CYTOGENETIC STUDIES OF *ORYZA OFFICINALIS* COMPLEX

2. Meiotic Studies of Induced Autotetraploids of *O. officinalis*[1]

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The so-called "Officinalis Complex" in genus *Oryza* L. is the diploid *O. officinalis* Wall. (2n=24) and its close relatives, *i.e.*, *O. minuta* Presl. (2n=48), *O. latifolia* Desv. (2n=48), *etc.*; They constitute a part of Section *Sativa* Roschev. and similar in characteristics. However, the differences in morphologies between any two species in this complex and between the diploid and tetraploid strains of the same species are rather insignificant (Tateoka, 1962, 1965). Several plant taxonomists (Hooker, 1897: Bor, 1960; *etc.*) considered *O. latifoiia, O. officinalis* and *O. minuta* to be synonymous. Rice cytogeneticists (Morinaga, 1943; Morinaga and Kuriyama, 1960; *etc.*) based on the chromosome pairing at metaphase-I of meiotic division of F_1 hybrids, assumed that these species are closely related and *O. officinalis* has genome C, *O. minuta* and *O. latifolia* have genomes BC and CD. But the genome B and/or D species have not been found at the present time.

From the cytological studies of interspecific hybrid, Nandi (1936 1938), and Okura (1937) considered that one of the genomes in *O. minuta* is similar to that of *O. sativa*. Recently, Kihara and his coworkers proposed that genome B in *O. minuta* is partially homologous with genome A of cultivated species *O. sativa* (Nezu, *et al.*, 1960; Kihara, *et al.*, 1961). Li, *et al.*, (1962) noted that *O. latifolia* and *O. sativa* might have one genome in common. Later, studies on various interspecific hybrids of *O. latifolia* and Li (1964) concluded that the bivalents could be occur between two sub-genomes of *O. latifolia* by meaning of autosyndesis. Katayma (1965, 1966a, b), however, considered C and A genome to be partially homologous.

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An alternative hypothesis is that genome B, C and D might have originated from the same source (O^2, O^1 and O^3 were symbolized by Richharia, 1960) in which gene mutations, such as complete controlling of chromosome pairing, have occurred in these species (Sampath, 1962) during the process of differentiation. The purpose of this study is to investigate whether genomic differentiation has occurred in this species complex. The first paper of this study has indicated that high hybrid-sterility can be found in geographic race crosses and multivatents and unpairing univalents were found within the *O. officinalis* (Hu and Chang, 1967). The present paper reports the results of observations of chromosome pairing in meiosis of induced autotetraploid plants of *O. officinalis*.

Materials and Methods

Two induced autotetraploid plants which were derived from the diploid of *O. officinalis* by colchicine treated with different concentrations of colchcine solution by diffierent methods. A plant whose chromosome number being completely doubled, was induced in strain W002 (Bangkok, Thailand origin). The other one was kindly supplied by Mr. S. Sampath of the Central Rice Research Institute of India. According to Mr. Sampath this tetraploid plant was induced from an inter-racial hybrid of *O. officinalis* (perhaps is an F_1 hybrid between two strains of Ceylon and India).

Meiosis of pollen mother cells of the above two induced autotetraploid plants was studied by aceto-carmine smearing method. The young panicle were fixed with Farmer's solution, to which a trace of ferric chloride was added. The freshly prepared slides were used for observation and photography.

Various characteristics of diploid and induced autotetraploids were measured with the pot-cultured plants in the spring season of 1967.

