CYTOGENETICAL STUDIES ON THE GENUS *ORYZA* XI. ALIEN ADDITION LINES OF *O. SATIVA* WITH SINGLE CHROMOSOMES OF *O. OFFICINALIS*

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From crosses between allotriploid plants with genomes AAC and diploid *O. sativa* plants (AA), twelve types of alien addition lines with single C-genome chromosome were distinguished on the basis of morphological characteristics of the addition type plants. Of the twelve chromosomes of *O. officinalis* (W0065), six appeared to be homoeologous with those of *O. sativa* in varying degrees. The occurrence of many univalents in the PMCs of two alien addition lines, G and J, suggests that two chromosomes of *O. officinalis* (W0065) have genes which disturb the pairing of homologous *sativa* chromosomes.

INTRODUCTION

Since Sears (1956) transferred the leaf-rust resistance of *Aegilops umbellulata* to common wheat, the transfer of useful genes from wild to cultivated plants has been attempted by many workers. When the chromosomes of wild and cultivated species fail to pair normally, establishing alien chromosome addition lines has been a way of approach. There are some reports on the alien addition lines of common wheat with *Secale cereale* chromosomes (Riley and Chapman 1958; Evans and Jenkins 1960; Mukade *et al.* 1970), those of tetraploid wheat with *Aegilops squarrosa* (Alston 1970) and with *Agropyron elongatum* chromosomes (Mochizuki 1962) and those of *Avena sativa* with *A. hirtula* chromosomes (Thomas 1968).

To explore the possibility of transfer of disease and insect resistance genes from wild *Oryza* species to *O. sativa*, the authors have tried to isolate alien addition lines from crosses between allotriploid plants with different genomic constitutions (AAB and AAC, as reported by Shin *et al.* 1978) and *O. sativa* (AA). The wild species used for this experiment were *O. officinalis* Wall. (CC), *O. collina* (Trimen) Sharma et Shastry (CC, Ceylonese *officinalis*), an intermediate form (CC, between *O. punctata* and *O. eichingeri*) and diploid *O. punctata* Kotschy et Steus. (BB).

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From the cross of AAC×AA, the authors have succeeded in isolation of twelve different types of $2n=25$ (AA+C_x) plants. Presumably, each of them has a different chromosome of *O. officinalis*. Cytological and morphological investigations of those plants are reported in this paper.

MATERIALS AND METHODS

The initial cross was made between a tetraploid strain of an *O. sativa* cultivar (Sekitori, from National Institute of Agricultural Sciences, AA) and a diploid strain of *O. officinalis* (W0065, from National Institute of Genetics, CC) as reported by Shin *et al.* (1978). The allotriploid F₁ plants obtained from this cross were backcrossed with Sekitori (1974-76). Plants having $2n=25$ chromosome were obtained from the backcrosses as well as in the selfed progenies of $2n=26$ or 27 B₁ plants.

Their seeds were sterilized and sown on the artificial culture medium in test tubes. The cultured seedlings were transplanted into pots. In order to promote floral initiation, the plants were subjected to short-day conditions (about 9 h).

The root tips were pretreated with 0.002M 8-oxyquinoline at 18-20°C for 2-3 h. After fixing with acetic-alcohol (1:3), the root-tip and pollen-mother-cells (PMCs) were stained with acetocarmine.

The electrophoretic analysis using mature leaf blades was used for isolating alien chromosome addition lines having an acid phosphatase zymogram as of *O. officinalis* (W0065). The procedure of the electrophoresis was described by Katayama and Chern (1973).

RESULTS

Results of crossing and selfing: From the backcrosses, 1,218 seeds were obtained during three years, and they were sown under a sterile condition. From 688 germinated seeds, 405 plants grew up to the adult stage. Besides this, 96 plants were obtained from the selfing of $2n=26$ and 27 B₁ plants. Their somatic chromosome numbers

Table 1. Somatic chromosome numbers of plants from AAC×AA crosses and in the selfed progeny of $2n=26$ and $2n=27$ plants

	Chromosome number												Total
	24	25	26	27	28	29	30	31	32	33	36	54	
Backcross													
1974	1			2			1		1				5
1975	10	10	5	8	4		3		2		1		43
1976	40	23	28	50	77	69	46	12	8	3		1	357
Selfing													
$2n=26$	16	32	8										56
$2n=27$	7	17	16										40
Total	74	82	57	60	81	69	50	12	11	3	1	1	501

varied from $2n=24$ to 54 (Table 1). The numbers of plants with $2n=25$ (alien chromosome addition lines), 26 and 27 chromosomes were 82, 57 and 60, respectively.

