ON THE SECONDARY BALANCED KARYOTYPES OF $M-m_4+s_1M-m_4+s_1$ AND $M-m_4+a_4M-m_4+a_4$

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Introduction

More than forty sorts of karyotypes derived from the interspecific hybrid between *Vicia sativa* and its related species [1, 5, 7, 8, 9].

However, these karyotypes were heterozygously except both parental karyotypes, and chromosome paring at meiosis was irregulary and low fertility. On the other hand, stable new karyotype which substituted the satellite chromosome s_1 of *V. sativa* or a_4 of *V. amphicarpa* for the same chromosome m_4 of *V. macrocarpa* homozygously in the MM (*V. macrocarpa*) karyotype derived from the hybrids between the species. These new karyotypes were secondary balanced type. Characteristic and cytological studies were reported in this paper.

Materials and Methods

The strains used were introduced from Dr. D. Mettin in 1960 and Dr. C. Barnard in 1963. These strains were as follows: *Vicia sativa* var. *alba* (S_W), *V. amphicarpa* (A) and *V. macrocrpa* (M). And the other strain of *V. sativa* was strain No. 4 (S_4) introduced from the National Institute of Animal Industry in 1954.

Some F_1 seeds of interspecific hybrids were obtained from the combinations of $S_W \times M$, $S_4 \times M$, $M \times A$ and $A \times M$ in 1964. These seeds were sown in the October of the same year, and the later generations grew up. Besides, $S_W \times M$ F_1 were backcrossed with S_W and the later generations were examined. In the F_2, two plants of $M-m_4+s_1M-m_4+s_1$ and $M-m_4+a_4M-m_4+a_4$ karyotypes derived.

The root tip of these plants was pretreated in low temperature at 0-1°C in 8 hours and fixed in Fermer's solution and stained with acetic orcein. Karyotype was observed by the squash method.

Results and Discussions

1. On the stable new karyotypes of $M-m_4+s_1M-m_4+s_1$ and $M-m_4+a_4M-m_4+a_4$

Both karyotypes derived in the F_2, F_3 and B_1F_2 of the interspecific hybrids between *V. sativa* and *V. macrocarpa*, *V. amphicarpa* and *V. macrocarpa*.

The parental karyotype was shown in Fig.1, and each length of satellite chromosome of parental species was indicated in table 1. Besides, microphotograph of somatic chromosomes of $M-m_4+s_1M-m_4+s_1$ and $M-m_4+a_4M-m_4+a_4$ karyotypes was shown in Fig. 2. Total length of satellite chromosome was the shortest in m_4 of *V. macrocarpa* (ca. 0.6μ, next was s_1 of *V. sativa* (ca. 0.9μ) and the longest was a_4 of *V. amphicarpa* (ca. 1.1μ)

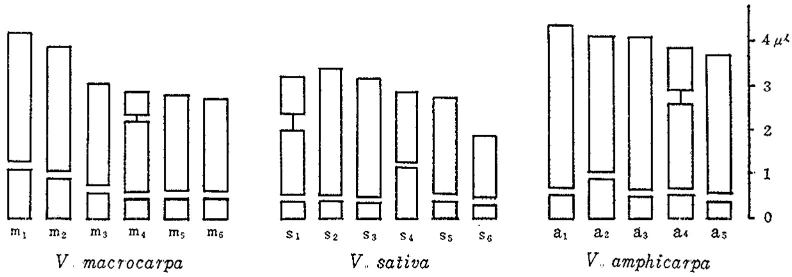

Fig. 1. Karyotype of *V. macrocarpa*, *V. sativa* and *V. amphicarpa*.

Table 1. Length of satellite chromosome of *V. macrocarpa* (m_4), *V. sativa* (s_1) and *V. amphicarpa* (a_4). (1/10μ)

Satellite chromosome	Short arm	Long arm	Interval	Satellite	Total length
m_4	5.05	17.70	—	5.90	28.65
s_1(Sw)	3.95	15.10	3.15	9.15	31.35
s_1(S_4)	3.20	15.70	4.20	9.35	32.45
a_4	5.41	19.14	3.18	11.27	39.00

The substitution of satellite chromosome s_1 or a_4 for the m_4 was indicated that the small satellite chromosome of *V. macrocarpa* was filled up by the larger same chromosome of *V. sativa* and *V. amphicarpa*, and it was also presumed that the $M-m_4+s_1$ and $M-m_4+a_4$ karyotype gametes were fertile. The same phenomena was also observed in the hybrid between *V. sativa* and *V. angustifolia*, but no plant derived homozygously[(6)].

