Karyological Studies in Haploid Rice Plants

IV. Chromosome morphology and intragenome pairing in haploid plants of *Oryza glaberrima* Steud., as compared with those in *O. sativa* L.

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Rice varieties grown in most countries of the world belong to *Oryza sativa* L., while another cultivated species, *O. glaberrima* Steud. is known to be distributed in a rather limited area in West Africa (Porteres 1956). *O. glaberrima* is differentiated from *O. sativa* by its glabrousness of spikelets, short tough ligules, relative brittleness of leaves, stems and panicles, and death after maturity. These two rice species have the same number of chromosomes ($2n=24$) which normally pair in the F_1 , though the F_1 plants show high sterility.

Since 1957, the writer has been engaged in cytological studies of *Oryza* species, taking partial charge of the research project "Studies on the origin of cultivated rice" which the National Institute of Genetics of Japan has planned and is conducting with international cooperation. The haploid plants of *O. glaberrima* used in this study were obtained from a twin seedling, which was found when seeds of the strain were germinated for a field experiment in 1958.

The twin seedling might possibly have occurred from a poly-embryonic seed. A similar case in *O. sativa* has been reported by Ramiah et al (1933). In *O. glaberrima*, however, the writer's may be the original. Cytological observations of the haploid plants thus obtained showed that the morphological characteristics of chromosomes as well as the mode of intra-genome pairings were consistent with those previously observed in haploid plants of *O. sativa* by the writer, suggesting that the two rice species might have structurally the same chromosomes.

Materials and methods

The strain of *O. glaberrima* from which the haploid plants were found, W040, was one of several strains received from Dr. C. R. Adair of the Agricultural Research Service, Beltsville, United States, by Dr. H. I. Oka of National Institute of Genetics of Japan (P7, 232854 in Dr. Adair's list). A twin seedling was found among seven seeds germinated in a petridish. The

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two plants from the seedling were both determined to be haploid by a preliminary observation of the root tip cells. They were carefully grown in a pot, and after being separated, were transplanted in a paddy field. Then, by separating the tillers, several plants were obtained.

The techniques employed for cytological observations are as follows: For the fixation of root tips, the low temperature method (4° – 5° C, 24 hrs.), which had been used for haploid plants of *O. sativa* (Hu 1958), was chosen in view of its producing well stretched configurations of metaphase chromosomes. Root tips were taken in distilled water in fixation tubes between 3 and 4 PM when the temperature was high, and were immediately put in a refrigerator. After 24 hours, they were hydrolysed in 1N HCl for 7 to 8 minutes at 60° C. They were then stained in 1% aceto-orcein, and were squashed on a slide.

For observing meiotic divisions of PMCs, young panicles taken between 10 and 11 AM were fixed in Farmer's fluid (absolute alcohol 3 : glacial acetic acid 1) for 24 hours, and were stored in 75% alcohol. Slides were prepared by the ordinary aceto-carmin smear method. In both cases, only freshly prepared slides were used for observation and photography.

Results of observations

1. Growth of the haploid plants

Though the twin seedling was very slender when young, the plants separated from it grew well after being transplanted to the paddy field. They headed in October at approximately the same time as the diploid plants of the same strain which were raised as control. The photographs taken at 75 days after germination and at heading time are presented in Figs. 1 and 2, respectively. The two haploid plants from the twin seedling showed the same stature throughout their growing period, and no difference could be found between them. Some measurements for the haploid plants as compared with those for diploid ones are given in Table 1.

In Table 1, the ratios of $x/2x$ in *O. glaberrima* seem to be of the same order as those found in *O. sativa*.

The haploid plants of *O. glaberrima* were completely sterile, and died after heading. It seems that their death is due to the non-regenerating habit of the species, since haploid plants of *O. sativa* can survive through the winter in Taichung, even though left outdoors.

