Cytogenetic Studies on the Artificial Polyploids in the Genus *Oryza*

2. Colchicine-induced octoploid plant of *Oryza minuta* PRESL.

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Synopsis. A colchicine-induced octoploid plant of wild rice, *Oryza minuta* PRESL. was studied cytogenetically. In Diakinesis and MI of PMCs, the average number of tetravalents formed was 9.14 and 7.26, respectively. Very few trivalents or univalents were observed in both stages. As the meiotic stages proceeded from AI to Pollen Tetrad, the percentage of irregularities observed increased remarkably. Most of such abnormalities seemed to be due to the non-orientation of bivalents in MI. As a result of such irregularities, this plant produced only abortive pollens, thus the seed fertility being extremely low.

The female gamete, however, seemed to function rather normally, giving rise to a number of amphiploids with the pollens of octoploid-*latifolia*. Some amphiploids were also secured between tetraploid-*sativa* and octoploid-*minuta* taking the former species on the female side, though it was rather difficult to make cross.

Introduction

The authors are attempting chromosome multiplication of species in the genus *Oryza* and their F_1 hybrids with the view to synthesize new polyploid types of rice. The results of cytogenetic studies on the colchicine-induced octoploid-*latifolia* were reported in the previous paper (WATANABE and ONO, 1965). In *O. minuta* PRESL., an octoploid plant was also artificially induced by treating the germinated seeds with colchicine.

This paper deals with the cytogenetic studies on this octoploid-*minuta*.

Material and Methods

Oryza minuta PRESL. is an allotetraploid wild species indigenous to the Philippines, for which the genomic formula, BBCC, was given by Dr. T. MORINAGA (1943). The material used in this experiment is the original line with which the chromosome number was first decided by him in 1934, and has been carefully maintained at the Department of Physiology & Genetics, National Institute of Agricultural Sciences.

The method of the colchicine treatment and the cytogenetic observations are much the same as in the previous paper.

Results

I. Colchicine Treatment

In the spring of 1963, 20 germinated seeds of *O. minuta* PRESL. were treated with colchicine. Out of those 20 young seedlings, 12 survived the treatment and reached maturity. To prevent the loss of matured seeds, each panicle was kept in a paraffin

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paper bag. From the seeds collected, comparatively large 20 grains were selected and sown in the next spring. Among the seedlings obtained, one had dark green leaves and showed somewhat slow growth from the beginning. The plant was soon ascertained as an octoploid by the examination of the somatic chromosome number.

II. Karyological Observations

(A) Somatic Chromosomes

Original species, *Oryza minuta* PRESL., is an allotetraploid of which somatic chromosome number is 48, and the genomic constitution is BBCC. In RTCs of the dark green plant above mentioned, the authors counted clearly 96 chromosomes, that is twice as many as of its original species (Fig. 1-2). Therefore, this is, no doubt, an octoploid plant of which genomic constitution is the

Fig. 1. Somatic chromosomes in the root-tip cells of the original 4x and its colchicine-induced 8x *O. minuta*.

1 : 4x (2n=48) 2 : 8x (2n=96)

Table 1. Chromosome configurations and their frequencies in PMCs of octoploid *Oryza minuta* PRESL. (2n=96)

(A) In Diakinesis :		(B) In MI :	
Chromosome configuration	Frequency	Chromosome configuration	Frequency
4 _{IV} +40 _{II}	1	3 _{IV} +42 _{II}	6
5 _{IV} +38 _{II}	2	4 _{IV} +40 _{II}	2
5 _{IV} +37 _{II} +2 _I	1	4 _{IV} +39 _{II} +2 _I	2
6 _{IV} +36 _{II}	6	5 _{IV} +38 _{II}	6
6 _{IV} +35 _{II} +2 _I	1	5 _{IV} +3 _{III} +33 _{II} +1 _I	1
7 _{IV} +34 _{II}	3	6 _{IV} +36 _{II}	4
8 _{IV} +32 _{II}	4	6 _{IV} +35 _{II} +2 _I	2
9 _{IV} +30 _{II}	8	7 _{IV} +34 _{II}	7
10 _{IV} +28 _{II}	8	7 _{IV} +32 _{II} +4 _I	1
11 _{IV} +26 _{II}	6	8 _{IV} +32 _{II}	4
12 _{IV} +24 _{II}	5	9 _{IV} +30 _{II}	3
13 _{IV} +22 _{II}	5	10 _{IV} +28 _{II}	2
Total	50	11 _{IV} +26 _{II}	4
		12 _{IV} +24 _{II}	3
		14 _{IV} +20 _{II}	2
		15 _{IV} +17 _{II} +2 _I	1
		Total	50