These secondary balanced karyotypes derived in the F_2 and F_3 generations were shown in Fig. 3. Total 14 plants of both types derived from the $M-m_4+s_1M-m_4+s_1$, $M-m_4+s_1M+s_6$, SM, $SM-m_4+s_1$, $SM-m_4+s_1+s_6$, $MM-m_4+s_1$, $M-m_4+s_1M-m_4+s_1$, $A+stM-m_4+a_4$, $MM-m_4+a_4$ and $M-m_4+a_4M-m_4+a_4$ karyotypes. In the next of $M-m_4+s_1$ $M-m_4+s_1$ and $M-m_4+a_4M-m_4+a_4$ karyotype plants, only the same karyotype derived. From the appearance of the substitution above mentioned, it is presumed that $M-m_4+s_1$ and $M-m_4+a_4$ type gametes will be able to guide from the karyotype of before generation. The frequency of both substituted $M-m_4+s_1$ $M-m_4+s_1$ and $M-m_4+a_4M-m_4+a_4$ karyotypes derived in the next generation was considerable high, and about 20% in each combinations.

2. Characters of substituted type plant

The morphological chararacters of $M-m_4+s_1M-m_4+s_1$ type plant were indicated in the table 2. As shown in the table, these characters were classified in three groups.

a. The character with which no variation was observed among the $M-m_4+s_1M-m_4+s_1$ karyotype plants, and the same character was appeared in the other karyotype plants. 1. twist of leaflet 2. type of branch 3. crease of surface of pod 4. hollow between the loculus of pod.

Combination	F_1 No.	F_1 Karyotype	F_2 No.	F_2 Karyotype		F_3 No.	F_3 Karyotype
$S_W \times M$	6-2	SM	4- 8	$M-m_4+s_1M-m_4+s_1$	(82.5)*	18-1	$M-m_4+s_1M-m_4+s_1$ (99.0)*
			4-27	$M-m_4+s_1M+s_6$	(48.7)	23-2	$M-m_4+s_1M-m_4+s_1$ (60.3)
						23-4	$M-m_4+s_1M-m_4+s_1$ (72.7)
$S_W \times M$	S_W ↓ F_1		3-1-2	SM	(6.6)	1- 5	$M-m_4+s_1M-m_4+s_1$ (99.0)
			12	SM	(17.4)	3- 2	$M-m_4+s_1M-m_4+s_1$ (99.0)
			3-1-3	$SM-m_4+s_1$	(11.1)	5- 2	$M-m_4+s_1M-m_4+s_1$ (59.9)
			3-23	$SM-m_4+s_1+s_6$	(15.2)	10- 5	$M-m_4+s_1M-m_4+s_1$ (82.6)
$S_4 \times M$	8-4	SM	1-18	$MM-m_4+s_1$	(92.5)	10- 4	$M-m_4+s_1M-m_4+s_1$ (98.4)
	8-2	SM	4-23	$M-m_4+s_1M+s_6$	(83.3)	23-10	$M-m_4+s_1M-m_4+s_1$ (90.0)
			4- 3	$M-m_4+s_1M+s_6$	(91.1)	24- 5	$M-m_4+s_1M-m_4+s_1$ (95.0)
$M \times A$	3-4	AM	5- 1	$A+stM-m_4+a_4$	(15.5)	14- 1	$M-m_4+a_4M-m_4+a_4$ (15.5)
						14- 2	$M-m_4+a_4M-m_4+a_4$ (36.9)
$A \times M$	2-3	AM	4-28	$MM-m_4+a_4$	(36.8)	17- 1	$M-m_4+a_4M-m_4+a_4$ (89.0)
			4-19	$M-m_4+a_4M-m_4+a_4$	(21.7)	18- 1	$M-m_4+a_4M-m_4+a_4$ (43.2)

Fig. 3. $M-m_4+s_1M-m_4+s_1$ and $M-m_4+a_4M-m_4+a_4$ karyotype plants derived from the interspecific hybrid between *Vicia sativa*, *V. amphicarpa* and *V. macrocarpa*.
* Good pollen %

b. The character with which some variation was observed among the $M-m_4+s_1M-m_4+s_1$ type plant. 1. color of main vein, leaflet and stem 2. flower color 3. pod color 4. hair of pod.

c. The character with which no variation was observed among the $M-m_4+s_1M-m_4+s_1$ type plant, and no plant was observed in the other karyotype plant. 1. shape of leaf apex 2. width of pod.

Although almost of these characters above mentioned were not clarified by the gene analysis, it was presumed that the characters belong above mentioned were not controlled by the genes which located the satellite chromosome. The genes which controlled the characters belong b, located on the chromosome which cannot be identified by the observation of somatic chromosome. And the characters belong c, located the satellite chromosome s_1 and a_4. In almost all the qualitative characters of the plants, considerable variation was observed among the same karyotype plants, for example flower color, pod color, hair of pod. The same phenomena was reported by Yamamoto in *Vicia sativa* and its related species[5]. On the character of $M-m_4+a_4M-m_4+a_4$ type, the same inclination was observed.

