CYTOGENETIC STUDIES OF *ORYZA OFFICINALIS* COMPLEX

I. F_1 Hybrid Sterility in Geographical Races of *O. Officinalis*

CHAO-HWA HU and CHIA-CHENG CHANG[1]

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Introduction

Oryza officinalis Wall. is a wild rice species distributed in Southeast Asia. Based on the surface structure of spiketlets and other characteristics, Roschevicz (1931) placed this species in his Section *Sativa*. Several workers considered that it could have played a role in the evolution of *O. sativa* (Watt, 1891; Roschevicz, 1931; Chatterjee, 1951; Shastry *et al.*, 1961). However, cytological studies showed that it has C genome, which differs from genome A of the cultivated rice, *O. sativa* and *O. glaberrima*, and their wild relatives *O. perennis* and *O. breviligulata* (Morinaga and Kuriyama, 1960; Nezu *et al.*, 1960; Li *et al.*, 1962; IRRI, 1964).

O. officinalis is a diploid (2n=24), but other species closely related to it, *O. minuta*, *O. eichingeri*, *O. punctata* (distributed in Asia and Africa, genome BBCC) and *O. latifolia*, *O. alta*, *O. grandiglumis* (distributed in Central and South America, genome CCDD) are tetraploids. Because of minor character differences between these species, several taxonomists (Hooker, 1897; Bor, 1960; *etc.*) considered *O. latifolia*, *O. officinalis* and *O. minuta* to be synonymous. Sasaki (1935) considered *O. punctata* to be an African form of *O. officinalis*. To distinguish between *O. minuta* and *O. officinalis*, a chromosome count is often needed to supplement the morphological studies (Tateoka and Pancho, 1963). Recently, Tateoka (1965 a, b) reported that *O. eichingeri* used in cytogenetic studies in various countries should be identified as *O. punctata*. True *O. eichingeri* showed 2n=24, while *O. punctata* species had both 2n=24 and 2n=48. One of the problem areas in these species group is where does genome B and/or D come from?

This study was undertaken to investigate whether or not some genomic

1. Professor of Agronomy and Research Assistant, College of Agriculture, Provincial Chung-Hsing University, Taichung, Taiwan.

differentiation could be found among strains of *O. officinalis*. The preliminary report of this study has indicated highly sterility in the F_1 hybrids of parent plants from widely separated geographical sources (Hu and Chang, 1965a). This paper gives detailed results both in plant morphology and cytological studies.

Materials and Methods

A number of strains of *O. officinalis*, collected from different Asian countries, were kindly supplied by Dr. H. I. Oka of the National Institute of Genetics, Japan. Seven of them, one or two from each country, were selected from the collection as test-strains for crosses. Their accession number, place of origin and records of some characters are given in Table 1.

Table 1. *Characteristics of seven strains used of O. officinalis*

No.	Origin	Ligule hairiness	Leaf trichome	Anthocyanin coloration*			
				Pulvinus	Sheath outside	Sheath inside	Stem node
W002	Bangkok, Thailand	+	+	++	+	+	+
W012	India (from CRRI)	—	—	—	—	—	—
W066	Srisanrong, Thai.	—	+	±	±	+	±
W564	Johore, Malaya	+	+	—	—	+	—
W614	Prome, Burma	—	—	+	—	+	—
W1132	Assam, India	—	—	—	—	+	—
W1213	Bacolod, Philippines	+	—	++	+	+	—

*++ deep purple; + purple; ± light purple; — no color.

For hybridization, emasculation was made by the hot-water method (43°C for 7 minutes) at about 4 A.M. For cytological observation of the PMC's young panicles were fixed in aceto-alchohole (1:3), to which a trace of ferric chloride had been added. Temporary preparations were used for observation and photomicrographs. The percentage of normal pollen grains was determined by counting pollen grains stained with iodine potassium iodide solution.

