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Cytogenetical Studies on the Genus *Oryza*

X. Cytogenetics of Tetraploid F_1 Plant between Amphiploid *punctata-eichingeri* and BBCC Genome Species

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In 1967. Katayama proposed that diploid *punctata* strain is the B genome donor based on the cytogenetical evidences of the diploid F, plants between diploid *punctata* strain and its related species. To corroborate the result, the tetraploid F_1 plants were produced from the crosses between amphiploid *punctata-eichingeri* and the species having the BBCC genomes. The results of the cytogentical observations of both the diploid F_1 (diploid *punctata* x C genome species) and tetraploid F_1 (synthesized amphiploid strain x BBCC genome species) plants support the opinion that diploid *punctata* is the donor of the B genome.

INTRODUCTION

There are three different opinions to date on the genome constitution of diploid ***punctata*** strain. They are BB (Katayama, 1967), CC (Sampath, 1962 ; Gopalakrishnan and Sampath, 1966) and homologous to one of BBCC and partially homologous to AA and CC (Hu, 1970).

In our previous reports (Katayama and Ogawa, 1974; Ogawa and Katayama, 1973, 1974), the authors have also made clear that diploid ***punctata*** strain could be the species having B genome from the cytogenetical standpoints of the diploid F_1 plants between diploid ***punctata*** strain and the C genome species, 0. *officinalis*, ***0. eichingeri, 0.*** *collina* and an intermediate ***punctata-eichingeri.***

In our further work, the facts described above were also confirmed by the chromosome pairing at meiosis of the tetraploid F_1 plants between synthesized amphiploid and the species with the BBCC genomes (Katayama, 1977).

For further corroboration in these facts, another experiment was designed.

The present paper deals with the results obtained from morphological and cytogenetical observations of the tetraploid F_1 plants of amphiploid ***punctata-eichingeri x*** species having the BBCC genomes.

MATERIALS AND METHODS

As shown in Table 1, amphiploid ***punctata-eichingeri*** was used as the female parent, while 0. ***punctata (4x,*** BBCC, W 1145), 0. ***minuta (4 x,*** BBCC, W 0016, Y22), 0. *malabarensis* ***(4x,*** BBCC, W 021) and 0. ***malampuzhaensis (4x,*** BBCC, W 1159) were used as male parents. The amphiploid ***punctata-eichingeri*** was artificially

Table 1. Materials used in this experiment.

Acc. no.	Species	2n	Genome constitution
4x-l	amphiploid *punctata-eichingeri*	48	BBCC[1)]
w1145	0. *punctata*	48	BBCC[2)]
Y22	*0. minuta*	48	BBCC[1)]
W0016	0. *minuta*	48	BBCC"
wo21	0. *malabarensis*	*48*	BBCC[2)]
W1159	*O. malampuzhaensis*	*48*	BBCC[2)]

[1)] National Institute of Agricultural Sciences, Hiratsuka.
[2)] National Institute of Genetics, Mishima.

induced by Sampath (CRRI) with colchicine treatment of the sprout from the diploid F_1 plant of 0. *punctata (2 x) x 0. eichingeri (2x)* and introduced to Japan in 1971 by Watanabe (Watanabe and Sampath, 1972; Watanabe, 1975).

0. *eichingeri* has *2* n=24 chromosomes (Tateoka, 1965a) and its genomic constitution was similar to that (CC) of 0. *officinalis* (Hu, 1970; Ogawa and Katayama, 1973). All these strains were obtained from the genetic stocks preserved by the National Institute of Genetics, Mishima. A strain of 0. *minuta (Y 22)* was from the National Institute of Agricultural Sciences, Hiratsuka.

Castration was made by the clipping method. Seeds were grown in test-tubes under sterilized conditions. Heading was accelerated under the controlled day length of 8 hours.

After fixation with Farmer's solution (alcohol 3 : acetic acid 1), root-tips and PMCs were stained with aceto-carmine solutione as usual.

RESULTS AND CONCLUSION

Percentage of seed setting in each cross combination was considerably high as shown in Table 2. Plants from these seeds were almost true hybrids in their appearance.

The amphiploloid *punctata-eichingeri* and the tetraploid F_1 plants from the cross between the amphiploid *punctata-eichingeri* and the species with BBCC genomes were morphologically similar to the species having the BBCC genomes, *i. e., 0. punctata (4 x), 0. minuta, 0. malabarensis* and 0, *malampurhaensis* (Fig.

Table 2. Cross-combinations and the results.