The growth of $M-m_4+s_1M-m_4+s_1$ was also indicated in table 2. The plant height was the highest in *V. macrocarpa*, next was *V. sativa*. On the other hand, the number of branch was not differed from these species. In the $M-m_4+s_1M-m_4+s_1$, plant height and

Table 2. Character and growth of $M-m_4+s_1M-m_4+s_1$ type plant and the parents

Character	Plant no. 1-5	3-2	5-2	10-5	18-1	23-2	23-4	M	Sw
Twist of leaflet	n	n	n	n	n	n	n	twist	n
Color of main vein	rp	rp	rp	g	rp	rp	rp	rp	g
Shape of leaf apex	ne	ne	ne	ne	ne	ne	ne	wide and emarginate	intermediate and truncate
Type of branch	el	el	el	el	el	el	el	el	intermediate
Flower color	red	red	red	white	red	red	red	red	white
Hair of pod	hairy	hairy	hairy	hairy	hairy	hairy	hairy	smooth	hairy
Crease of surface of pod	crease	crease	crease	crease	crease	crease	crease	crease	smooth
Color of pod	yellow	black	yellow	black	brown	black	black	black	yellow
Hollow between the loculus of pod	plane	plane	plane	plane	plane	plane	plane	plane	hollow
Width of pod	im	im	im	im	im	im	im	wide	narrow
Plant height (cm)	66	70	87	70	73	73	73	83.6	56.8
Number of branch	22	12	27	20	13	14	20	17.6	12.4
Number of seeds per pod	6.1	4.9	6.5	4.5	3.8	4.8	4.6	6.2	5.7

Note n : normal, rp : reddish purple, g : green, ne : narrow and emarginate
el : elect, im : intermediate

number of branch were intermediate between the parental species in plant No. 5-2. It is very interest for practical use. The good pollen % and number of seeds per pod were high except the few plants as shown in Fig. 3 and table 2.

3. Meiosis of $M-m_4+s_1M-m_4+s_1$ and $M-m_4+a_4M-m_4+a_4$ type plant

Chromosome configuration at MI of PMCs of $M-m_4+s_1M-m_4+s_1$ and $M-m_4+a_4M-m_4+a_4$ type plant was shown in table 3 and the photographs of chromosome configuration were shown in Fig. 4 and 5. In almost of $M-m_4+s_1M-m_4+s_1$ type plant, normal 6 bivalents were observed and at AI, 6 daughter chromosomes separated regularly. But in a few of $M-m_4+a_4M-m_4+a_4$ type plants, considerable irregularity of conjugation was observed. It is presumed that the irregularity of chromosome conjugation was due to the partial homologous of parental chromosomes which cannot be identified. The partial homology observed in the parental species was deduced from the Navashin's hypothesis[2] on the differentiation of species. This deduction was studied in *Vicia* species by Sveschnikova [4], Rousi [3] and Yamamoto [5]. The substitution of large satellite

Table 3. Chromosome configuration at MI of PMC of $M-m_4+s_1M-m_4+s_1$ and $M-m_4+a_4M-m_4+a_4$ type plants.

Type of configuration	Sw×M F_2 4-8	Sw×M F_3 23-2	(Sw×M)×Sw B_1F_3 1-5	(Sw×M)×Sw B_1F_3 5-2	S_4×M F_3 10-4	M×A F_3 14-1	A×M F_3 17-1
$2_{II}+8_{I}$						5	
$4_{II}+4_{I}$						4	2
$5_{II}+2_{I}$						16	35
6_{II}	53	37	48	29	24	7	
$1_{III}+3_{II}+3_{I}$						10	
$1_{III}+4_{II}+1_{I}$						34	
$2_{III}+3_{II}$						6	
$1_{VI}+4_{II}$						2	

chromosome of s_1 or a_4 for the small satellite chromosome of m_4 was observed, but in the opposite direction, the substitution of m_4 for s_1 or a_4 did not derive. This phenomena indicate that the function of large satellite chromosome s_1 or a_4 is able to carry out that of small satellite chromosome m_4, and this fact is also presumed that the m_4 of *V. macrocarpa* is the minimum unit of satellite chromosome in the parental species.

It is interest phenomena that the secondary balanced karyotypes derived in the interspecific hybrid between *Vicia* species.