2. Morphology of chromosomes in root tip cells

In haploid rice plants, chromosomes display their size, relative arm length, and position of constrictions relatively clearly, and identification of individual chromosomes is possible, to some extent, in good preparations. A few such good preparations of root tip cells were obtained from the haploid plants of *O. glaberrima*. Micro-photographs of chromosomes from those preparations are shown in Figs. 3, 10 and 11. For the sake of comparison, a photo-

graph obtained from a haploid of *O. sativa*, which was already presented in the writer's previous paper (Hu 1958), is shown in Fig. 4. The following features can be pointed out in Fig. 3 for *O. glaberrima*, while the same can also be recognized in Fig. 4 for *O. sativa*, as already pointed out by the writer.

Fig. 1. A twin plant of *O. glaberrima*, 75 days after germination.

Fig. 2. Haploid and diploid plants of *O. glaberrima* at the time of heading.

Table 1. Measurements for haploid and diploid plants of *O. glaberrima*

Character	Haploid (x)	Diploid (2x)	Ratio of x/2x	x/2x ratio in <i>O. sativa</i> *
Plant height (cm)	56.0	164.0	.341	.557
Panicle length (cm)	14.0	21.4	.654	.659
Spikelet number per panicle	84.0	115.0	.730	.875
Top leaf length (cm)	15.5	29.7	.522	.755
Top leaf width (cm)	1.5	1.6	.940	.710
Top leaf sheath length (cm)	15.0	27.7	.560	.608
Spikelet length (mm)	6.48	8.46	.766	.720
Spikelet width (mm)	1.74	2.62	.665	.783
Stoma cell length (μ)	7.6	9.7	.783	.687

* Taichung no. 65.

1) A sub-median chromosome is the largest of the complement, (designated as No. 1).

2) The second largest one, which has sub-terminal constriction (No. 2), shows a prominent secondary constriction.

3) Two median chromosomes, one (No. 3) being as large as No. 2, and the other (No. 4) being a little smaller, are found in the complement.

4) Three sub-median chromosomes of medium size (smaller than No. 4) are found, (Nos. 5, 6 and 9).

5) A sub-terminal chromosome similar to the former in size, but showing a secondary constriction and a satellite, is recognized, (No. 7).

6) A sub-terminal chromosome, slightly smaller than No. 7, also has a satellite, (No. 8).

7) Two median chromosomes apparently smaller than Nos. 3 and 4 are recognized, (Nos. 10 and 11).

8) A sub-median chromosome is the smallest of the complement, (No. 12).

Based on the similarity in these respects, it may be assumed that *O. glaberrima*

Figs. 3, 4. 3, metaphase chromosomes in a root tip cell of haploid *O. glaberrima*. A, microphotograph ($\times 3,000$). B, camera lucida drawing. 4, metaphase chromosomes in a root tip cell of haploid *O. sativa*. A, microphotograph ($\times 3,000$). B, camera lucida drawing. The small photograph attached is to show that chromosome No. 7 has a satellite and a secondary constriction.

has the same karyotype as that of *O. sativa*. Their karyotypes may be diagrammatically shown in Fig. 5.

Which of the twelve chromosomes is the nucleolus-forming one, may then become a question. The aceto-orcein technique employed for root tip cells did not bring about a good staining of nucleoli, so that the nucleolar chromosome could

not be determined from the above observations. In this connection, a late prophase figure in an anther tapetal cell, which was stained by aceto-carmin, may be worthy of discussion. In that cell (shown in Fig. 9), the nucleolar

Fig. 5. Diagrammatic representation of the karyotype, common to *O. glaberrima* and *O. sativa*.

chromosome (shown by a short arrow) had a satellite, but no secondary constriction was seen. Apart from the nucleolus, a chromosome with two round heads (shown by a long arrow), which may possibly correspond to No. 7 in the diagram in Fig. 5, is found. It may then be assumed that the Sat-chromosome, designated as No. 8, might be the nucleolus-forming one. This assumption is supported by the photograph of a PMC in Fig. 6, in which the nucleolar chromosome also showed no secondary constriction. However, some diakinetid cells showed two nucleolar chromosomes, both being characterized by the presence of a satellite. It is known in *O. sativa* that one

or two pairs of nucleolar chromosomes occur depending on the strain (Sakai 1938, Oka and Kao 1956). It then follows that the Sat-chromosome with no secondary constriction (No. 8) might be the first nucleolar-forming one, and the other Sat-chromosome with a secondary constriction (No. 7) might be the second one.