Table 2. Meiotic behaviour of chromosomes in PMCs of octoploid *Oryza minuta* PRESL. (2n=96)

(A) In Diakinesis :

Type of chromosome association Items researched	I	II	III	IV	Total number of bivalents calculated*	Number of PMCs observed
Mode	0	28 : 30	—	9 : 10	48	50
Range	0~2	22~40	—	4~13	47~48	
Average	0.08	29.68	—	9.14	47.96	

(B) In MI :

Type of chromosome association Items researched	I	II	III	IV	Total number of bivalents calculated*	Number of PMCs observed
Mode	0	34	0	7	48	50
Range	0~4	17~42	0~3	3~15	46~48	
Average	0.30	32.60	0.06	7.26	47.82	

* Calculated a trivalent as one bivalent, a tetravalent as two, respectively.

duplication of BBCC.

(B) Meiosis in PMCs

As was the case in octoploid-*latifolia* previously reported, anther development of the octoploid-*minuta* was also delayed, and meiosis in PMCs was observable only when the top floret of the panicle was about to come out of the flag-leaf sheath. Chromosome configurations were examined on 50 PMCs, each in Diakinesis and in MI. The results obtained were given in Tables 1 and 2.

In Diakinesis, tetravalents ranging from 4 to 13, and bivalents from 22 to 40 appeared together with one nucleolus (Fig. 2-3,4 and Fig. 3-1). Univalent chromosomes were observed in only 2 cells out of 50 PMCs examined, and no trivalent chromosome was met with. The mode of tetravalents was 9 and 10, and the average number of tetravalents per PMC was 9.14. On the other hand, the mode of bivalent chromosomes was 28 and 30, and the average number of bivalents per PMC was 29.68 (Tables 1-A, -B and Figs. 2, 3).

In MI, the number of tetravalents ranged from 3 to 15, and the number of bivalents ranged from 17 to 42, the range of numbers being somewhat wider than in Diakinesis. Univalent chromosomes, 1 to 4 in number, were also observed in 7 cells, and trivalents, 3 in number, were met with only in one cell. The mode of tetravalent chromosomes was 7, and the average number of tetravalents per PMC was 7.26, while the mode of

Fig. 2. Diakinesis and MI plates in the original 4x and its colchicine-induced 8x *O. minuta*. ($\times 1,500$)

1-2 : 4x *O. minuta*

1 : Diakinesis (24_{II})

3 : Diakinesis ($8_{IV} + 32_{II}$)

5 : MI ($4_{IV} + 39_{II} + 2_{I}$)

7 : MI ($15_{IV} + 17_{II} + 2_{I}$)

3-7 : 8x *O. minuta*

2 : MI (24_{II})

4 : Diakinesis ($11_{IV} + 26_{II}$)

6 : MI ($6_{IV} + 35_{II} + 2_{I}$)

bivalent chromosomes was 34, the average number of bivalents per PMC being 32.60 (Tables 2-A and -B).

In either stages, PMCs having only tetravalents (24_{IV}) or bivalents (48_{II}) have never been observed. The tetravalent chromosomes are almost always shaped as 0 (ring), and the 3 trivalents observed assumed the shape as I (chain). In about one-fourth of the PMCs examined (25.75%), a few bivalents were not enough orientated to the equatorial plane when the main group of bivalents and multivalents were going to disjoin. Though only once, there was observed an unexpected PMC with only 43 chromosomes (Fig. 4-1). The chromosome configuration in this haploid PMC, however, was $6_{IV}+12_{II}$, instead of 24_{II} which was the case in tetraploid-*minuta* (Figs. 2-1 and -2). In AI or TI, lagging chromosomes ranging from 1 to 5 or more were often observed, and tri-polar cells

Fig. 3. Microphotographs of PMCs in diakinesis and MI of the colchicine-induced $8x$ *O. minuta*.

1 : Diakinesis ($11_{IV}+26_{II}$)

2 : MI ($3_{IV}+42_{II}$)

3 : MI ($5_{IV}+38_{II}$)

4 : MI ($5_{IV}+3_{III}+33_{II}+1_{I}$)

5 : MI ($7_{IV}+34_{II}$)

6 : MI ($12_{IV}+24_{II}$)

and chromosome bridges were occasionally met with.