The origin and number of each type of alien chromosome addition lines with $2n=25$ chromosomes, thus obtained, are presented in Table 2.

Table 2. Numbers of 12 types of alien chromosome addition lines obtained and their sources

Origin	Plant type												Total
	A	B	C	D	E	F	G	H	I	J	K	L	
Allotriploid (AAC)													
×	20	3	2	1	1	1	1	1		1	2		33
Sekitori (AA)													
Selfing													
AD 1 ($2n=27$)				5					1			8	14
AD 11 ($2n=27$)	1								1			1	3
AD 24 ($2n=26$)	20	12											32
Total	41	15	2	6	1	1	1	1	2	1	2	9	82

These may be compared with the alien addition lines having *O. australiensis* chromosomes reported by Wu *et al.* (1967).

Morphological characteristics: Eighty-two alien chromosome addition lines in total were classified into the following twelve types on the basis of their vigor, growth habit, stature, rolling of leaves, panicle and spikelet shapes, awnedness, seed setting and other characteristics (Table 3, Fig. 1).

Type A: Long grain, thick stem, horizontal stretch of flag-leaf, slight reduction in height, and high fertility.

Fig. 1. Panicles of alien chromosome addition lines and their parents. P₁: Sekitori (2x). P₂: *O. officinalis* (W0065, 2x). A-L: Twelve alien chromosome addition lines (cf. Table 3).

Table 3. Morphological characteristics of alien chromosome addition lines (2*n*=25)

Parent and addition line	Awn	Spikelet*		Apiculus color	Ligule	Flag-leaf		Leaf	Plant height (cm)	Panicle		Seed fertility (%)
		Length (cm)	Length / width			Angle	Length Width (cm) (cm)			Habit	Length (cm)	
Sekitori (AA)		5.5	1.8			15	33 1.4		95		18	99
W0065 (CC)		3.6	1.8			120	14 1.4		104	spreading	28	36
AA+Cx												
A		6.0	1.9			90	28 1.4		85		20	84
B		5.2	2.0			60	33 1.0		75		18	80
C		5.3	2.4		short	10	24 0.9	dark-green	66		17	11
D		5.1	1.9			120	23 1.2	drooped, soft	84	semi-spreading	18	56
E		5.9	2.4		long	90	29 0.9	drooped, soft	69		18	40
F		4.8	1.7			15	17 1.1		72		15	23
G	+	—	—			120	29 1.1	dark-green, rolled	70		19	0
H	+	5.7	2.1		long	90	28 1.3	soft	91		20	27
I	+	5.5	1.9	purple		90	47 1.5		100		27	48
J	+	—	—	purple		120	30 1.1	light-green, soft	71		21	0
K	+	5.2	2.0			90	36 1.1		62		20	13
L	+	5.2	1.8			30	24 1.5	dark-green	64		17	1

*: Brown rice.

+: Spikelet awned.

Type B: Slight reduction in height, thin uppermost internode, and high fertility.

Type C: Large grain, erect panicle, short ligule, narrow angle of flag-leaf, narrow and dark green leaves, reduced height, and low fertility.

Type D: Hanging of flag leaf, drooped and soft leaves, thin stem, semi-spreading panicle, and semi-sterility.

Type E: Long grain, long ligule, horizontal stretch of flag-leaf, narrow, drooped and soft leaves, thin stem, and semi-sterility.

Type F: Small grain, short and narrow angle of flag-leaves, short and compact panicles, and low fertility.

Type G: Hanging, drooped, slightly rolled and dark green leaves, awnedness, and sterility.

Type H: Long ligule, awnedness, horizontal stretch of flag-leaf, soft leaf, low fertility, and the same acid phosphatase zymogram as that of *O. officinalis* (W0065) (Shin 1977).