Results of Observations

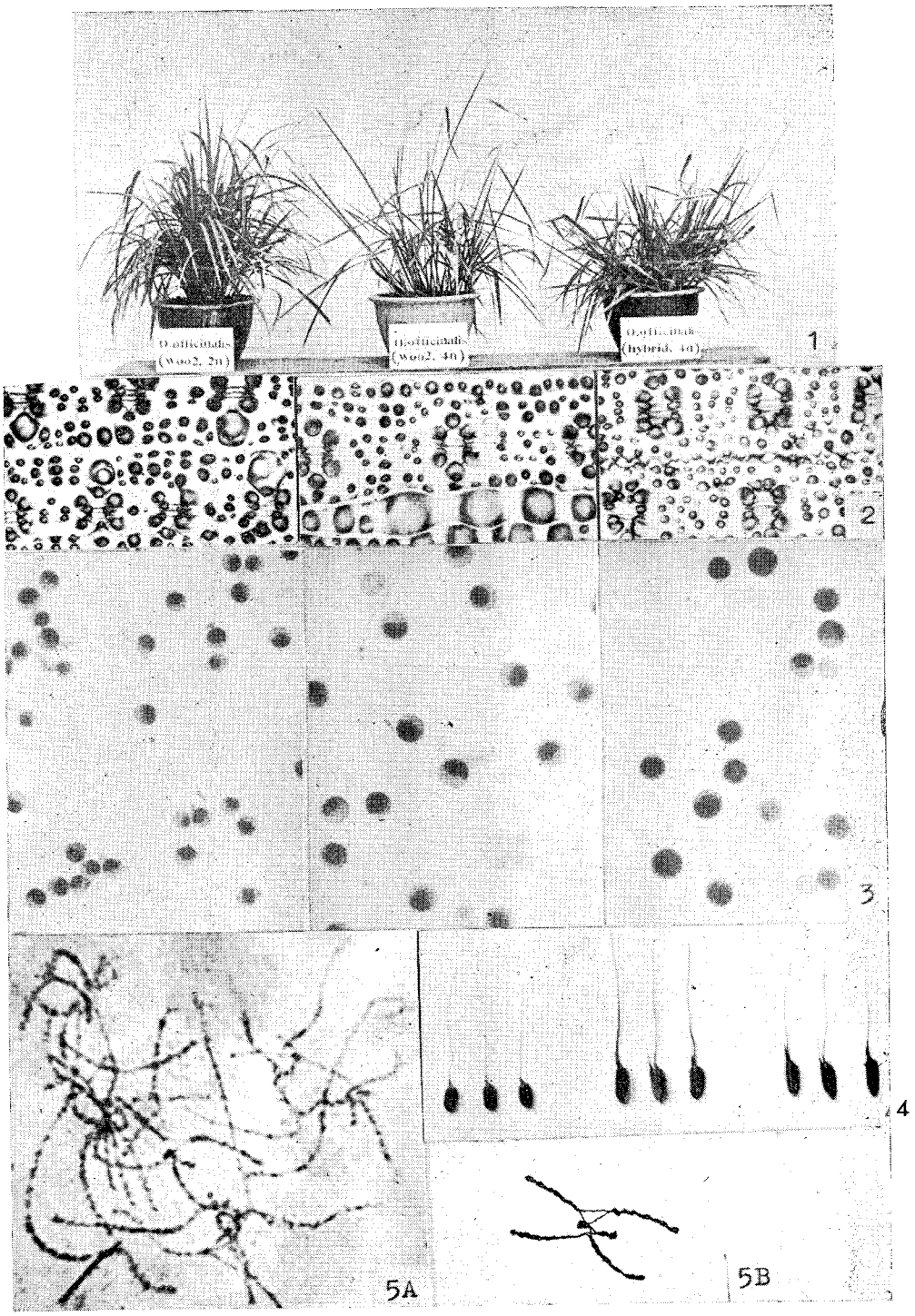
1. *Plant morphology of induced autotetraploids*

