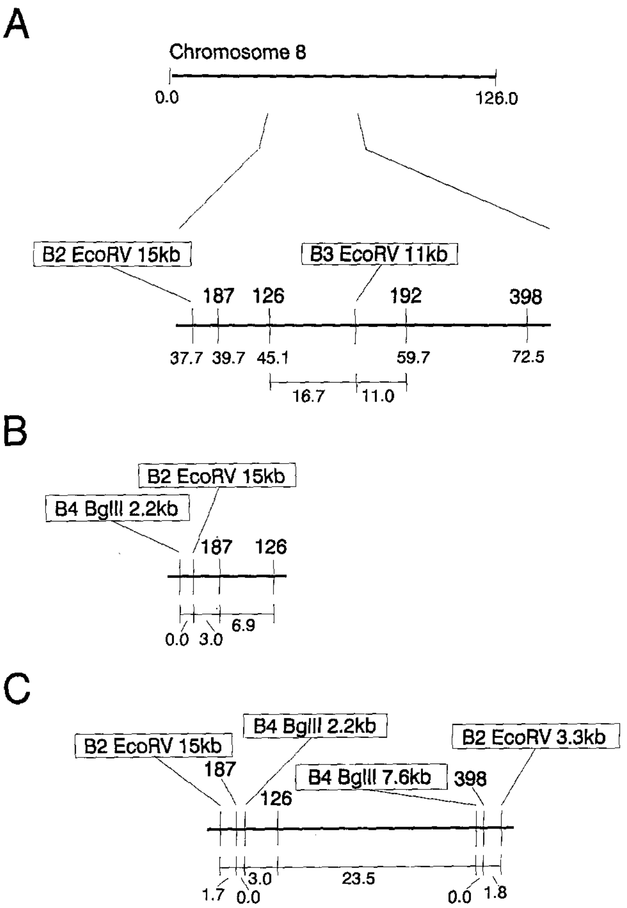


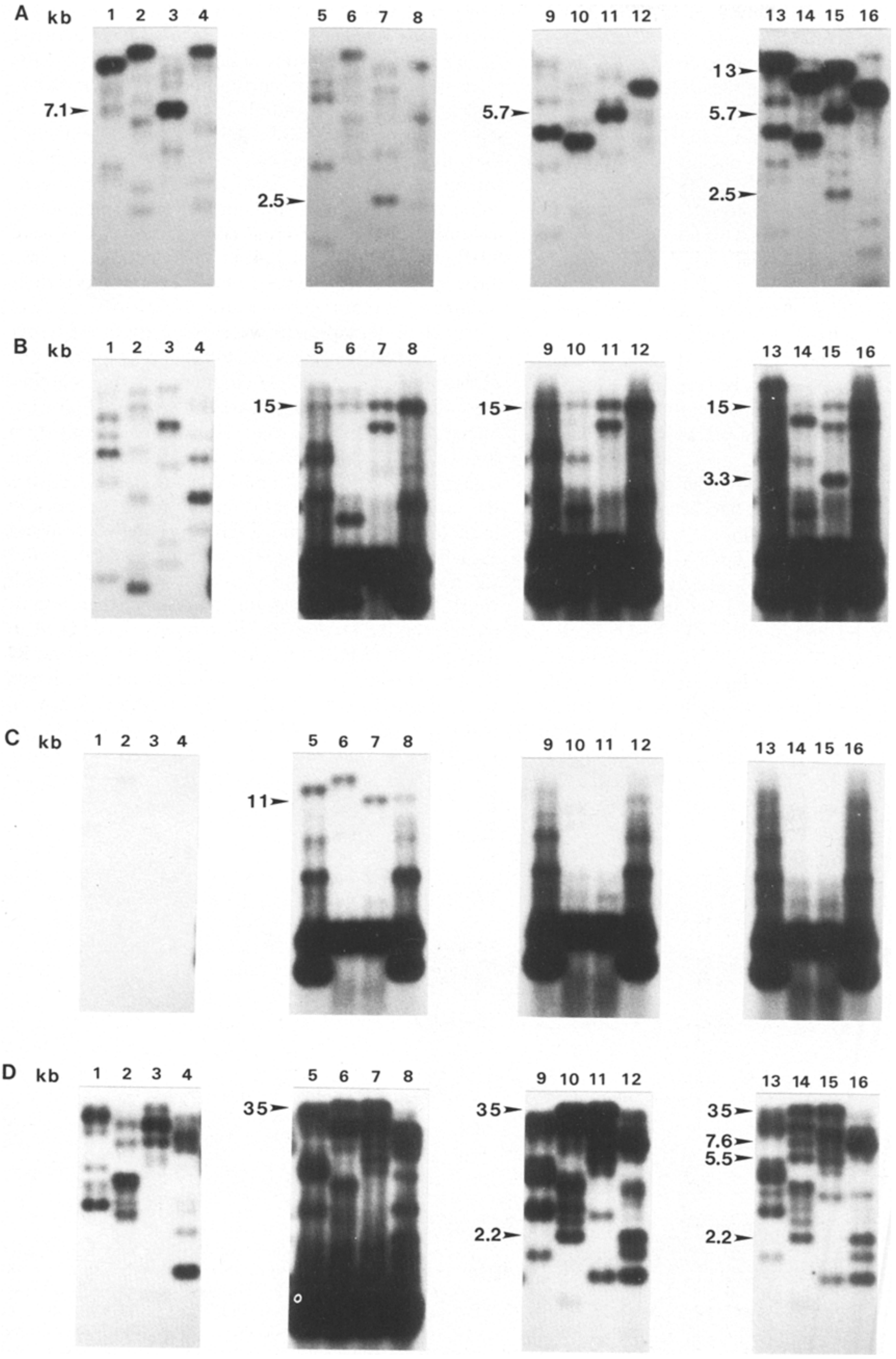
Fig. 3. Linkage map of the nuclear homologues of mitochondrial plasmid-like DNAs and their neighboring RFLP markers on chromosome 8, constructed using the data from F_2 populations derived from 'Kasalath' × 'FL134' (**A**), from 'Liuzhou baoya zao' × '74 F4-9' (**B**) and from 'Hong xie nuo' × 'FL27' (**C**). For map distances, see legend to Fig. 1