Type I: Purple apiculus, awnedness, horizontal stretch of flag-leaf, tall height,

Table 4. Chromosome configurations at MI in the PMCs of alien monosomic addition lines

Chromosome configuration	Plant type											
	A	B	C	D	E	F	G	H	I	J	K	L
1III+ 11II	38	19		8	1	1		1	7	6		3
1III+ 10II+ 2I										2		
1III+ 9II+ 4I										2		
1III+ 8II+ 6I										1		
1III+ 7II+ 8I										1		
1III+ 6II+ 10I										2		
1III+ 5II+ 12I							1			3		
1III+ 4II+ 14I										1		
1III+ 3II+ 16I										3		
1III+ 2II+ 18I										1		
12II+ 1I	19	19	111	67	46	39	1	72	56	11	85	52
11II+ 3I		2	4	3	1		1	11		10	2	
10II+ 5I							7	2		24		
9II+ 7I							9			20		
8II+ 9I							6			24		
7II+ 11I							11			15		
6II+ 13I							9			16		
5II+ 15I							10			11		
4II+ 17I							8			6		
3II+ 19I							2			7		
2II+ 21I							1			1		
1II+ 23I							2			1		
25I							1					
Total	57	40	115	78	48	40	69	86	63	168	87	55

long and semi-spreading panicles, wide and long leaves, and semi-sterility.

Type J: Hanging of flag-leaf, awnedness, purple apiculus, drooped, soft and light green leaves, imperfect panicle emergence, late heading, and sterility.

Type K: Awnedness, horizontal stretch of flag-leaf, reduction in height, exerted stigma after flowering, and low fertility.

Type L: Small angle and shortness of flag-leaves, slightly wide and dark green leaves, awnedness, imperfect panicle emergence, slightly late heading, and sterility.

The morphological characteristics such as leaf color, awnedness, leaf width, plant height and leaf drooping found in the alien chromosome addition lines were similar to those described in the trisomics of *O. sativa* (Hu 1968). But in such characteristics as purple coloration of the apiculus (types I and J), semi-spreading panicle (type D), exerted stigma (type K) and the occurrence of the same acid phosphatase zymogram as *O. officinalis* (W0065, type H), the alien chromosome addition lines differed from the trisomics.

Cytological observations: The chromosome configurations observed at MI of PMCs of the twelve types of alien chromosome lines (Fig. 2-a) are summarized in Table 4. The twelve types were classified into three groups on the basis of chromosome configurations, i.e., 1) showing 1 trivalent and 11 bivalents with a high frequency (types A and B, Fig. 2-b), 2) showing a disturbance in the pairing of homologous chromosomes of genome A (types G and J, Fig. 2-d), and 3) having 12 bivalents and 1 univalent (all other types, Fig. 2-c).

The frequencies of trivalents at MI of the types A and B were 67% and 48%,

Fig. 2. Somatic chromosomes and chromosome configurations at MI of alien monosomic addition lines.

a: $2n=25$. b: $1\text{III}+11\text{II}$ (A type). c: $12\text{II}+1\text{I}$ (C type).
d: $3\text{II}+19\text{I}$ (G type).

respectively. The number of trivalents per cell ranged from 0 to 1 with a mean of 0.7 in the type A, and 0 to 1 with a mean of 0.5 in the type B, as shown in Table 5. The frequencies of trivalents found in the types D, I, J, and L were 10, 11, 4, and 6 percent, respectively.

Table 5. Range and mean of chromosome pairing per PMCs at MI of alien monosomic addition lines

Plant type	No. of cells observed	Chromosome pairing at MI					
		III		II		I	
		Range	(Mean)	Range	(Mean)	Range	(Mean)
A	57	0-1	(0.67)	11-12	(11.3)	0-1	(0.3)
B	40	0-1	(0.48)	11-12	(11.5)	0-3	(0.5)
C	115	0	(0.00)	11-12	(12.0)	1-3	(1.1)
D	78	0-1	(0.10)	11-12	(11.9)	0-3	(1.0)
E	48	0-1	(0.02)	11-12	(12.0)	0-3	(1.0)
F	40	0-1	(0.03)	11-12	(12.0)	0-1	(1.0)
G	69	0-1	(0.01)	0-12	(6.5)	1-25	(11.9)
H	86	0-1	(0.01)	10-12	(11.8)	0-5	(1.3)
I	63	0-1	(0.11)	11-12	(11.9)	0-1	(0.9)
J	168	0-1	(0.13)	1-12	(7.9)	0-23	(8.9)
K	87	0	(0.00)	11-12	(12.0)	1-3	(1.0)
L	55	0-1	(0.06)	11-12	(11.9)	0-1	(0.9)

The chromosome configurations observed in the types C, D, E, F, H, I, K, and L were $12_{II}+1_I$ (Tables 4 and 5). No difference in the chromosome configurations was observed within these eight types except for occasional trivalent formation.