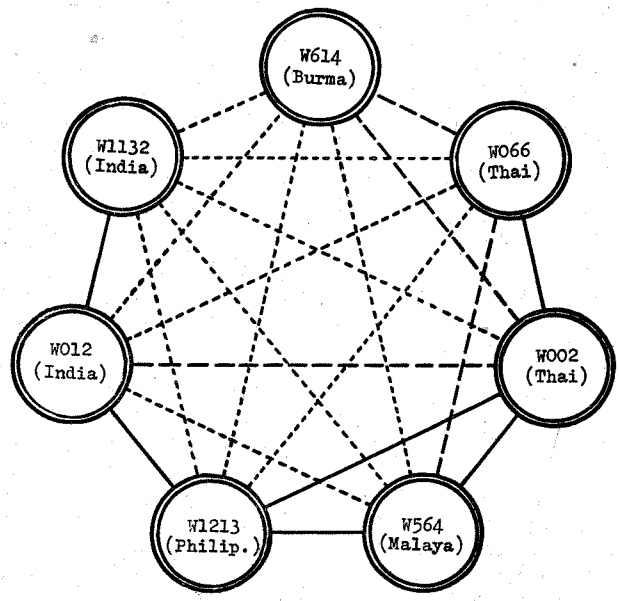
Results of Observations

1. *Character variations among the test-strains*

Some of the character differences found between the seven test-strains are given in Table 1. In addition, it was found that the strains from Bangkok, Thailand (W002) had narrow, dark green and semi-rolled leaf blades. The strain from Srisanrong, Thailand (W066) had flat and broad leaf blades. The Indian strain (W012) had yellowish leaves. The Malayan (W564) and the Philippine (W1213) strains had light-green leaves with thick mid-ribs in the sheaths. The Burmese strain (W614) had spreading tillers when young but

was erect at the flowering time. Its leaves were soft and the plants were small. The strain from Assam, India (W1132) was tall (over 2 meters) and had stiff but spreading culms. It also had twisted flag-blades and well developed rhizomes.

The Thai (W002 and W066) and the Burmese (W614) strains were found to be non-sensitive to photoperiod. They flowered in summer months at Taichung. The Malayan and Philippine strain, flowering in November, were highly sensitive to photoperiod. The other strains, sensitive in different degrees, flowered from September to April.



Percentage of normal pollen grains
0% ------ 3-10% ——— 50-70% ———

Figure 1. Diagram of fertility relationships (F_1 fertility) among seven strains of *O. officinalis*.

2. *F_1 sterility between strains*

All of the seven strains showed, when self-pollinated, a high percentage of normal pollen and a good seed set. Their F_1 hybrids were generally sterile (Table 2). Reciprocal crosses showed no significant differences in fertility. The fertility of the pollen could be classified into three grades, 50–70%, 3–10% and 0%. F_1's between strains from the same country (W002×W066 and W012×W1132) were partly fertile (50–70%). Those between strains from different countries ranged in fertility from about 60 to 0%. The sterility relationships are shown diagrammatically in Figure 1. In the digram, it is found that six strains can be connected by partial-fertility relationship if orderly arranged by geographic distribution, but many cross-combinations among them produce

Table 2. *Pollen fertility of F_1 hybrids and parental strains (in %).*

Female parent	Male parent						
	W002	W066	W564	W1213	W012	W1132	W614
W002 (Thai.)	98.0	66.6	55.7	—	8.9	0.0	3.2
W066 (Thai.)	66.4	94.9	—	0.0	0.0	0.0	3.0
W564 (Malaya)	60.4	6.3	95.4	51.2	0.0	0.0	0.0
W1213 (Philip.)	53.8	0.0	50.7	97.8	56.6	0.0	0.0
W012 (India)	7.6	0.0	—	52.3	96.0	50.1	0.0
W1132 (India)	0.0	0.0	0.0	—	54.9	90.7	0.0
W614 (Burma)	3.6	3.0	—	0.0	0.0	0.0	90.0

highly or completely sterile F_1 hybrids. Three of them (W002, W564 and W1213) appear to a partly interfertile group. The seventh, *i.e.* the Burmese strain (W614), did not show partial-fertility relationships with any other strain.