Total DNA from these cultivars was digested with *Bam*HI, *Bgl*II, *Eco*RV and *Hin*dIII, and then each digest was probed with each of four cloned plasmid-like DNAs to analyze the RFLP of the nuclear homologues (Fig. 4). The discrimination of nuclear homologues from digested plasmid-like DNAs (Figs. 4B, C, lanes 6, 7, 10, 11, 14, 15; Fig. 4D, lanes 6, 7) or from undigested plasmid-like DNAs (Figs. 4B, C, lanes 5, 8, 9, 12, 13, 16; Fig. 4D, lanes 5, 8) in mitochondria has been described in previous reports (Sakamoto et al. 1991; Kanazawa et al. 1991). The nuclear locations were confirmed by F_2 analysis. Multiple hybridization signals were obtained for each homologue, with the various intensities depending on the probe: 5–7 signals were found when digested DNA was probed with B1, 4–7 with B2, 3–5 with B3, and 5–9 with B4, including weakly hybridizing bands. The RFLPs found among three *Indica* cultivars were of a lower degree of polymorphism than those found between *Indica* and *Japonica* cultivars. '74 F4-9' and 'FL27' showed 98.9% and 90.0% similarity to 'FL134' in terms of banding patterns, respectively, for about 90 hybridization signals obtained with four enzymes and four probes. Some of the nuclear homologues were polymorphic with respect to their presence or absence among the three *Indica* cultivars. This phenomenon was distinguishable from the variation in fragment size caused by changes in restriction site because of the conspicuous differences in signal intensity: the B1-homologous 5.7-kb *Eco*RV fragment and the B4-homologous 2.2-kb *Bgl*II fragment were found in both 'Liuzhou baoya zao' and 'Hong xie nuo', but not in 'Kasalath'; the B1-homologous 13-kb *Eco*RV fragment, the B2-homologous 3.3-kb *Eco*RV fragment and the B3-homologous 11-kb *Eco*RV fragment were found in only 'Hong xie nuo', 'Hong xie nuo' and 'Kasalath', respectively. This result suggests that the numbers and kinds (i.e., sequences) of nuclear homologues of plasmid-like DNAs did not remain constant during the varietal differentiation of cultivated rice.