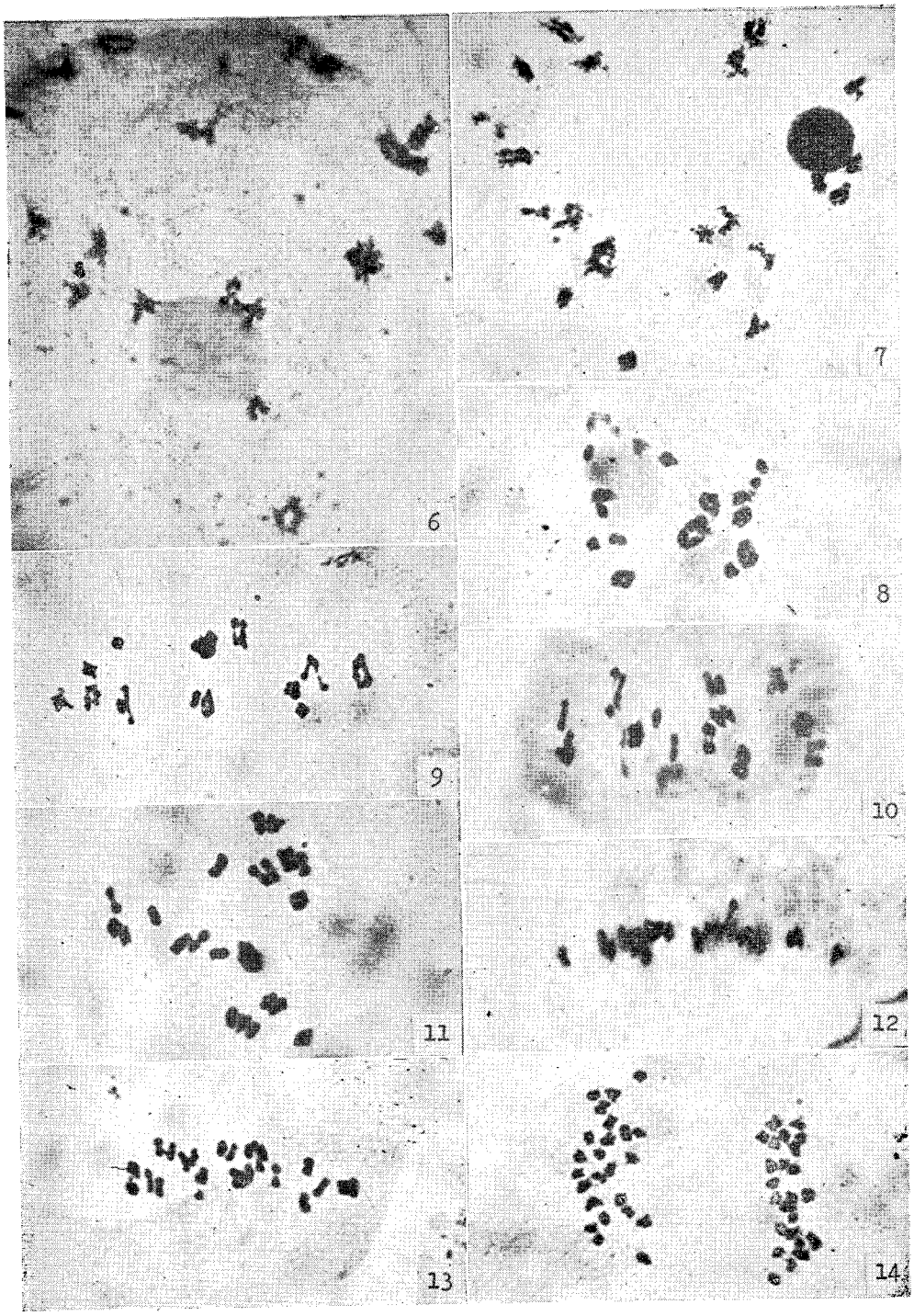
The plant of diploid and artifically induced autotetraploids of *O. officinalis* are shown in Fig. 1. Comparision of stomata in leaf, pollen grains and the

Explanation of figures

Comparision of diploid and its induced autotetraploids of *O. officinalis*. Left: Strain of W002 (2n); Center: Induced autotetraploid of W002 (4n); Right: Inter-racial hybrid (4n).

Fig. 1. Morphologies of plant.
Fig. 2. The size of leaf stoma.
Fig. 3. The size and fertility of pollen grain.
Fig. 4. The size of spikelet (full size).
Fig. 5. 5A: A pachytene cell of meiotic division of W002 (4n) an X type quadrivalent is found in the center of the cell. 5B: Camera lucida drawing indicated the quadrivalent is formed by two zigag chromatids and two parallels.