In the second division, laggards or micronuclei were observed in nearly 50% of MII, and 76% of AII or TII. As the result of such irregularities, various numbers of micronuclei were formed in pollen quartets, though they were normal in size and shape. Normal pollen quartets without micronuclei were rather few, and "Meiotic

Table 3. Frequency of laggards of micronuclei in each meiotic stage of octoploid *Oryza minuta* PRESL. ($2n=96$)

No. of laggards or micronuclei	Stage	MI	AI-TI	Interk.	MII	AII-TII	Pollen Tetrad	
0		124	33	109	73	47	0-0-0-0	93
1		29	31	47	28	52	0-0-0-1	73
2		9	19	11	22	36	0-0-1-1	58
3		0	5	3	9	24	0-1-1-1	15
4		0	5	0	3	11	1-1-1-1	4
5		0	4	0	4	9	0-0-0-2	9
Others		5	9	0	5	11	0-0-1-2	24
Bridges		0	4	0	0	2	0-1-1-2	24
							1-1-1-2	8
							Others	67
Total		167	100	170	144	192	Total	375
Percentage of irregularities		25.75%	67.00%	35.88%	49.31%	75.52%	Meiotic Index	24.80

Note: The value recorded in each stage indicates the number of PMCs with laggards (in Anaphase or Telophase of 1st and 2nd division, and in MII) or micronuclei (in Interkinesis or Pollen Tetrad), except MI, in which the number of non-orientated bivalents is given.

Table 4. Measurements on the original tetraploid and the induced octoploid *Oryza minuta* PRESL.

Characters Plants	Height of plant (cm)	Length of Panicle (cm)	Number of Panicle	Number of sterile tillers	Flag-leaf		Thickness of culm (mm)
					Length (cm)	Width (cm)	
4x-minuta	73.5	18.0	63	18	13.5	1.1	3.9
8x-minuta	85.5	17.2	40	9	15.4	1.4	5.5

Number of spikelets per panicle	Density of panicle*	Length of awn (cm)	Length of stoma**	Size of pollen grain		Size of spikelet	
				Long diameter	Short diameter	Length (mm)	Width (mm)
135.3	7.5	1.2±0.30	7.2±0.23	14.5±0.03	14.2±0.03	4.7±0.03	1.8±0.003
72.7	4.2	1.8±0.28	12.9±0.33	18.2±0.03	17.6±0.11	5.7±0.004	2.1±0.02

Size of hulled grain		Weight of hulled grain (mg)	Shape of hulled grain (length/width)	Fertility (%)	
Length (mm)	Width (mm)			Pollen	Seed
3.6±0.04	1.7±0.03	3.1±0.05	2.2±0.06	89.62	79.09
3.2±0.05	1.6±0.03	3.5±0.03	2.0±0.05	16.67	near 0***

* number of spikelets ÷ length of panicle

** readings of the ocular micrometer (one score corresponds to circa 2.58 μ)

*** 34 seeds were obtained from a whole plant

Index" as was expressed by the percentage value of normal quartets was 24.80%. There were also observed such quartets as III form or IIII form quite often (Figs. 4-5 and -6). No polyad or monad was, however, met with.

In conclusion, the nuclear division proceeds on the whole regularly, and the cytokinesis which follows is carried out without interruption.

III. Morphology

Oryza minuta PRESL. is characterized by its small spikelets, poorly spreading small panicles and low sensitivity to day-length. The morphological difference of tetraploid and octoploid is not so clear at a glance in *minuta* as is in *latifolia* previously

Fig. 4. Some examples of meiotic irregularities in $8x$ *O. minuta*.

- 1 : Haploid PMC ($2n=48$) observed in $8x$ *O. minuta* ($2n=96$) (6_N+12_{II} in diakinesis)
- 2-3 : Non-orientation of bivalent in MI
- 4-6 : Abnormal pollen quartets
- 4 : Pollen quartet with a microcyte
- 5 : Pollen quartet of III type
- 6 : Pollen quartet of IIII type

year. Therefore, it can be said that the octoploid-*minuta* as well as the original *minuta* has very low sensitivity to day-length, though the former headed about 10 days later than the latter. Both tetraploid and octoploid panicles begin to flower early in the morning, at 6 to 7 o'clock. Anthers easily dehisce and pollen grains are shed easily.