Verification of the conservative nature of the loci of nuclear homologues in different rice cultivars

Since most of the fragments homologous to plasmid-like DNAs that were mapped as described above were also found in 'Liuzhou baoya zao' and 'Hong xie nuo' (Fig. 4 and Table 1), we analyzed whether they were also present at the same loci in the nuclear genomes of these cultivars. For this purpose, we investigated the linkage between the nuclear homologues and their neighboring markers on the linkage map in two different F_2 populations of which these *Indica* cultivars mentioned above were the maternal parents. Among the five nuclear homologues of plasmid-like DNAs that had been mapped using the cross of 'Kasalath' × 'FL134', four nuclear homologues, namely, the 2.5-kb *Eco*RV B1 homologue, the 7.1-kb *Eco*RV B1 homo-

Fig. 4A–D. Polymorphism of nuclear homologues of mitochondrial plasmid-like DNAs among rice cultivars. Total DNAs from FL134 (*lanes 1–4*), 'Kasalath' (*lanes 5–8*), 'Liuzhou baoya zao' (*lanes 9–12*) and 'Hong xie nuo' (*lanes 13–16*) were digested with *Bam*HI (*lanes 1, 5, 9* and 13), *Bgl*II (*lanes 2, 6, 10* and 14), *Eco*RV (*lanes 3, 7, 11* and *15*) and *Hin*dIII (*lanes 4, 8, 12* and *16*) and hybridized with cloned B1 (**A**), B2 (**B**), B3 (**C**) and B4 (**D**). Sizes of the nuclear homologues analyzed in this study are indicated on the *left side* of each panel


A
kb
1 2 3 4
7.1
5 6 7 8
2.5
9 10 11 12
5.7
13 14 15 16
13
5.7
2.5
B
kb
1 2 3 4
5 6 7 8
15
9 10 11 12
15
13 14 15 16
15
3.3
C
kb
1 2 3 4
5 6 7 8
11
9 10 11 12
13 14 15 16
D
kb
1 2 3 4
5 6 7 8
35
9 10 11 12
35
2.2
13 14 15 16
35
7.6
5.5
2.2

logue, the 15-kb *Eco*RV B2 homologue and the 35-kb *Bgl*II B4 homologue, could be analyzed for their segregation in the F_2 populations from the cross of 'Liuzhou baoya zao' × '74 F4-9' and from the cross of 'Hong xie nuo' × 'FL27'. The 11-kb *Eco*RV B3 homologue was not found in 'Liuzhou baoya zao' and 'Hong xie nuo', as mentioned earlier. Although not found in the lane representing 'Liuzhou baoya zao' in Fig. 4A, the 2.5-kb *Eco*RV B1 homologue was found in the F_2 plants of which this cultivar was a parent. This phenomenon was perhaps due to heterogeneity with respect to the presence of this homologue among individuals within this cultivar. The association of such individual differences with this homologue have also been found in another *Indica* cultivar, 'Surjumkhi' (data not shown). Linkage to a fertility-related gene might be responsible for the "unfixed" state of this locus in some cultivars since this homologue and its neighboring markers showed distorted segregation (see below).

We analyzed, simultaneously, the segregation of other nuclear homologues that had been found either in 'Liuzhou baoya zao' or 'Hong xie nuo', but not in 'Kasalath' (Table 1): the 5.7-kb *Eco*RV B1 homologue and the 2.2-kb *Bgl*II B4 homologue in the F_2 plants from 'Liuzhou baoya zao' × '74 F4-9', the 5.7-kb and the 13-kb *Eco*RV B1 homologues, the 3.3-kb *Eco*RV B2 homologue and the 2.2-kb, 5.5-kb and 7.6-kb *Bgl*II B4 homologues in the F_2 plants from 'Hong xie nuo' × 'FL27' (Fig. 5). The segregation ratios for these nuclear homologues and RFLP markers in these two F_2 populations are shown in Table 2. In both populations, the 2.5-kb *Eco*RV B1 homologue and its neighboring RFLP markers on the linkage map, namely, 74 and 234, showed distorted segregation, due perhaps to a specificity of this chromosomal region. Other nuclear homologues and their neighboring markers segregated with a ratio of 3:1. This result indicates that nuclear homologues, unlike transposable elements, are transmitted stably from generation to generation. Therefore, common and polymorphic nuclear homologues in the parental cultivars appear to have been integrated into the genome before and during the differentiation, respectively, of these cultivars or their ancestors.