O. officinalis (W002, 2n)
O. officinalis (W002, 4n)
O. officinalis (hybrid, 4n)
1
2
3
4
5A
5B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14

size of spikelets of diploid and tetraploids as shown in the figures 2-4, there was no differences between the two tetraploids but they were being larger than those of diploid. Measurements of several characteristics both diploid and induced autotetraploids are shown in Table 1.

Table 1. *Measurements of various characteristics of diploid and induced autotetraploids of* ***O. officinalis***

Character	W002(2n)	W002(4n)	Hybrid(4n)
plant heigt (cm)	91	103	101
length of			
panicle (cm)	19	27	25
flag leaf (blade/sheath) (cm)	27/22	33/23	32/23
ligule (mm)	3.3	5.6	3.4
stoma (μ)	25.6	29.7	29.8
anther (mm)	1.67	2.25	2.27
awn (cm)	1.13	1.38	1.51
width of leaf blade (cm)	1.25	1.35	1.40
pollen diameter (mm)	0.32	0.42	0.42
fertilization of			
pollen (%)	98.0	83.5	69.5
seed (%)	93.2	88.5	84.7
spikelet			
length (mm)	4.76	6.83	6.82
width (mm)	2.00	2.75	2.44
length/width	2.38	2.45	2.78
grain per panicle	72	64	75

Explanation of figures

Meiotic division of PMC's of induced autotetraploids *O. officinalis*. (ca. × 1,500)

Fig. 6. A diakinesis cell (hybrid, 4n). Showing 6 quadrivalents, 1 trivalent, 10 bivalents and 1 univalent. The end to end fusion quadrivalents can be seen in this cell.

Fig. 7. Diakinesis (hybrid, 4n). 24 separated bivalents are found.

Fig. 8. Late diakinesis (hybrid, 4n). Showing 5 ring-type quadrivalents, 2 trivalents, 10 bivalents and 2 univalents.

Fig. 9. Metaphase-I. (W002, 4n). Showing 4 quadrivalents and 16 bivalents in this cell and forming 8 groups of 2 (bivalents) and 2 groups of 3 (one quadrivalent and one bivalent) secondary association.

Fig. 10. Metaphase-I (hybrid, 4n). 24 bivalents forming 10 groups of 2 bivalents secondary association are seen in this cell.

Fig. 11. Metaphase-I (hybrid, 4n). Showing 1 quadrivalent (center) and 22 bivalents. The secondary association of bivalents also can be found in this cell.

Figs. 12-13. Metaphase-I (W002, 4n). Showing 24 bivalents.

Fig. 14. Anaphase-I (W002, 4n). Showing 24 separated chromosomes move toward the poles.

The measurements in Table 1 were taken in the spring season or pot-cultured plants, plant size will be large if grown in the field and in fall. In addition to differences of various agronomic traits shown in the table, the development of rhizome of the induced autotetraploids is also different from diploid. The diploid Thai strain W002 has less rhizome, but the rhizome become strong after chromosome doubling. The induced autotetraploid plant of *O. officinalis* from India, showed spreading tiller at young stage and less rhizome at heading as compared with the autotetraploid of strain W002.

The panicle development and flower blooming of induced autotetraploids of *O. officinalis* are normal. Short awn developed in palea (inner) lemma is a peculiar characteristics found in these strains (Fig. 4).

2. *Meiotic division of pollen mother cells*

In the early stages of prophase-I, chromosomes two by two longitudinally pairing can be seen in most cells. Fig. 5 is one of the pachytene cells found in W002 (4n), showing one quadrivalent formed by non-homologous pairing.