Fig. 6. Chromosomes at early diakinesis of haploid *O. glaberrima* ($\times 1,450$). Numbering of chromosomes was made tentatively as shown in B.

Fig. 7. Frequency distributions of number of pairings in a haploid cell compared between *O. glaberrima* and *O. sativa*.

3. Intra-genome pairing of chromosomes in meiosis

Each chromosome is represented once in a haploid plant. However,

Fig. 8. Histograms showing frequencies of meta-anaphasic cells with different numbers of bivalents and trivalents, compared between *O. glaberrima* and *O. sativa*.

Figs. 9-19. All show chromosomes of haploid *O. glaberrima*. 9, late prophase cell in anther tapetal tissue. $\times 2,500$. Shown by a long arrow—A secondary-constricted chromosome with a satellite (two round heads), possibly corresponding to No. 7. Shown by a short ar-

intragenome pairings of chromosomes were observed in haploid plants of *O. sativa* (Hu 1957). The haploid plants of *O. glaberrima* also showed pairings of chromosomes in the same manner, as described below.

In early diakinesis, as the chromosomes become clearly recognizable, it was frequently found that some of them were connected end to end forming a telosynaptic bivalent or trivalent. A photograph showing such pairings is shown in Fig. 13, in which a Y-shaped chromosome, which is apparently a trivalent, and a V-shaped one, which is a bivalent, are recognized.

In late diakinesis, in addition to terminally paired chromosomes connected by a thin chromatic thread, ones located close to each other with no visible connection were found. It appeared as if two univalents were in secondary association. The photograph in Fig. 14 shows a typical configuration of late diakinesis, in which two trivalents, one bivalent (very close to one of the two univalents attached to the nucleolus, but confirmed to be apart from it), and an assembly of two univalents, are recognized.

At metaphase-I, seemingly real bivalents or trivalents, and groups of closely gathered univalents, were both found in the same manner as in late diakinesis. It seemed that the "real" bivalents or trivalents tended to be located in the central part of the spindle, while univalents, though forming assemblies, were scattered in the cell.

The above-mentioned patterns of intra-genome pairings observed in diakinesis through Metaphase-I of haploid *O. glaberrima* are in complete accord with what has been observed in haploid plants of *O. sativa*. Thus, the writer distinguishes bivalents or trivalents and assemblies of univalents in the same manner as in his previous paper (Hu 1957), considering that the former is due to true pairing and the latter is due to secondary association.

Frequencies of these two types of pairings found in diakinesis through metaphase-I are given in Tables 2 and 3. Table 2 shows the frequencies of true bivalents and trivalents, and Table 3 shows the total frequencies includ-

row—Nucleolus-attached chromosome with a satellite, possibly being No. 8. 10, metaphase of a root tip cell, showing two Sat-chromosomes. (Shown by a long arrow—No. 7, by a short arrow—No. 8). Two large chromosomes may possibly be Nos. 1 and 2, while two other chromosomes are gathered together and appear to be one in the lower part of the figure. $\times 3,000$. 11, metaphase chromosomes in a root tip cell, tentatively numbered. $\times 3,000$. 12–20, all show PMCs. $\times 2,000$. 12, early diakinesis. The chromosome indicated by an arrow is No. 7. 13, diakinesis. A Y-shaped trivalent and a V-shaped bivalent are seen. 14, late diakinesis. 2 trivalents, 1 bivalent located close to the nucleolus, 1 group of 2 univalents, and 2 univalents attached to the nucleolus, are recognized. 15, a polar view of metaphase-I. 1 trivalent, 1 univalent attached to a bivalent, and 6 univalents scattered, are recognized. 16, metaphase-I. 1 trivalent, 1 univalent attached to a bivalent, 1 group of 2 univalents, and 4 univalents scattered, are recognized. 17, metaphase-I. Univalents are in secondary association showing a $1(3)+4(2)+1$ configuration. 18, metaphase-I. 2 stretched bivalents, 2 groups of 2 univalents, and 4 univalents scattered, are recognized. 19, metaphase-I. The pairing type seems to be $2\text{II}+1(3)+1(2)+1+1+1$. 20, meta-anaphase-I. The pairing type seems to be $2(3)+2(2)+1+1$.