3. *Pairing of chromosomes in F_1 hybrids*

The results so far obtained are given in Table 3 and 4. The following points are worth mentioning:

Table 3. *Chromosome configurations at metaphase-I in parents and F_1 hybrids*

Parent of F_1	12II	11II+2I	10II+4I	10II+1I+1III	10II+1IV	No. of PMC's observed
W002	64	1				65
W066	74	5				79
W564	51	3				54
W1213	66	4				70
W012	47	6	1			54
W1132	65	4				69
W614	56	9	1			66
W002 × W614	59	3				62
W066 × W002	66	10				76
W614 × W066	55	1				56
W564 × W012	72					72
W012 × W1213	75		1			76
W1132 × W564	87	2				89
W1132 × W012	178	6				184
W066 × W1213	40	3		6	7	56
W066 × W012	214	31	3	43		291
W066 × W1132	37	5		13		55
W564 × W066	87	26		20		133
W1213 × W614	37	10	1	12	2	62
W012 × W066	40	7		18	3	68

Table 4. *Pairing of chromosomes in the F_1's of Indian and Burmese strains*

Cross and plant no.	Preparation	12II	10II + 2I	11II + 4I	9II + 6I	8II + 8I	7II + 10I	6II + 12I	5II + 14I	4II + 16I	3II + 18I	2II + 20I	1II + 22I	24I	10II + 1I + 1III	10II + 1IV	Total
W012 × W614 -1	1 (MI)	23															23
	2 (MI)	32	1														33
	3 (MI)	1	1	2	1	3	2	2	1	3	3	2	1	1			23
	4 (MI)	2							1	1			1	7			12
	5 (MI)			1				1			2	2	2	6			15
	6 (Dia)						2	1	3	2		3	6	8	1		48
	6 (MI)					1		1	2	3	4	4	2	5	1		
No. of PMC's observed		58	2	3	1	4	4	5	7	9	9	11	12	27	2		154
W1132 × W614 -1	(MI)	20	11	1	3	1			3	1	2	3	2	12	5	2	66
-2	(MI)	9	12	2		1			1			1		2	5	1	34
-3	(MI)	9	1		1	1				1		1		2	4		20
No. of PMC's observed		38	24	3	4	3			4	2	2	5	2	16	14	3	120

a) The partly fertile F_1 hybrid, W002×W066, showed normal pairing. At pachytene and diplotene some loose pairing or unpaired segments were found (Figs. 4 and 5) but the same phenomena were also found in one of the parents W066 (Fig. 3).

b) Highly or completely sterile hybrids could be divided into two types, one showing no particular disturbance in chromosome pairing, as the above-mentioned partly fertile hybrid, and the other type showing a quadrivalent or a trivalent chromosome (Figs. 8, 9, 13 and 14). Crosses between Thai and Burmese strains (W002, W006 and W614) and those between Indian, Malayan and Philippine strains (W012, W1132, W564 and W1213) belong to the former type, while W066×W012, W066×W1213, *etc.* belong to the latter, as shown in Table 3. The occurrence of one single tri- or quadri-valent might be due to the presence of a reciprocal translocation. On this basis, the seven strains observed can be classified into two groups, one consisting of W002, W 066 and W614, and the other of W012, W1132, W564 and W1213.

c) As shown in Table 4, two crosses between Indian and Burmese strains (W012×W614 and W1132×W614), besides the normal pairing found in metaphase-I, occasionally showed various degrees of chromosome pairing ranging frome 0 to 12 bivalents from pachytene through metaphase-I (Fig. 6, 10–12) in different preparation (each preparation was made from one spikelet). Furthermore, some of their PCM's had 48 or more chromosomes (Fig. 15).