In diakinesis, 0 to 9 quadrivalents were found in strain W002 (4n) and 1 to 8 in the inter-racial hybrids (4n). The chromosome pairing in genetically pure autotetraploid seems more variable than that of the hybrid. Most of the quadrivalents show ring type and/or end to end fusion (Figs. 6, 8). The number of the quadrivalents, however, was reduced at metaphase-I and some of them formed bivalents. Secondary association of the bivalents was common. The maximum secondary association at metaphase-I was found to be 10 groups of two bivalents or 8 groups of two bivalents and 2 groups of three bivalents (Figs. 9–10) so far as the present expriments are concerned. Table 2 gives the frequencies of different chromosome configurations observed at diakinesis and metaphase-I. The secondary association of bivalents was not included in this table.

As Table 2 shows at metaphase-I, about one fourth (25%) of the cells observed formed bivalents only (24 bivalents) and 23 bivalents blus 2 univalents in both genetically pure and hybrid antotetraploids. The mean number of bivalents per cell of induced autoteraploids was found to be 15.78 (W002, 4n) and 18.89 (hybrid, 4n) at diakinesis and 19.37 (W002, 4n) and 20.47 (hybrid, 4n) at metaphase-I, respectively. The mean number of bivalents in hybrid (4n) appeared to be higher than that of strain W002 (4n); and the bivalents were increased at metaphase-I in both strains. The bivalents dividing into 24: 24 at anaphase-I were recognized in most cells (Table 3). The normal behavior of the chromosomes in anaphase-I and later stages, as will be mentioned later, may be the formation of fertile pollens and the production of normal seeds.

Although several lagging chromosome can be seen in anaphase-I and

Table 2. *Frequencies of PMC's with different chromosome configurations observed in induced autotetraploids* ***O. officinalis***

Chromosome configuration						W002 (4n)		Hybrid (4n)	
I	II	III	IV	VI	X	Dia.	MI	Dia.	MI
	24						13	4	6
2	23						8		9
	22		1			6	2	4	7
1	22	1							2
4	22							1	7
	21	2					1	1	
2	21		1			1	6	1	3
3	21	1					1	1	1
6	21								1
	20		2			6	2	5	3
1	20	1	1				2		3
2	20	2				1	1		1
4	20		1				3	1	2
5	20	1							2
8	20								4
2	19		2				3		
3	19	1	1					1	3
4	19			1			1		
6	19		1				4		2
7	19	1					1		2
10	19						1		
	18		3			4	4	1	
1	18	1	2				1	4	
4	18		2			1	3	2	1
8	18		1				1		2
9	18	1					2		
	17		2	1		1			
2	17		3			1	4	1	1
3	17	1	2				1		
4	17	2	1				2		
4	17		1	1			1	1	
6	17		2				3		
7	17	1	1				3		
10	17		1				1		
	16		4			4	1	2	
1	16	1	3					1	
2	16	2	2					1	
2	16		2	1				1	
4	16		3			1			
5	16	1	2				1		
10	16	2							1
	15	2	3				1		
	15		3	1		1			
1	15	1	2	1		1			
2	15		4			1			
8	15	2	1				1		
	14		5			6			
4	14		4				1		
8	14		3				1		
12	14		2					1	
	13		3		1	1			
2	13		5				1		
	12		6			7			
2	12	2	4				1		1
	11		5			1			
7	11	1	4	1			1		
	10		7			1			
1	10	1	6			1		1	
14	10	2	2				1		
	8		8					1	
1	7	1	5			1			
	6		9			2			
Number of PMC's observed						49	85	36	64

telophase-I in induced autoteraploids of *O. officinalis* but such aberrations were also observed in the diploid strains of *O. officinalis* and other rice species. The second meiotic division is generally normal.