ing both of the two pairing types, arranged according to the "number of pairings" (viz., one pairing makes a bivalent, and two make a trivalent or

Table 2. Frequency of cells with bivalents and trivalents in PMC's of haploid *O. glaberrima*

Configuration	Diakinesis	Diametaphase	Meta-anaphase	Total	%
Univalents only	60	32	173	265	65.8
1II	16	6	72	94	23.3
2II	5	3	13	21	5.2
3II		3		3	0.7
1III	2	1	9	12	2.9
1II+1III	1	1	2	4	1.0
2II+1III		1	1	2	0.5
2III		1		1	0.3
1II+2III		1		1	0.3
Total	84	49	270	403	

Table 3. Frequency of cells with different numbers of pairings, including both primary and secondary ones, in haploid *O. glaberrima*

No. of pairings*	Types of pairing			Diakinesis	Dia-metaphase	Meta-anaphase	Total	%
	(3)	(2)	(1)					
0			12	15		25	40	9.9
1		1	10	23	6	53	82	20.4
2	1	0	9	2	1	6	9	25.3
"		2	8	18	6	69	93	
3	1	1	7	4	2	15	21	21.6
"		3	6	11	8	47	66	
4	2	0	6	1	1	2	4	14.4
"	1	2	5	3	6	12	21	
"		4	4	4	5	24	33	
5	2	1	4		2	4	6	6.0
"	1	3	3	1	2	6	9	
"		5	2	1	5	3	9	
6	2	2	2	1	2	2	5	2.5
"	1	4	1		3	2	5	
7	2	3						0.0
No. of cells observed				84	49	270	403	
Average no. of pairings				1.86	3.53	2.33		

* Number of pairings needed to make up bivalents, trivalents, an assembly of univalents, etc.; for instance, one pairing makes a bivalent, and two secondary pairings and a pairing of three chromosomes make two bivalents, as shown in the column titled "types of pairing".

two bivalents). As the data in Table 3 show, the "number of pairings" ranged from zero to six, and the number of chromosome groups (including both true and secondary pairings, each consisting of two or three) ranged from zero to five. The maximum pairing found in the data was the cases

in which one group of three and four groups of two were formed, or two groups of three and two groups of two were formed.

Examining statistically the difference in pairing frequency between stages, it is found that frequencies at diakinesis and meta-anaphase (late metaphase-I) do not differ significantly, but the frequency at dia-metaphase (early metaphase-I) is higher than that at the former two stages. This could be due to the tendency for chromosomes to be gathered in early metaphase. At diakinesis as well as at meta-anaphase, the frequency distribution had its mode at two pairings forming two groups of two, as shown in Table 3. This distribution was found not to fit a Poisson distribution, calculated assuming random pairings of chromosomes.

The frequency distribution in Table 3 was then compared with the similar datum for haploid plants of *O. sativa*, which the writer had obtained previously (Hu 1957). The results are diagrammatically shown in Fig. 7. In the figure, the distributions for *O. sativa* and for *O. glaberrima* appear to be comparable to one another. A t-test for the average number of pairings showed no significant difference between them. The same comparison was made further using the frequency of true bivalents and trivalents. It was found that the distributions for the two species were quite similar, as shown in Fig. 8. Though *O. glaberrima* showed higher frequencies of cells with two or more bivalents or trivalents, this difference was not significant.

The various types of chromosome distribution at anaphase-I are given in Table 4. The frequencies of 6:6, 1:11 and 0:12 were more than expected,

Table 4. Types of chromosome distribution at anaphase-I, in haploid *O. glaberrima*

	Mode of distribution							No. of cells
	6:6	5:7	4:8	3:9	2:10	1:11	0:12	
Observed	35	47	18	12	5	4	5	126
Expected*	29.4	48.8	29.0	13.3	4.4	0.8	0.1	125.8

* Assuming random distribution.

and that of 4:8 was less. Chromosome bridges, "stretched bivalents", lagging univalents causing separation of sister chromatids, and restitution nuclei, were seen in a few cells. The second division seemed to proceed with relative regularity, but the resulting tetrads and microspores were irregular in size and shape. These data for anaphase-I and later stages also agree with the corresponding data from haploid plants of *O. sativa*.