IV. Fertility

As was expected from the irregularities in the behaviour of chromosomes, pollen fertility was very low, showing 16.67% as against 89.62% of the tetraploid-*minuta*, and the seed fertility of the octoploid-*minuta* was extremely low. Only 34 seeds in total were obtainable from 40 panicles of the octoploid.

With an aim to make amphiploids, crossing was tried between tetraploid-*sativa* and octoploid-*minuta*, taking the former species on the female side. Tetraploid-*sativa* varieties used were tetraploid-

Table 5. Cross-ability between tetraploid-*sativa* and octoploid-*minuta*

Cross combination		Number of florets pollinated	Number of seeds set	
Female parent	Male parent		Perfect	Imperfect
4x-KAMENOO	8x- <i>minuta</i>	47	0	4
4x-SHINRIKI-MOCHI	"	130	0	10

KAMENOO (non-glutinous) and tetraploid-SHINRIKI-MOCHI (glutinous), the same varieties used in the case of octoploid-*latifolia*. The results obtained are given in Table 5. Out of 177 florets pollinated, only 14 ovaries showed slight development (Table 5), and the immature embryos of about 15 days old were subjected to embryo-culture. Most of those embryos germinated, and reached maturity. The cross-ability between tetraploid-*sativa* and octoploid-*minuta* was lower than that between tetraploid-*sativa* and octoploid-*latifolia*. This may be due to the defect of pollens of octoploid-*minuta*.

Discussion

Artificial polyploid reported here is the allooctoploid plant induced from an allotetraploid wild species, *Oryza minuta* PRESL. of which genomic constitution has been determined as BBCC. Accordingly, the genomic constitution of this octoploid-*minuta* will be expressed as BBBBCCCC. Since this material is "di-genomic octoploid", having 2 kinds of different genomes in quadruplicate condition respectively, the chromosome configuration of 24_{IV} will be expected in MI. Actually, however, the maximum number of tetravalent chromosomes observed was only 15, though such PMC showing 48_{II} , as was observed rarely in octoploid-*latifolia*, was not met with at all.

Autotetraploids may be classified into the following 4 types:

- (1) the type forming tetravalents only,
- (2) the type without forming tetravalents,
- (3) the type forming both tetravalents and bivalents,
- (4) the type forming trivalents and univalents in addition to both tetravalents and bivalents.

Then, the present material belongs to the fourth category, though trivalents were noticed only in one cell and univalents in 9 cells through both stages, Diakinesis and MI. The observed number of tetravalent chromosomes per cell decreased from 9.14 in

Diakinesis to 7.26 in MI, and the observed number of bivalent chromosomes increased in the same course from 29.68 to 32.60. In autotetraploid, it is generally stated that the frequency of occurrence of tetravalent chromosomes is higher in Diakinesis than in MI. The average number of tetravalent chromosomes found in MI was 4.11 in octoploid-*latifolia*, and the corresponding number for octoploid-*minuta* was 9.14 and 7.26 respectively, in Diakinesis and MI, the average being 8.20. Since no multivalent chromosomes higher than tetravalent were found in octoploid-*latifolia* and octoploid-*minuta*, it is simply assumed that the heterogenetic association did not occur between C and D, or B and C. As is shown by octoploid-*latifolia*, the total number of tetravalents formed within C and within D is 4, so the number of tetravalents formed within C only is probably less than 4, and may be 3 or 2. Thus, it is supposed that the number of tetravalents formed only by the 4 sets of B in octoploid-*minuta* will be more than 4, and may be 5 or 6, the difference of the total number of tetravalents in octoploid-*minuta* and the supposed number of tetravalents produced within C.

It is already reported by several workers that 4 sets of A genome produce about 9 tetravalents in autotetraploid-*O. sativa*. The diploid ancestral species with B genome has not been discovered yet. Though *O. officinalis* WALL. ex WATT. and *O. asustraliensis* DOMIN are diploid species with C and E genomes respectively, no report dealing with the autotetraploid type of these species has been published as far as the present authors know. It is interesting, therefore, to know how many tetravalent chromosomes the autotetraploid-*officinalis* does form actually. It remains still unknown, however, why 12 tetravalent chromosomes are not formed when the same genome is present in quadruplicate condition.

In the present material, octoploid-*minuta*, anthers dehisced well and pollen grains were shed abundantly, but the fertility of both pollens and seeds was lower comparing with octoploid-*latifolia* previously reported. The seed was easily produced, however, when octoploid-*minuta* was pollinated with the pollen of octoploid-*latifolia*, and a number of amphiploid plants were secured by the present authors between those two octoploid types.