Table 3. *Separation of chromosomes at anaphase-I of PMC's in induced autotetraploids of* ***O. officinalis***

Separation ratio	W002 (4n)	Hybrid (4n)
24 : 24	23	30
23 : 25	8	5
22 : 26	1	0
23 : 1 : 24	5	2
22 : 1 : 25	1	0
23 : 2 : 23	2	7
22 : 2 : 24	1	1
22 : 3 : 23	1	0
22 : 4 : 22	1	0
Number of PMC's observed	43	45

3. *Pollen and seed fertility*

The highly fertile pollen grain as well as good seed setting are found in induced autotetraploids of *O. officinalis*. In strain W002 (4n) the values were found to be 83.5% (good pollen) and 88.5% (seed setting). The good pollen and high percentage of seed setting suggest that the multivalents in early stages of meiosis separated regularly in the succeeding stages. The fertile pollen (69.5%) of inter-racial hybrid (4n) being less than that of strain W002 (4n) may be due to gene segragation and recombination of chromatids in which structural differences may be present in the two parental strains, although the seed setting is good (84.7%). The duplicated chromosome sets of female gamete may be effective in fertilization.

Discussion

Induced autotetraploids of *O. officinalis* are of great interst in the study of genome interrelationships and speciation of the *O. officinalis* complex by the following reasons. 1) There are highly developed polyploids in *O. officinalis* species complex but no spontaneous or induced autotetraploid has been reported so far. 2) Allotetraploid species of *O. minuta, O. latifolia, etc.* were thought to have diffeerent genomes BBCC and CCDD but no diploid BB and/or DD genome species was found. 3) If there were an allotetraploid of *O. minuta, O. latifolia, etc.* were not the hybrid between species of BB and CC or CC and DD but due to chromosome doubling occurred in *O. officinalis*-like plants which

carried meiotic pairing controlling genes. 4) An amphidiploid can be made by crossing an autotetraploid *O. officinalis* (CCCC) with *O. sativa* (AAAA). Which may be useful to test the hypothesis that genome A of the cultivated species may be one of the genomes of the tetraploid wild species. The problems to be discussed in this paper are mainly on the pairing of meiotic chromosomes in induced autotetraploids of *O. officinalis* as compared with those formerly investigated in *O. sativa, O. latifolia* and *O. minuta.*

Spontaneous and induced autotetraploids of *O. sativa* showed 4 to 12 quadrivalents in metaphase-I with a mode at 9-10 and a mean 8 (Morinaga and Fushima, 1936, Mashima, 1940; Cua, 1952; Oka, *et al.*, 1953). In the induced autotetraploids of *O. officinalis* a maximum pairing of 9 quadrivalents can be found at diakinesis and 5 at metaphase-I. The mean frequency of quadrivalents per cell was found to be 3.71 (W002, 4n) and 1.86 (hybrid, 4n) at diakinesis and 1.33 (W002, 4n) and 0.91 (hybrid, 4n) at metaphase-I. One or two of the quadrivalents formed in metaphase-I of induced autotetraploids of *O. officinalis* may not be due to pairing among sets of homologous chromosome but to the autosyndesis of homeologous ones within the genome. It was known that 1 to 2 bivalents can be found in haploid plants of *O. sativa* and *O. glaberrima* (Morinaga and Fukushima, 1934; Hu, 1958, 1960), and one quadrivalent in diploids of *O. sativa* (Sakai, 1935), *O. officialis* as well as *O. australiensis* (Hu, 1962). A hexavalent and extra bivalents were also found in autotriploid of *O. sativa* (Hu and Ho, 1963). It may be assumed that pairing between homologous chromosomes may be controlled by certain unknown factors in induced autoteraploids of *O. officinalis.* These factors may or may not increase the pairing between non-homologous chromosomes.

The average number of quadrivalents in allo-octoploid (2n=96) of *O. latifolia*×*O. minuta* (genome BBCCCCDD) reported by Morinage *et al.* (1963) was two; and that in *O. latifolia* (CCCCDDDD) and *O. minuta* (BBBBCCCC) found by Watanabe and Ono (1965, 1966) was four and nine, respectively. The low frequency of quadrivalent formation of these studies and in the present studies of induced autotetraploids of *O. officinalis* may be attributable to the same factors.