Discussion

Character variation among strains of *O. officinalis*, though more may be disclosed by careful observation, seem to be relatively small if compared with those in *O. perennis* or *O. sativa*. In contrast, the F_1 fertility barriers seem to be highly developed in *O. officinalis*. Strains from different countries of Asia generally showed high F_1 sterility, and those of the same country partial fertility.

In eight of the fifteen crosses observed cytologically, one single tri- or quadri-valent chromosome was found in the PMC's. As already mentioned, a reciprocal translocation might be involved in these crosses, and most of them were completely sterile. However, a reciprocal translocation can only partly account for the high sterility of these F_1 hybrids. The other seven crosses showing normal chromosome behavior were also sterile in varying degrees, two of them being completely sterile. The sterility in these F_1 hybrids may not be due to differences in gross structure of the chromosome of the parents. In contrast the meiosis in partial sterile hybrids of *O. sativa*, *e. g. japonica* and *indica* variety combinations in essentially normal. The situation *O. officinalis* in this study may be compared with that found between *O. sativa* and *O.*

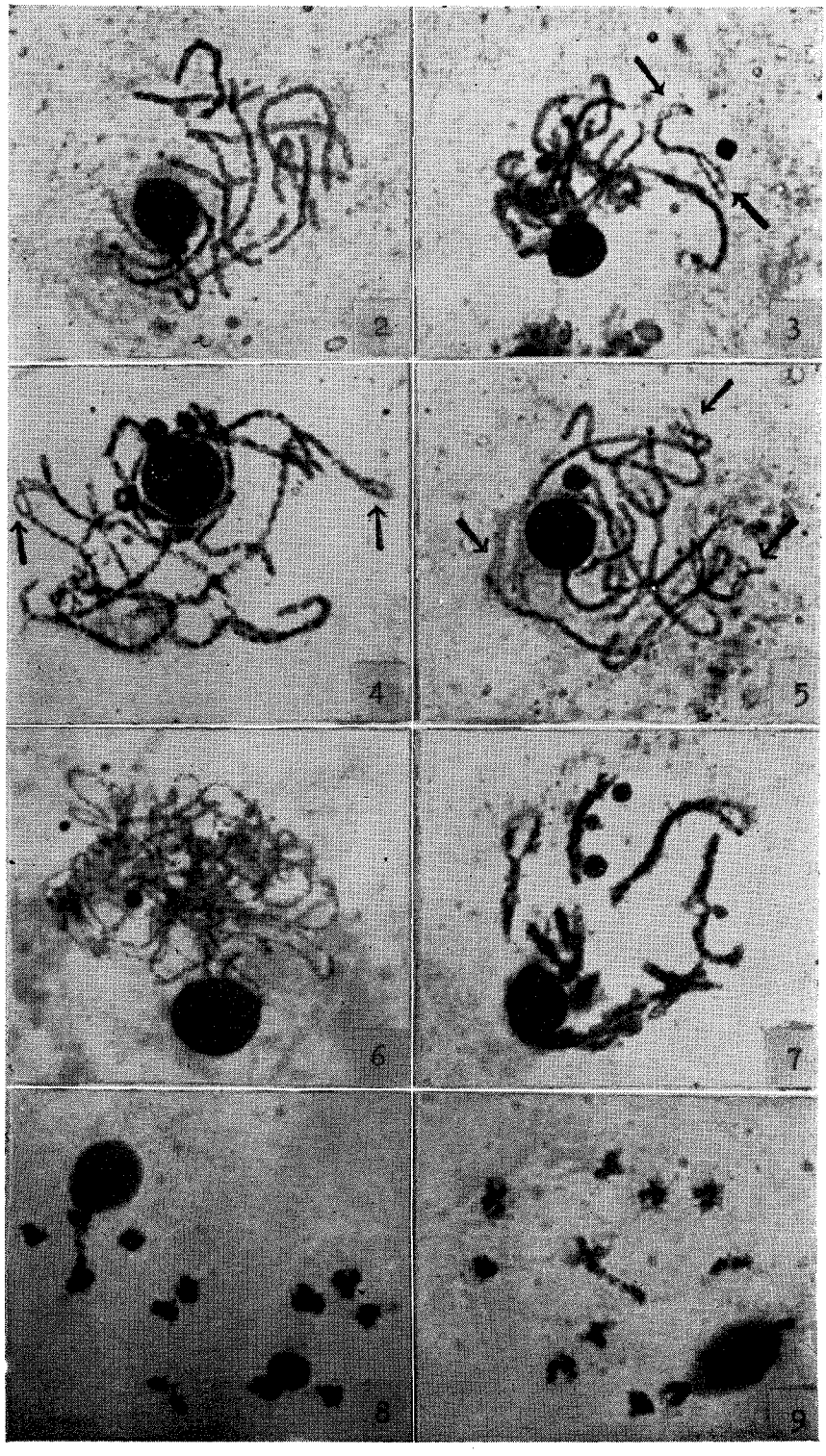
glaberrima, in which the chromosomes normally pair and divide in meiosis, but pollen as well as seed fertility is almost nil. The F_1 sterility without visible chromosomal abnormalities, which has been reported for different plant groups (Stebbins, 1950), may be due either to the so-called cryptic structural differences or to disharmonious interactions of parental genes.