In both F_2 populations derived from the cross of 'Liuzhou baoya zao' × '74 F4-9' and from the cross of 'Hong xie nuo' × 'FL27', four nuclear homologues that were mapped using 'Kasalath' and 'FL134' were linked to the neighboring markers, an indication that the loci of these nuclear homologues have been conserved (Figs. 1, 2, 3). The 5.7-kb and 13-kb *Eco*RV B1 homologues were not linked to other nuclear homologues. Accordingly, they should be localized elsewhere on the genome. However, the 3.3-kb *Eco*RV B2 homologue and the 2.2-kb, 5.5-kb and 7.6-kb *Bgl*II B4 homologues were linked to other homologues: the 5.5-kb *Bgl*II B4 homologue was linked to the 7.1-kb *Eco*RV B1 homologue and the 35-kb *Bgl*II B4 homologue on chromosome 1 in 'Hong xie nuo'; others were linked to the 15-kb *Eco*RV B2 homologue on chromosome 8 in 'Liuzhou baoya zao' and 'Hong xie nuo' (Figs. 1, 3).

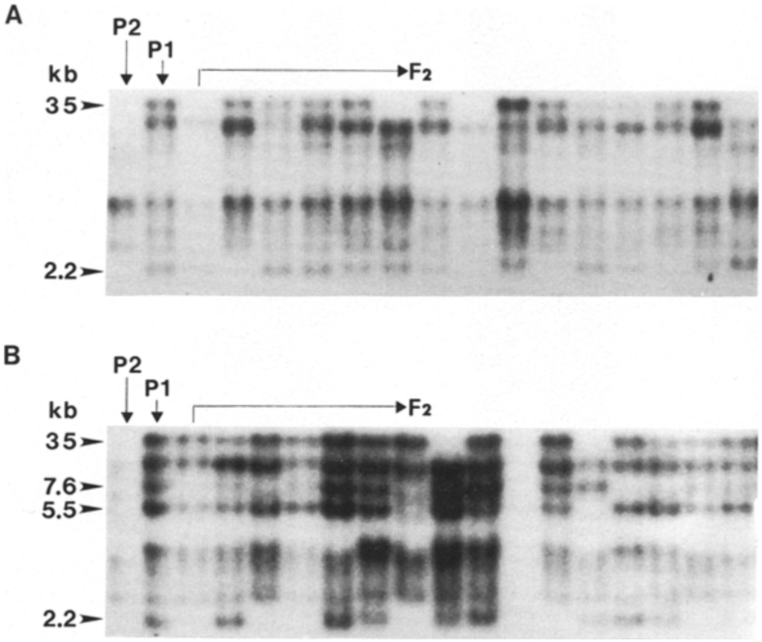


Fig. 5A, B. Segregation of nuclear homologues of plasmid-like DNA in F_2 populations. Total DNA from the F_2 individuals derived from crosses of 'Liuzhou baoya zao' × '74 F4-9' (**A**) and from 'Hong xie nuo' × 'FL27' (**B**) were hybridized with cloned B4. *P1* and *P2* indicate the maternal and paternal parents, respectively

Table 2. Segregation analysis of the nuclear homologues of mitochondrial plasmid-like DNAs and their neighboring RFLP markers in the F_2 populations derived from crosses of 'Liuzhou baoya zao' × '74 F4-9' (A) and 'Hong xie nuo' × 'FL27' (B)