The gene or genes controlling chromosomal pairing in allohexaploid common wheat, *Triticum vulgare* (2n=42), resulting in diploid meiotic behavior were demonstrated by Sears and Okamoto (1958) as well as by Riley and Chapman (1958). According to Riley (1960), allopolyploids should not always be considered to have originated from species-hybrids. A number of natural tetraploid species which only form bivalents at meiosis could posses a similar genetic mechanism as in common wheat for control of regular bivalent formation. The allotetraploid species of *O. officinalis* complex, therefore, may be

the case originating from autotetraploids of *O. officinalis*-like plants and carrying a diploidizing gene which controls regular bivalent formation.

The number of quadrivalents in induced autotetraploids of *O. officinalis* as well as its related allo-octoploid, *O. latifolia* and *O. minuta*, is generally, very low as mentioned above. Pairing of four of homologous chromoosomes in induced polyploid Officinalis Complex, therefore, must be suppressed by certain genetic mechanisms. The suppression may not be complete in the early stages of meiosis in induced autotetraploids as spontaneous autotetraploids. Or the reduction achieved in number of quadrivalents is due to selective elimination of modified chromosome pairing during the process of evolution.

Summary

Two colchicine induced autotetraploid plants of *O. officinalis* (2n=48) were studied cytologically. It was found that only 25% of observed PMC's showed all bivalents or two univalents and the rest bivalents. The mean pairing of bivalents was found to be 15.78 (W002, 4n) and 18.89 (hybrid, 4n) in diakinesis and 19.37 (W002, 4n) and 20.47 (hybrid, 4n) in metaphase-I, respectively. Most of the anaphasic chromosome showed regular separation. The good pollen and seed setting (about 85%) indicated that induced autotetraploids of *O. officinalis* might be due to certain genetic factors which controlling chromosome pairing and showed allotetraploid-like meiotic behavior.

稻屬 *Oryza officinalis* Complex 細胞遺傳學的研究

第二報 人工誘變 *O. officinalis* 同質四倍體之減數分裂

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人工誘變 *Oryza officinalis* 同質四倍體植物對於研究稻屬種間類緣及進化關係頗有價值其原因如後：(1) 稻屬中 *O. officinalis* 及其近緣稻種具有最多四倍體，但在本研究前未有自生或人爲誘變同質四倍體之研究報告；(2) *O. minuta* 及 *O. latifolia* 據前人報告係具有 BBCC 及 CCDD 染色體的異質四倍體，但具有 BB 或 DD 染色體組植物從未發現；(3) 假如 *O. minuta* *O. latifolia* 等並不是一般所想像來自 BB 與 CC 種，或 CC 與 DD 種雜種染色體倍加，而係從類似 *O. officinalis* 之植物的同質四倍體同携有某種遺傳因子控制減數分裂的染色體的配對；(4) 同質四倍體的 *O. officinalis* (CCCC) 與同質四倍體的 *O. sativa* (AAAA) 行雜交應可產生複二倍體 (amphidiploid, AACC) 時，此複二倍體可用於有效的檢驗一部分學者所主張四倍體野生稻種染色體組內具有栽培種稻染色體組的假說 (hypothesis)。

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本文主要係用秋水仙精（colchicine）處理 *O. officinalis*, 得到染色體倍加的同質四倍體植物兩品系，以細胞學的方法研究其減數分裂時染色體的接合情形。結果發現約有 25% 的花粉母細胞在第一中期（metaphase-I）現出全爲二價染色體或 2 個單價染色體餘爲二價染色體。平均每一個細胞的二價染色體數在親交期（diakinesis）爲 15.78（W002 四倍體）及 18.89（種內雜種四倍體），第一中期的二價染色體數增加爲 19.37（W002 四倍體）及 20.47（種內雜種四倍體）。第一後期（anaphase-I）細胞染色體的分離大多正常。花粉稔性及種子結實亦近正常。由種種的研究分析啓示 *O. officinalis* 的同質四倍體稔性之良好或基於攜帶有某種遺傳因子控制減數分裂行爲，使同質四倍體染色體配對形成類似異質四倍體。（中文摘要）

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