It may be noticed that in two crosses with W614, cells with 0 to 12 bivalents were found and a few cells had 48 or more chromosomes. This suggests the existence of complementary genes interfering with chromosome association. The seven strains used are supposed to have the same C genome. The data from the present study have, however, shown that they can be divided into a number of inter-sterile groups. And the strains of Thailand and Burma appeared to be genetically differentiated by one reciprocal translocation from those of India, Malaya and Philippines. It is known that many other species of the *Officinalis* complex contain the C genome and are tetraploids. Gopalakrishnan (1962) reported that *O. malampuzhaensis* found in South India could be a segmental allotetraploid originated from a hybrid between different reces of *O. officinalis*. Tateoka (1962) in view of similarities in various characters, considered it to be a sub-species of *O. officinalis*. Studies on the interspecific hybrids of *O. punctata* by the present authors have shown there are one or more translocation differences between BBCC genomes species (Hu and Chang, 1965b). The similar situation may also be present among CCDD genomes species (the authors unpublished data). It may be infered that in this plant group there could be some factor causing instability of chromosomal mechanisms which may in a way promote genomic differentiation and polyploidization.

Summary

Oryza officinalis (genome CC) and its relatives, because of similarity, usually are difficult to classify by phenotypical characteristics. The C's sib genome B and/or D in tetraploid species of this complex are yet to be found. It may show genomic differentiation among geographical races of *O. officinalis*. Seven