Discussion

Haploid plants are of great theoretical interest to students of genetics for the following three reasons, i.e., 1) the pattern of intra-genome pairings of chromosomes, which suggests the inner structure of the genome, can be observed, 2) since each chromosome is represented only once, a favorable

material for a detailed observation of the karyotype is provided, and 3) diploid plants obtained from a haploid can be used as a genetically pure line of the species. The problems to be discussed in this paper are the karyotype and intra-genome pairings of chromosomes in *O. glaberrima*, as compared with those formerly investigated by the writer (Hu 1957, 1958) in *O. sativa*.

First, the karyotype of *O. glaberrima* is a new problem so far as the writer knows. It has only been suggested in this connection that *O. glaberrima* might have a genome similar to *O. sativa*, since chromosomes of the two species normally pair in the F_1 (Morinaga and Kuriyama 1956, Indian Council of Agricultural Research 1956). The present study has shown that the karyotype of *O. glaberrima* is the same as that of *O. sativa*, and those of both species can be tabulated as follows:

Size	Median	Sub-median	Sub-terminal, 2nd constriction being	
			Present	Absent
Large ($>2\mu$)	No. 3	No. 1	No. 2	
Medium ($1.5-2\mu$), without Sat. with Sat.	No. 4	Nos. 5, 6 & 9	No. 7	No. 8
Small ($<1.5\mu$)	Nos. 10 & 11	No. 12		

The numbers for respective chromosomes in the above table were first chosen for those of *O. sativa* (a strain of "Japonica" type) in the order of their size, while the same were found to be applicable to *O. glaberrima*. Thus, nine types of chromosomes could be distinguished in both species, though the difference in size between Nos. 3 and 4 was not always apparent.

As mentioned before, it seems that No. 8 is a nucleolar chromosome. In some diakinetic cells, however, two nucleolus-attached chromosomes are seen, and No. 7 also appears to have nucleolus-forming ability. In *O. sativa*, there are one or two pairs of nucleolar chromosomes in different strains (Selim 1930, Sakai 1938), and the second one seems to have a low nucleolus-forming ability relative to the first, differing according to strains (Oka and Kao 1956). Whether such a situation as in *O. sativa* occurs in *O. glaberrima* or not is a problem for further study.

Secondly, regarding the frequency of pairings in a haploid set of chromosomes, it was also found that *O. glaberrima* and *O. sativa* do not differ significantly. The average numbers per cell of truly paired and secondarily associating chromosomes can be summarized from the data as follows:

	Bivalent	Trivalents	Group of two	Group of three
<i>O. sativa</i>	0.381	0.008	0.245	0.040
<i>O. glaberrima</i>	0.378	0.044	0.470	0.056

A haploid plant of a strain of *O. sativa* f. *spontanea* Roschev. from

India may also have a similar distribution of intra-genome pairings, though its observation is still under way.

We should consider the reason for intra-genome pairings in haploid plants. The bivalents and trivalents found were generally due to "loose pairings" of chromosome ends. Morinaga and Fukushima (1934) regarded such pairings as coincidence or artifact. However, the writer has formerly demonstrated with haploid plants of *O. sativa* that the distribution of frequencies of cells with different numbers of pairings did not fit a Poisson distribution calculated assuming random pairing. The distribution in *O. glaberrima* also wasn't found to fit such a Poisson distribution. This suggests that the pairings tend to occur between definite chromosomes.

In diploid plants, the cause of secondary association between bivalents has been a subject of dispute. However, in haploids, it is apparent that chromosomes first pair in a stage before diakinesis, and secondary association occurs between those which have failed to pair. Thus, the primary and secondary pairings together make up a certain pattern of intra-genome pairings.