Acc. no.	Cross combination	No. of flowers crossed	No. of seeds obtained	% of seeds setting	No. of seeds sown	% of true F, plant
A (4x-l)	amphi. *punctata-eichingeri*	*27*	*10*	*37*		
F, plant						
E	4x-1 × W1145	48	26	54	19	100.0
D	4x-l x Y 22	44	24	54. 5	24	88.0
H	4x-1 × W0016				11	87. 5
F	4x-l x wo21	53	40	75.5	12	100.0
G	4x-1 × W1159	43	35	81	11	100.0

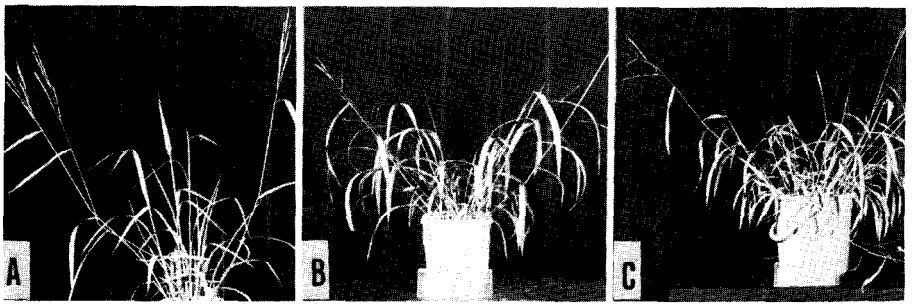

Fig. 1. Adult **plants** of BBCC genome species and F_1 plant between amphiploid punctata-eichingeri and BBCC genome species.

A: 0. *punctata* (4x, W1145).
B: F_1 plant (D) of amphiploid *punctata-eichingeri* × *0. minuta* (Y22).
C: 0. minuta (Y22).

l-A, -B, -C), except grain size of the F_1 plants and anthocyanin colorations of node, internode and leaf-sheath in some F_1 plants, as shown in Table 3.

The ears of the amphiploid *punctata-eichingeri* and the tetraploid F_1 plants, D, E, G and H, are of spreading type with the spikelets being large and deciduous, consisting of long and double awns (Table 3).

Seed fertilities of the amphiploid *punctata-eichingeri* (plant A) and the tetraploid F_1 plants (E, D, H and G) were very high as compared to those of the diploid F_1 *punctata* (2x)*-eichingeri* (2x) which are completely sterile (Table 4).

In all plants used in the present experiment, 48 chromosomes were counted (Fig. **Z-A).**

Chromosome configurations were examined at MI of PMCs in the amphiploid *punctata-eichingeri,* and the results were given in Table 4. This table shows that almost all chromosomes observed at MI were bivalents.

All of the tetraploid F_1 plants (D, E, F, G and H) which were produced from the crosses between amphiploid *punctata-eichingeri* and the species with the BBCC genomes, showed similar patterns of chromosome pairing at MI of PMCs (Table 4). Number of bivalents per cell at MI of PMCs ranged from 18 to 24 with a mean of about 22–24. These chromosome configurations show that the genomes of the amphiploid *punctata-eichingeri* are homologous with those of the species with the BBCC genomes, such as 0. *punctata* (4x) and 0. *minuta.*

In addition to the normal bivalent formation as described above, these tetraploid F_1 plants (D, E, F, G and H) rarely formed univalents, trivalents and/or tetravalents (Table 4). Tetravalents appeared as o-type (Fig. 2-B) or zigzag type (Fig. Z-C). This fact suggests that one reciprocal translocation should have occurred between 2 nonhomologous chromosomes concerned. Hu and Chang (1965) observed such chromosome configurations in the F_1 plants between 0. *punctata* (4x) and its related species, and assumed that these species would be differentiated through translocation.

From the morphological characteristics, Sampath (1962) supposed that Ceylonese *officinalis* **(0.** *collina*) and diploid *punctata* strain have the B and C genomes, respectively. Morinaga and Kuriyama (1960) reported already that

Tabl 3. Comparison of morphological

Characteristics	A (4x-l)	F_1 plants			
		E	D	H	F
Plant height	high	inter mediate	inter mediate	high	inter mediate
Panicle length	inter mediate	long	inter mediate	short	long
Habit	semi-erect	semi-erect	semi-erect	semi-erect	semi-erect
Awn length	long	long	long	long	long
Color					
stigma	purple	purple	purple	purple	purple
node	purple	purple	purple	colorless	colorless
internode	purple	colorless	colorless	colorless	colorless
leaf-sheath	dilute purple	purple (under part)	purple (under part)		colorless
Spikelet					
length	0. 65	0. 61	0.70	0. 68	
width	0. 25	0. 24	0. 24	0. 22	
L/W	2. 60	2. 60	2. 90	3.09	
Hulled rice					
length	0. 48	0.46	0. 52	0. 56	
width	0. 20	0. 21	0. 19	0. 19	
L/W	2.46	2.19	2. 74	3.00	

Table 4. Chromosome pairing at MI and fertility of F_1 plants between amphiploid *punctata-eichingeri* and BBCC genome species.

Acc. no.	2n	No. of cells observed	Chromosome pairing at MI				Fertility	
			IV	III	II	I	pollen (%)	seed (%)
A (4x-1)	48	18			23. 9(22-24)	0. 1 (O-l)	98.00	40.91
F_1 plant		25						
E-6	48	30	0.32 (O-l)		23. 0(21-24)	0. 8(0-2)	93.33	73.53
D-3	48	39	0.23 (O-2)	0.1 (O-l)	23. 0(18-24)	0. 8(0-4)	54.29	29.17
H-l	48	27		0.5 (O-1)	23. 0(22-24)	0.5 (O-l)	93.40	68.63
F-5	48		0.67 (O-2)		22. 0(20-24)		50.32	22. 81
G-l	48	23	0. 22 (O-2)	0. 04(0-1)	23. 0(20-24)	0. 2(0-3)	20.97	14.40

Ceylonese *officinalis* has a homologous genome with 0. *officinalis* from the cytogenetical investigations. The authors also obtained a similar result to Morinaga and Kuriyama (Ogawa and Katayama, 1973).