Fig. 2. Late pachytene chromosomes in W002. 12 bivalents can be distinguished.
Fig. 3. Late pachytene or early diplotene chromosomes in W066. A chromosome with unpaired terminal region is seen, as indicated by arrows.
Figs. 4 & 5. Pachytene chromosomes in the F_1 of W066×W002. Loose pairing, unpaired segments and unpaired terminal regions are seen, as indicated by arrows.
Fig. 6. Showing unpaired pachytene chromosomes in the F_1 of W012×W614-1.
Fig. 7. Mid-diplotene chromosomes in the F_1 of W002×W614. Separated segments and unpaired terminal regions are seen in some chromosomes.
Figs. 8 & 9. Diakinesis in the F_1 of W012×W066, showing a quadrivalent attached to the main nucleolus (Fig. 8), and a trivalent in the central part (Fig. 9). An univalent is seen attached to the nucleolus. (All ca. 2000 X).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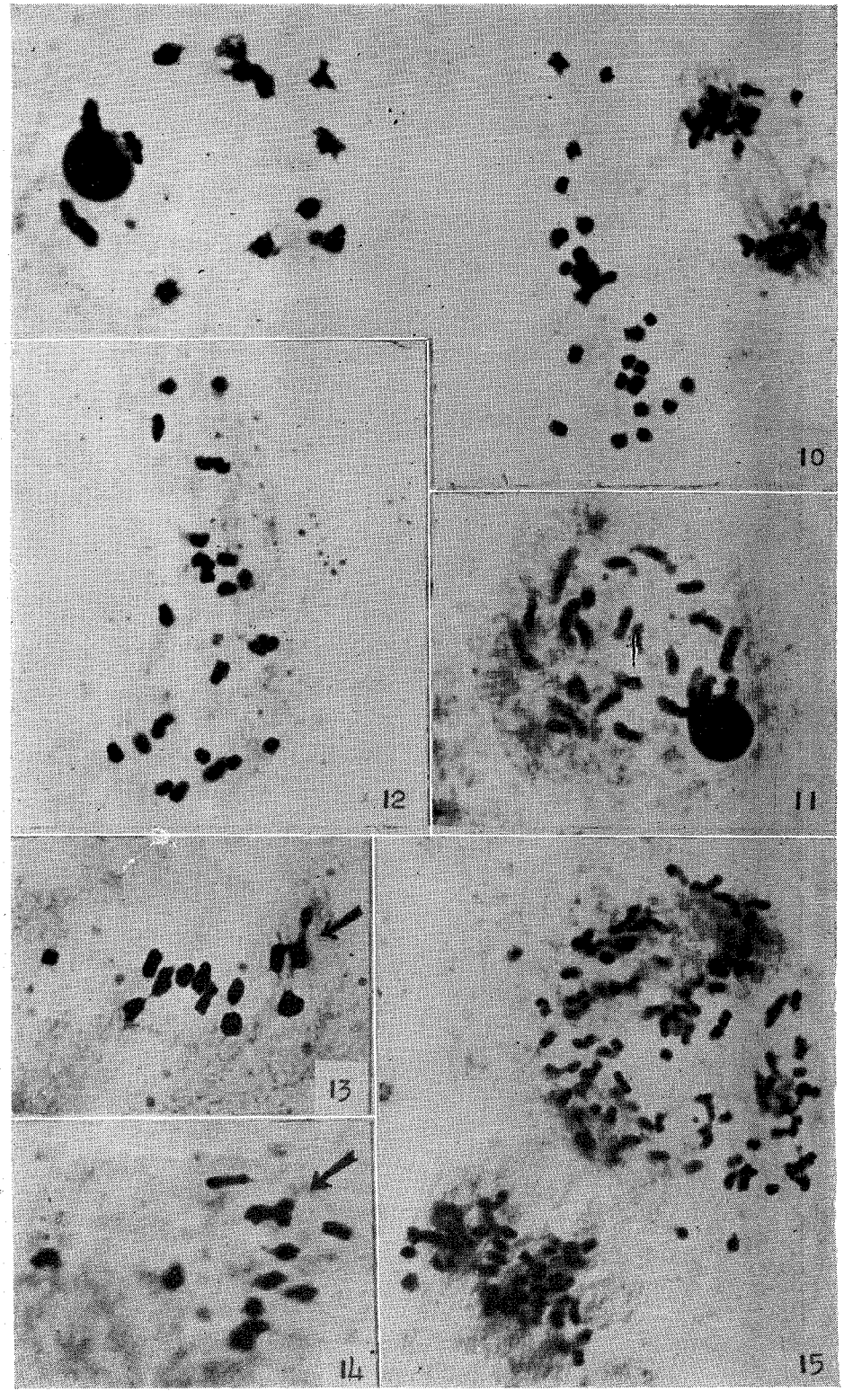
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strains indigenous to Thailand, India, Burma, Malaya and the Philippines were diallel inter-crossed and their F_1 hydrids were studied. The results are:

1. Though their phenotypical differences in characters are relatively of minor importance, they showed pronounced F_1 sterility.