Fragment	Segregation ratio			Chi-squared for 3:1 or 1:2:1	Chromosome
	P1	Het	P2[a]		
(A) Liuzhou baoya zao × 74 F4-9					
B1 7.1-kb *Eco*RV	20		49	0.58	1
B4 35-kb *Bgl*II	49		18	0.12	1
368	17	31	21	1.17	1
165-2	49		19	0.31	1
B1 2.5-kb *Eco*RV	64		5	11.60**	3
74	31	34	4	21.14**	3
234	30	31	4	20.94**	3
B2 15-kb *Eco*RV	48		20	0.71	8
B4 2.2-kb *Bgl*II	49		21	0.93	8
187	20	30	18	1.06	8
126	19	28	22	2.71	8
(B) Hong xie nuo × FL27					
B1 7.1-kb *Eco*RV	20		48	0.71	1
B4 35-kb *Bgl*II	52		16	0.08	1
B4 5.5-kb *Bgl*II	54		15	0.39	1
368	51		14	0.42	1
364	21	33	14	1.50	1
165-2	51		16	0.04	1
252	22	31	16	1.75	1
B1 2.5-kb *Eco*RV	66		2	17.65**	3
74	25	41	2	18.44**	3
234	29	36	2	22.13**	3
B2 15-kb *Eco*RV	50		18	0.08	8
B4 2.2-kb *Bgl*II	52		16	0.08	8
B2 3.3-kb *Eco*RV	46		15	0.01	8
B4 7.6-kb *Bgl*II	51		14	0.42	8
187	20	32	15	0.88	8
126	19	32	19	0.51	8
398	21	33	14	1.50	8

** Significant at the 1% level

[a] P1, P2 and Het: number of F_2 individuals that gave the RFLP pattern of the maternal type, the paternal type and both types, respectively

Heterogeneity of nuclear homologues

Southern analysis indicated that there were several homologues of plasmid-like DNAs in the nuclear genome (Fig. 4). To characterize roughly those parts of mitochondrial plasmid-like DNAs that corresponded to nuclear homologues, we divided plasmid-like DNA B1 into three parts and used each as a probe for Southern blots of 'Kasalath' and 'FL134' (Fig. 6). The hybridization signals obtained with each B1 subclone were quite different, suggesting that each nuclear homologue has different portions in common with mitochondrial plasmid-like DNAs. With respect to the nuclear homologues analyzed in this study, the 2.5-kb *Eco*RV B1 homologue of 'Kasalath' hybridized mainly with the 0.73-kb *Stu*I-*Xho*I subclone, faintly with the 1.02-kb *Hae*III-*Xho*I subclone and not at all with the 0.36-kb *Xho*I-*Stu*I subclone, whereas the 7.1-kb *Eco*RV B1 homologue of 'FL134' hybridized with all three B1 subclones. This analysis also showed that the lengths of integrated sequences in the nuclear genome contributed to the variations in signal intensity observed when entire sequences of plasmid-like DNAs were used as a probe, and it confirmed that different homologues were integrated in the different cultivars.

Discussion

Chromosomal location of nuclear homologues of mitochondrial plasmid-like DNAs

In this study we determined the locations of restriction fragments that were homologous to mitochondrial plasmid-like DNAs on the linkage map of rice. Putting all of