From the cytogenetical standpoints in which the genome of diploid ***punctata*** strain is homologous with one of the BC genomes of 0. ***minuta,*** and also partially

characteristics of F, plants and their parents.

G	w1145	*Y22*	wo21	W1159
high	high	*low*	inter mediate	high
inter mediate	inter mediate	short	inter mediate	inter mediate
semi-erect	semi-erect	procumbent	semi-erect	semi-erect
long	short	short	short	
purple	purple	purple	purple	purple
colorless	colorless	colorless	colorless	colorless
purple	colorless	colorless	colorless	colorless
colorless	colorless	colorless		dilute purple
0. 65	0. 50	0. 47	0.53	0. 56
0. 26	0. 21	0.16	0.21	0.23
2.50	2.38	2.94	2. 59	2. 43
0. 50	0. 40	0. 35	0.46	0. 49
0. 20	0. 17	0.14	0.17	0. 19
2. 50	2. 42	2. 50	2.43	2. 55

homologous with the A genome of 0. ***sativa.*** Hu (1970) proposed that the genome of the diploid ***punctata*** strain could not be identified as A, B or C. The mean number of bivalents per cell in this datum, however, is only 2.3 in the cross with the C genome and 0.54-3.34 in the cross with the A genome, respectively. The results suggest that the genome of diploid ***punctata*** strain differs from C and A.

The amphiploid ***punctata-eichingeri*** used in the present experiment was artificially produced by Sampath and studied cytologically by Watanabe and Sampath (1972) based on the hypothesis in which African 0. ***punctata (4x)*** resulted from spontaneous polyploidization of the diploid F_1 plants between diploid ***punctata*** (BB) and 0. ***eichingeri*** (CC). The results described above show with reasonable certainty that the amphiploid ***punctata-eichingeri*** has homologous chromosomes with the BBCC genome species as supposed by Watanabe and Sampath (1972).

The zymograms for peroxidase, acid phosphatase and esterase in diploid ***punctata*** strain differed from those of the other related species or strain, 0. ***eichingeri, 0.*** *collina* and intermediate ***punctata-eichingeri*** strain (Katayama and

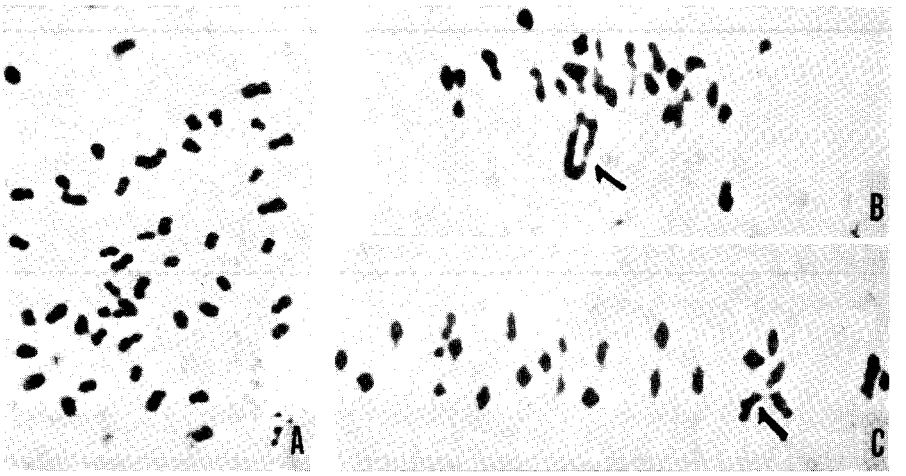

Fig. 2. Somatic chromosome and chromosome configurations at MI of plant of the cross, amphiploid *punctata-eichingeri* × BBCC genome species.

A: Somatic chromosome number showing 2n=48.
B: MI of F_1 plant (D) showing 1 IV (o-type, arrow) +21 II +2 I.
C: MI of F_1 plant (F) showiug 1 IV (zig-zag, arrow) +21 II +2 I.

Chern, 1973). The zymograms for peroxidase and acid phosphatase obtained in the amphiploid *punctata-eichingeri* are similar to those of the diploid F_1 plants of 0. *punctata* (W 1515) x 0. *officinalis* (W 0561) and of 0. *punctata* (W 1474) x *0. officinalis* (W 0561) (Chern and Katayama, 1977).

The present results are in good agreement with those of the description by one of the authors (Katayama, 1977) and support those described already by the authors (Katayama, 1967; Katayama and Ogawa, 1974; Ogawa and Katayama, 1973, 1974). The authors emphasize again that the diploid *punctata* strains are the donor of the B genome.

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