As shown in Table 3, far more lagging chromosomes and micronuclei than those expected simply from the number of univalents and trivalents were observed in AI or TI and in pollen quartets. Lagging bivalents often appeared in MI will be responsible to this fact. According to POWERS (1932 a, b), the phenomenon of non-orientation of bivalent, that is, lagging of bivalent not coming on to the equatorial plane during meiotic metaphase, is also one of the causal phenomena leading to the occurrence of micronuclei in the young pollen quartets. LOVE (1951) also superimposed this abnormality on the lagging of univalent. In the present material, octoploid-*minuta*, though univalent and trivalent were met with very rarely, lagging bivalents were observed in about a quarter of PMCs.

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Summary

1. An allooctoploid plant ($2n=96$, BBBBCCCC) was induced through colchicine treatment to the germinated seeds of allotetraploid species, *Oryza minuta* PRESL. ($2n=48$, BBCC).

2. In the octoploid-*minuta*, anther development is delayed, and the meiosis in PMCs begins only when the top floret of panicle is just coming out of the flag-leaf sheath. The same tendency is observed in octoploid-*latifolia*.

3. Meiotic chromosome behaviours in PMCs were studied in detail. In Diakinesis, the number of tetravalent chromosomes formed per PMC ranged from 4 to 13, and the mode was 9 and 10, the average being 9.14. In MI, the range of tetravalent chromosomes became somewhat wider, the number varying between 3 and 15, the mode and average per PMC being 7 and 7.26, respectively. Correspondingly, the average number of bivalent chromosomes increased from 29.68 in Diakinesis to 32.60 in MI. Lagging bivalents were observed frequently in MI. Very few trivalent chromosomes were observed in Diakinesis and MI. The irregularities became more obvious as the meiotic stages proceed from AI to Pollen Tetrad, producing finally many abortive pollens. Most of such abnormalities seemed to be due to non-orientation of bivalent in MI. On the female side, however, gametes seemed to function rather normally.

4. The seed fertility was extremely low even in open pollination condition. The cross-ability between tetraploid-*sativa* and octoploid-*minuta* taking the former species on the female side was lower comparing with that between the undoubled original species.

5. Octoploid-*minuta* gave gigas-type in most characters as compared with the original tetraploid-*minuta*, though not so conspicuous as in the case of *O. latifolia*. The size of stomata, pollen grains and spikelets increased, while the number of panicles and panicle density decreased remarkably. Like tetraploid-*minuta*, octoploid-*minuta* is also very low in the sensitivity to the day-length.

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イネ属の人為倍数体に関する細胞遺伝学的研究

第2報 *Oryza minuta* PRESL. のオクトプロイドについて

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イネ属の野生異質四倍種, *Oryza minuta* PRESL. ($2n=48$, BBCC) の発芽種子をコルヒチンで処理して1個体のオクトプロイド ($2n=96$, BBBBCCCC) を得た。PMC で観察される四価染色体の数は Diakinesis で 4~13 (平均 9.14), MI で 3~15 (平均 7.26) で、前報のオクトプロイド *Oryza latifolia* DESV. ($2n=96$, CCCCDDDD) に比べ約二倍の形成量を示した。三価あるいは一価染色体は両期を通じてほとんどみられない。分裂の時期が AI から花粉四分分子期にすすむにつれて異常率 (遅滞染色体, 染色体橋または小核などを有する PMC の百分率) が著しく増加し、不完全花粉粒の形成を結果する。この主因は MI における “bivalent の

non-orientation” によるものと思われる。しかし雌性側の配偶子はやや正常に機能するようであり、自殖または自然受粉による種子稔性は 0% に近かったが、オクトプロイド *latifolia* の花粉で多数の複二倍体を得られた。また、栽培イネのテトラプロイド品種との交雑能力は未倍加の両種間のそれより低かったが、未熟胚の胚培養によつて若干の複二倍体を得られた。本オクトプロイドは未倍加のテトラプロイドに比べ多くの形質においてギガス型を示すが、穂数、小穂数は著しく少なくなる。オクトプロイド *latifolia* と同様、葯の発達遅延が顕著であり、PMC の成熟分裂は穂の先端小穂が止葉葉鞘より抽出する頃にならなければ開始されない。