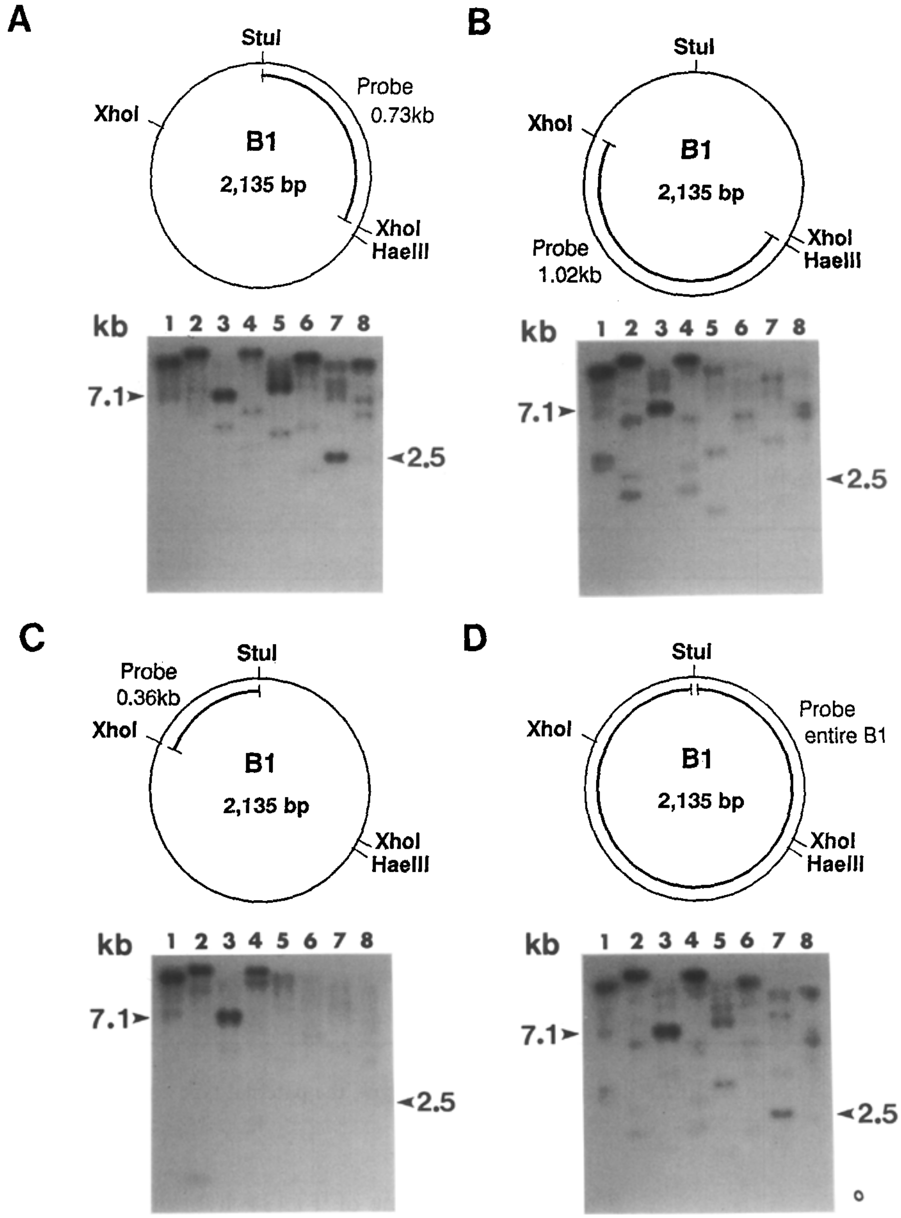


Fig. 6A–D. Heterogeneity exhibited by the nuclear homologues of plasmid-like DNA. Total DNA from 'FL134' (*lanes 1–4*) and 'Kasalath' (*lanes 5–8*) was digested with *Bam*HI (*lanes 1* and *5*), *Bgl*II (*lanes 2* and *6*), *Eco*RV (*lanes 3* and *7*) and *Hin*dIII (*lanes 4* and *8*) and hybridized with subcloned B1 (**A, B** and **C**) and the entire region of cloned B1 (**D**)

our results from mapping experiments together, we found that most of the nuclear homologues that were analyzed for their segregation in F_2 populations were localized within limited regions of chromosome 1 or 8. However, it does not seem likely that they were all integrated at once. With the restriction enzymes that we used for mapping 41 kinds of fragments homologous to plasmid-like DNAs were found in the cultivars studied. Among these fragments, 11 were polymorphic between *Indica* and *Japonica* cultivars and could be analyzed for their segregation in the F_2 populations. The fragments that were mapped to the limited regions of chromosomes 1 and 8 corresponded to 27.3% (3 out of 11) and 45.5% (5 out of 11), respectively, of the fragments that were analyzed. The regions on the linkage map in which the nuclear homologues were located, namely, 368–165.2 on chromosome 1 (24.3 cM; Fig. 1) and B2 *Eco*RV 15-kb-398 on chromosome 8 (34.8 cM; Fig. 3), in contrast, accounted for only 1.32% and 1.90% of the entire nuclear genome (1,836 cM), as estimated from map distances on the RFLP linkage map. The results suggest that sequences homologous to plasmid-like DNAs integrated non-randomly into these chromosomal regions. We cannot define characteristics that are common to the loci of nuclear homologues at present. However, if we assume the interor-ganellar transfer of these sequences from the mitochondrion to the nucleus or the introduction of foreign sequences from outside the cell, it seems likely that these chromosomal regions might have a higher capacity for the integration of DNA fragments than other parts of the genome. Fukuchi et al. (1991) reported finding nucleotide sequences that were homologous to parts of genes found in the chloroplast genome, namely, *rps12* and *psaA1*, and to parts of genes found in the mitochondrial genome, namely, *cox*II, *cob* and *nad*1; these sequences were contiguous to within 1 kb to B4-homologous sequences in the nuclear genome of a *Japonica* rice cultivar. Our analysis does not provide any direct information about the chromosomal location of these sequences because all of the B4 homologues mapped in the present study were those found in *Indica* cultivars. The concentration of nuclear homologues of plasmid-like DNAs on chromosomes 1 and 8 in our study implies, however, the existence of chromosomal regions into which DNA fragments are preferentially integrated from a genetic approach using RFLPs.