The maximum pairing found in haploid *O. sativa* was $1_{II}+2(3)+2(2)$, or $2(3)+3(2)$; That in *O. glaberrima* was $2_{III}+1_{II}+1(2)$, while the remaining two univalents were both attached to the nucleolus (Fig. 14). If we take the latter two as a group, we can enumerate five groups of chromosomes. However, so far as the writer's observation goes, no cell with six groups, or no group consisting of more than three chromosomes, was found in either *O. sativa* or *O. glaberrima*. Then the maximum haploid pairing in both species may be assumed to be five groups. This number agrees with the maximum number of secondarily paired groups of bivalents in diploid plants.

Christensen and Banford (1943) found in haploid plants from twin seedlings of pepper (*Capsicum annum* L.; $n=12$) that the number of groups of completely paired or at least associated chromosomes reached six. Thus the mode of intra-genome pairing may be characteristic of the species. We may assume that the tendency for pairing is as a whole controlled by the genotype. This may be true if we take the term "genotype" in its broad sense. However, if it is taken to be genic constitution, it is difficult to explain why *O. glaberrima* and *O. sativa*, in spite of their profound genic differences, have shown the same pattern of intra-genome pairings. In so far as we accept that pairing occurs between homologous segments of chromosomes (carrying the same gene loci), the intra-genome pairings in haploid plants should be an indication of the presence of homology between chromosomes of a genome.

The above discussion leads to the conclusion that in both *O. sativa* and *O. glaberrima*, there might be homologous segments among chromosomes of a haploid set, and these two species might have structurally the same chromosomes in the sense that the distribution of such homologous segments in their chromosomes is the same. This suggests that they might have arisen

from a common ancestral plant.

O. sativa and *O. glaberrima* have so far been considered to be distinct species. As mentioned in the introduction, they differ distinctly in several respects. Examining many strains of both species, Morishima and Oka (1959) have shown that the variation between them in the score of a discriminant function constructed out of measurements for ligule length and other five character was of discontinuous nature. The F_1 s between them show a high sterility, pollen fertility being usually less than 3%. In view of the normal pairing of chromosomes in the F_1 s, this sterility seems to be due to genic recombinations in the gametes, possibly the same mechanism as demonstrated for the semi-sterility of F_1 s between distantly related strains of *O. sativa* (Oka 1957). Among wild species of *Oryza*, it is known that *O. perennis* Moench. and *O. sativa* f. *spontanea* Roschev. are close to *O. sativa*, and *O. breviligulata* A Cheval. is close to *O. glaberrima* (Sampath 1951). F_1 s between all these species show no disturbance in chromosome pairing, while hybrid sterility occurs between *O. breviligulata* and *O. perennis* or *O. sativa* f. *spontanea*. Thus, the whole group comprises a net-work of species differentiated by hybrid sterility and other isolating barriers. The conclusion reached in the present study that *O. sativa* and *O. glaberrima* have structurally the same chromosomes, suggests that they and related wild species might have been differentiated largely as a result of genic changes, and gross changes of chromosomes have not been involved in the process. The creation of such a great deal of genetic diversity without chromosomal changes may be considered to be a characteristic feature of the genus *Oryza*.

Summary

Haploid plants were obtained from a twin seedling of a strain of *O. glaberrima*. The karyotype of the plants was investigated first using root tip cells. Nine types of chromosomes could be distinguished by their size, position of constriction, and presence or absence of satellite and secondary constriction. The karyotype of *O. glaberrima* was thus found to be identical with that of *O. sativa*, formerly observed by the writer.

Secondly, meiosis in PMCs was investigated with regard to intra-genome pairings of chromosomes. Seemingly true pairings to form bivalents and trivalents, and assemblies of two or three univalents which appeared to be due to secondary association, could be distinguished. The frequency distribution of cells with different numbers of pairings was similar to that found in haploid plants of *O. sativa*, and the maximum pairing found was five groups of chromosomes.

These observations lead to the conclusion that *O. glaberrima* might have structurally the same chromosomes as those of *O. sativa*, and these two species might have arisen from a common ancestral plant.

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