2. The F_1's between strains from the same country (Thailand and India) were partly fertile with pollen fertility 50 to 70%, but those between strains from different countries were highly sterile, the pollen fertility being 0% or 3–10%. The strains of Thailand, Malaya and the Philippines showed partial fertility among them. The Burma strain gave a high F_1 sterlity with all other strains.

3. Chromosome pairing in meiosis of PMC's were observed and three tyes were found; *i.e.* normal paring in MI, formation of tri- or quadrivalent chromosome, and non-pairing forming univalents. The hybrid showing these distrbances in chromosome pairing were highly sterile, but some of the crosses showing normal pairing were also highly sterile.

4. Cytological observation, in so far as cross-combination between two geographic groups (Burmese and Thai strains *vs.* Indian, Malayan and Philippine strains), showed one single tri- or quadri-valent chromosome, suggesting that a reciprocal translocation might be involved.

5. It was concluded that possibly genomic differentiation in the *O. officinalis* was due to certain genetic factors within the species and its relatives.

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Fig. 10. Diakinesis in the F_1 of W012×W614-1, showing two cells, one with 12 regular bivalents, while the other has 24 univalents.

Figs. 11 & 12. 24 univalents, at diakinesis of F_1 of W012×W614-1 (Fig. 11) and at metaphase-I of F_1 of W1132×W614-1 (Fig. 12), are seen.

Fig. 13. Metaphase-I in the F_1 of W066×W012. A trivalent (indicated by an arrow), one univalent and 10 bivalents are seen.

Fig. 14. Metaphase-I in the F_1 of W1132×W614-1. A quadrivalent is seen (indicated by an arrow).

Fig. 15. Two cells, one with 48 and the other with about 70 chromosomes, found in the F_1 of W012×W614-1. (All ca. 2000 X).

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

第一報 *O. officinalis* 異地系統間之雜種不稔性

胡兆華 張家成*

野生稻 *Oryza officinalis* 及其近緣種由於形態的相似常混亂不清，故有 Officinialis complex 之稱。已知 *O. officinalis* 染色體組爲 CC, 它的近緣四倍體種染色體組爲 BBCC 及 CCDD, 但 B 及 D 染色體組來源未明。設或 C 染色體組有分化。本文係報告原產泰國、印度、緬甸、馬來亞及菲律濱等地七系統進行輪互雜交（diallel inter-crosses）研究其雜種的結果。

1. 東南亞各地產 *O. officinalis* 系統間雖植株外部形態差異微小，但雜種第一代顯出明顯的不稔性。

2. 同一國內（泰國、印度）不同地區系統間雜種具有50–70%正常花粉，而不同國系統間雜種僅有3–10%正常花粉或完全不稔。但泰國、馬來亞與菲律濱系統間雜種的稔性花粉率較高，達到半不稔程度，而緬甸一系統與任何地系統雜交，其雜種均表現出近於完全不稔。

3. 觀察花粉母細胞的減數分裂，發現雜種的染色體接合可分爲三種型式，即：第一中期正常；形成三價或四價染色體；及產生不接合的單價染色體。具有染色體接合異常的植物固屬高度不稔，接合正常植物亦有高度不稔者。

4. 就已觀察的細胞學的記錄可指出泰國、緬甸與印度、馬來亞、菲律濱間系統的雜交，雜種可形成三價或四價染色體，啓示兩地域間此一野生稻遺傳的組成內有一染色體移位的差異。

5. 基於上述事實的發現可達到一初步結論，內在某種遺傳因素可能導至此一野生稻及其近緣種染色體組發生分化。（中文摘要）

* 臺灣省立中興大學農藝系教授及研究助理

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