Polymorphism of nuclear homologues of plasmid-like DNAs among cultivated rice

RFLPs of the nuclear homologues of mitochondrial plasmid-like DNAs have been identified in rice cultivars as variations in the number and intensity of hybridization signals (Sakamoto et al. 1991; this study). Sequence analysis of several B4-homologous genomic clones revealed that the sequences homologous to plasmid-like DNAs varied in length, although each exhibited a very high degree of homology to plasmid-like DNAs in mitochondria (Fukuchi et al. 1991). From the sequence data of Fukuchi et al. (1991) and our Southern analysis with B1- subclones as probes (Fig. 6), it seems that the intensity of signals due to nuclear homologues in the present study depended mainly on the length of the integrated regions that were homologous to plasmid-like DNAs rather than to the number of copies of each in the genome.

Some of the strong hybridization signals indicated that polymorphism exists with respect to the presence or absence of nuclear homologues in addition to the RFLPs caused by variations in restriction sites. Taking their stable transmission into account, we propose that the nuclear homologues that showed the former type of polymorphism were probably integrated independently during the varietal differentiation of rice. Recent work has shown that nuclear homologues also exhibit such polymorphism in the wild-rice ancestors of cultivated rice (unpublished data).

Origin of the sequences in the nuclear genome that are homologous to mitochondrial plasmid-like DNAs

Evidence for the interorganellar transfer of sequences between mitochondria and nucleus has been observed throughout the plant and animal kingdoms (Schuster and Brennicke 1988; Shay and Werbin 1992). Thorsness and Fox (1990) showed that in yeast the escape of an introduced plasmid from the mitochondria to the nucleus occurred at least 100,000 times more frequently than that in the opposite direction. A similar phenomenon is possible in rice. Until the present investigation, nuclear homologues of plasmid-like DNAs had been found universally in cultivated rice regardless of the presence or absence of plasmid-like DNAs in mitochondria (Sakamoto et al. 1990, 1991; Kanazawa et al. 1991; unpublished data). Therefore, if one considers the source of nuclear homologues to be plasmid-like DNAs in mitochondria, transfer of these sequences from the mitochondrion to the nucleus must have occurred during the early period of differentiation of rice before the sequences were lost from the mitochondria in some varieties of rice. We found variations in the relative number of copies of plasmid-like DNAs among several rice cultivars, which suggest their unequal distribution during mitochondrial division or variations in their efficiency of replication (Kanazawa et al. 1992). It is likely that they might be transmitted less stably than homologous sequences in the nuclear genome. However, we cannot exclude other possibilities, namely, that these sequences were introduced into these two organelles independently or that they were transferred in the reverse direction, possibilities that might more simply explain their distribution in these two organelles among the different varieties of rice.

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References

Abbot AG, O'Dell M, Flavell RB (1985) Quantitative variation in components of the maize mitochondrial genome between tissues and between plants with different male-sterile cytoplasm. Plant Mol Biol 4:233–240

Allard RW (1956) Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia 24: 235–278

de la Canal L, Crouzillat D, Flamand MC, Perrault A, Boutry M, Ledoigt G (1991) Nucleotide sequence and transcriptional analysis of a mitochondrial plasmid from a cytoplasmic male-sterile line of sunflower. Theor Appl Genet 81:812–818

Crouzillat D, Gentzbittel L, de la Canal L, Vaury C, Perrault A, Nicolas P, Ledoigt G (1989) Properties and nucleotide sequence of a mitochondrial plasmid from sunflower. Curr Genet 15:283–289

Flamand MC, Goblet JP, Duc G, Briquet M, Boutry M (1992) Sequence and transcription analysis of mitochondrial plasmids isolated from cytoplasmic male-sterile lines of *Vicia faba*. Plant Mol Biol 19:913–923