On the other hand, the number of univalents per cell ranged from 1 to 25 with a mean of 11.9 in the type G, and 0 to 23 with a mean of 8.9 in the type J.

DISCUSSION

To obtain alien chromosome addition lines, allotriploid plants were used as the male parent by some workers (Sears 1956; Thomas 1968; Alston 1970), and as the female parent by others (Riley and Chapman 1958; Evans and Jenkins 1960; Wu *et al.* 1967). In the present experiment, the authors used the allotriploid plant (AAC) as the female parent and made no castration because they were completely pollen sterile.

The chromosome added to an alien addition line can be specified by three criteria, namely, the morphology of the chromosome, morphological characteristics of the plants, and the meiotic behavior of the chromosomes in the F_1 plants between two alien chromosome addition lines. Although the karyotype of *O. officinalis* is not clear yet, the $2n=25$ plants obtained could be classified into 12 groups from their morphological characteristics.

Therefore, it was assumed that each of them had a different additional chromo-

some introduced from *O. officinalis* and each chromosome had a different effect on phenotypic expression.

Wu *et al.* (1967) observed an addition line of *O. sativa* (AAE) having the sat-chromosome of *O. australiensis* (EE). Ku *et al.* (1969a) used the technique of paper chromatography for identification of their alien addition lines with *O. australiensis* chromosomes. They concluded that all additional chromosomes except E_7 (australian chromosome no. 7) were almost indistinguishable by the chromatographic technique. In the present experiment, the type H only had the same acid phosphatase zymogram as of *O. officinalis* (Shin 1977).

As mentioned above six chromosomes of *O. officinalis* carried by the types A, B, D, I, J, and L formed trivalents with their homologous partners of *O. sativa*. This suggests that these six *officinalis* chromosomes have some homology with *sativa* chromosomes; the degree of homology between the chromosomes of genomes A (*O. sativa*) and C (*O. officinalis*) may be differently evaluated according to individual chromosome pairs. In the monosomic addition lines, Mochizuki (1962) recognized that six of the seven *Agropyron* chromosomes had homologous segments with *Triticum durum* chromosomes.

Based on the stainability and size of chromosomes at MI of PMCs, however, Ku *et al.* (1969b) found no AAE association in the alien addition lines ($AA+E_x$) of *O. sativa* $\times$ *O. australiensis*, although chromosome pairing was observed more frequently between genomes than within genomes in the F_1 plant of *O. sativa* $\times$ *O. australiensis*. From this fact, they considered that the E chromosomes failed in competition for pairing with its partially homologous A chromosomes.

The asynaptic and desynaptic genes controlling chromosome pairing at MI of PMCs have been reported in *Zea* (Beadle 1933), *Triticum* (Li *et al.* 1945; Riley *et al.* 1959) and other plants. The asynapsis or desynapsis was in most cases controlled by a single recessive gene.

Though in the present study the pachytene chromosomes were not observed and the genetic control of univalent formation in the types G and J remained unknown, two chromosomes of *O. officinalis* (W0065) may be considered to carry asynaptic or desynaptic genes disturbing the pairing of *sativa* chromosomes.

In the F_1 plants of *O. sativa* $\times$ *O. officinalis*, Shastry *et al.* (1961) as well as Katayama (1965) observed that the pachytene chromosomes did pair but there were no normal bivalents at MI. They assumed the presence of desynaptic genes controlling this phenomenon. But other workers observed in the same species hybrid that the chromosomes did not pair normally at both pachytene (Li *et al.* 1963) and MI stages (Morinaga *et al.* 1958; Nezu *et al.* 1960), and considered that there was no homology of chromosomes between the A and C genomes. This discrepancy found in the pairing of pachytene chromosomes among these workers might be due to their use of different strains of *O. officinalis*.

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