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Vicia 属の種間雑種に現われた2次平衡型 $M-m_4+s_1M-m_4+s_1$ および $M-m_4+a_4M-m_4+a_4$ について

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要　旨　*Vicia sativa* 2n=12 および *V. amphicarpa* 2n=10 と *V. macrocarpa* 2n=12 との雑種後代で，*V. macrocarpa* 型で付随体染色体 m_4が失なわれ，代りに *V. sativa* および *V. amphicarpa*の付随体染色体 s_1 または a_4 がホモに入つた $M-m_4+s_1M-m_4+s_1$ および $M-m_4+a_4M-m_4+a_4$ 型が出現した。これらは新らしい安定した二次平衡型である。したがって，小型の付随体染色体m_4の機能を大型の s_1 または a_4 で代行できることを示す。$M-m_4+s_1$ $M-m_4+s_1$ 型は小葉の先端の形，莢の幅などで特徴を有し，PMC での接合型は6_{II} で稔性は比較的高い。しかし$M-m_4+a_4M-m_4+a_4$ 型の1部の個体では，分裂の異常が認められる。これは識別不可能な両親染色体に若干の部分非相同性があることを示す。

(Received May 30, 1970)

Fig. 2. Somatic chromosome of $M-m_4+s_1M-m_4+s_1$ and $M-m_4+a_4M-m_4+a_4$ karyotype plants
upper : $M-m_4+s_1M-m_4+s_1$
lower : $M-m_4+a_4M-m_4+a_4$

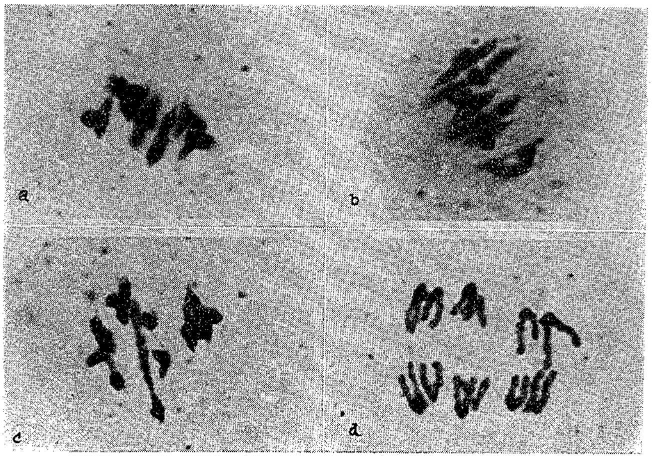

Fig. 4. Chromosome configuration at MI and AI of PMCs in $M-m_4+s_1M-m_4+s_1$ $(2n=12)$ type plants.

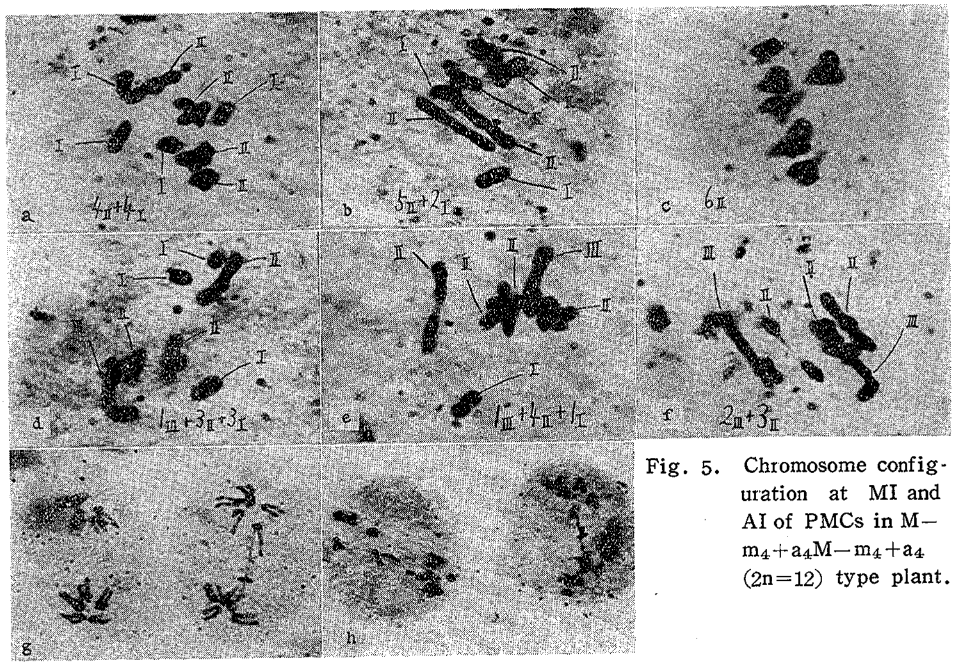

Fig. 5. Chromosome configuration at MI and AI of PMCs in $M-m_4+a_4M-m_4+a_4$ (2n=12) type plant.