Fukuchi M, Shikanai T, Kossykh VG, Yamada Y (1991) Analysis of nuclear sequences homologous to the B4 plasmid-like DNA of rice mitochondria; evidence for sequence transfer from mitochondria to nuclei. Curr Genet 20:487–494

Kanazawa A, Sakamoto W, Kishimoto N, Yano M, Tsutsumi N, Saito A, Tano S (1991) Linkage analysis of the nuclear homologues of mitochondrial plasmid-like DNAs in rice. Jpn J Genet 66:597–607

Kanazawa A, Sakamoto W, Nakagahra M, Kadowaki K, Tsutsumi N, Tano S (1992) Distribution and quantitative variation of mitochondrial plasmid-like DNAs in rice (*Oryza sativa* L.). Jpn J Genet 67:309–319

Kemble RJ, Mans RJ, Gabay-Laughnan S, Laughnan JR (1983) Sequences homologous to episomal mitochondrial DNA in the maize nuclear genome. Nature 304:744–747

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Murray T, Thompson WS (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4325

Nakagahra M (1977) Genetic analysis for esterase isozymes in rice cultivars. Jpn J Breed 4:1–14

Pring DR, Lonsdale DM (1985) Molecular biology of higher plant mitochondrial DNA. Int Rev Cytol 97:1–46

Saito A, Yano M, Kishimoto N, Nakagahra M, Yoshimura A, Saito K, Kuhara S, Ukai Y, Kawase M, Nagamine T, Yoshimura S, Ideta O, Ohsawa R, Hayano Y, Iwata N, Sugiura M (1991) Linkage map of restriction fragment length polymorphisms loci in rice. Jpn J Breed 41:665–670

Sakamoto W, Momose M, Tsutsumi N, Tano S, Yamaguchi H (1989) Analysis of homology of small plasmid-like mitochondrial DNAs in the different cytoplasmic male sterile strains in rice. Jpn J Genet 64:49–56

Sakamoto W, Kadowaki K, Tano S, Yabuno T (1990) Analysis of mitochondrial DNAs from *Oryza glaberrima* and cytoplasmic substituted line for *Oryza sativa* associated with cytoplasmic male sterility. Jpn J Genet 65:1–6

Sakamoto W, Kadowaki K, Kishimoto N, Yano M, Saito A, Tano S (1991) RFLP analysis of nuclear DNAs homologous with mitochondrial plasmid-like DNAs in cultivated rice. Theor Appl Genet 82:179–184

Schuster W, Brennicke A (1988) Interorganellar sequence transfer: plant mitochondrial DNA is nuclear, is plastid, is mitochondrial. Plant Sci 54:1–10

Shay JW, Werbin H (1992) New evidence for the insertion of mitochondrial DNA into the human genome: significance for cancer and aging. Mutat Res 275:227–235

Shikanai T, Yamada Y (1988) Properties of the circular plasmid-like DNA, B4, from mitochondria of cytoplasmic male-sterile rice. Curr Genet 13:441–443

Shikanai T, Yang ZQ, Yamada Y (1987) Properties of the circular plasmid-like DNA B1 from mitochondria of cytoplasmic male-sterile rice. Plant Cell Physiol 28:1243–1251

Shikanai T, Yang ZQ, Yamada Y (1989) Nucleotide sequence and molecular characterization of plasmid-like DNAs from mitochondria of cytoplasmic male-sterile rice. Curr Genet 15:349–354

Smith AG, Pring DR (1987) Nucleotide sequence and molecular characterization of a maize mitochondrial plasmid-like DNA. Curr Genet 12:617–623

Thorsness PE, Fox TD (1990) Escape of DNA from mitochondria to the nucleus in *Saccharomyces cerevisiae*. Nature 346:376–379

Ukai Y, Ohsawa R, Saito A (1990) Automatic determination of the order of RFLPs in a linkage group by multi-dimensional scaling method. Jpn J Breed 40 [Suppl 2]:302–303

Yamaguchi H, Kakiuchi H (1983) Electrophoretic analysis of mitochondrial DNA from normal and male sterile cytoplasms in rice. Jpn J Genet 58:607–611