Meiotic Chromosome Behaviours of Auto-pentaploid Rice Plant Derived from Anther Culture

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In cultivated rice, *Oryza sativa* L. ($2n=24$, AA), a number of reports on haploids ($2n=12$, A), triploids ($2n=36$, AAA), and tetraploids ($2n=48$, AAAA) have been published so far. No pentaploid ($2n=60$, AAAAA), however, has been reported up to date.

Nishi and Mitsuoka (1969) have first succeeded to induce a series of polyploids from haploid to pentaploid in rice through its tissue culture. The present author was kindly furnished one stubble of pentaploid rice plants from them, and was able to observe its meiotic chromosome behaviours. This paper deals briefly with its meiotic chromosome behaviours as well as its morphological features.

Material and methods

Nishi and Mitsuoka (1969) have raised seven pentaploid rice plants through anther culture using a cultivar, "Kyoto-Asahi". One of these as designated A4 was vegetatively multiplied, and subjected to cytogenetic observations.

Root-tip cells were fixed with 1:3 acetic-alcohol for one day, mordanted with 4.0% iron-alum solution for 2 hours after maceration with 1 N HCl for 7 minutes, and stained with aceto-carmin solution for more than 3 hours. PMCs were fixed and mordanted at the same time with 1:3 acetic-alcohol containing 0.5% ferric chloride, and stored in the refrigerator until used. For cytological observations, squashed preparations were made by staining with aceto-carmin solution.

Results

In root-tip cells, 60 chromosomes were invariably observed, showing that this plant is auto-pentaploid (Fig. 1). In PMCs, however, a number of aneuploid cells were observed ranging from 33 to 99 in $2n$ numbers when reckoned from the chromosome configurations in MI (Table 1, Fig. 2). Only 3 cells were observed possessing 60 chromosomes which was equal to the somatic number in RTCs. Their chromosome configurations were $2_{IV}+26_{II}$, $4_{IV}+22_{II}$, and $2_{III}+26_{II}+2_I$, respectively. Strictly speaking, this plant should be called mixoploid, and multivalents such as trivalents or tetravalents were frequently formed in most PMCs. In one cell, 4 pentavalents were met with. Univalents also were frequently observed ranging from 1 to 10.

Out of 30 PMCs examined, 4 cells possessed 56 chromosomes which was the

Figs. 1-2. 1, somatic chromosomes in MI of 5X plant ($2n=60$). 2, drawings of 1st metaphasic plates showing (a) $4_{IV} + 4_{III} + 11_{II} + 2_I$ ($2n=52$) and (b) $3_{IV} + 7_{III} + 10_{II} + 3_I$ ($2n=56$).

Table 1. Chromosome-mosaicism observed in PMCs of pentaploid rice plant

2n	chromosome configuration	frequency
33	$2_{IV} + 11_{II} + 3_I$	1
38	$1_{IV} + 6_{III} + 8_{II}$	1
39	$5_{III} + 12_{II}$	1
40	$1_{IV} + 16_{II} + 4_I$	1
41	$20_{II} + 1_I$	1
42	$6_{III} + 10_{II} + 4_I$	1
	$3_{IV} + 8_{III} + 2_{II} + 2_I$	1 } 2
44	$1_{IV} + 1_{III} + 17_{II} + 3_I$	1
45	$1_{IV} + 9_{III} + 7_{II}$	1
46	$22_{II} + 2_I$	1
47	$2_{IV} + 19_{III} + 1_I$	1
49	$22_{II} + 5_I$	1
50	$3_{III} + 18_{II} + 5_I$	1
51	$4_{III} + 18_{II} + 3_I$	1
	$1_{IV} + 23_{II} + 1_I$	1 } 2
52	$24_{II} + 4_I$	1
	$4_{IV} + 4_{III} + 11_{II} + 2_I$	1 } 3
	$1_{IV} + 2_{IV} + 1_{III} + 16_{II} + 4_I$	1
55	$5_{IV} + 1_{III} + 16_{II}$	1
	$1_{IV} + 3_{IV} + 7_{III} + 8_{II} + 1_I$	1 } 2
56	28_{II}	1
	$1_{IV} + 2_{III} + 18_{II} + 10_I$	1 } 4
	$3_{IV} + 7_{III} + 10_{II} + 3_I$	1
	$4_{IV} + 3_{IV} + 5_{III} + 4_{II} + 1_I$	1
57	$5_{IV} + 1_{III} + 16_{II} + 2_I$	1
	$7_{IV} + 1_{III} + 11_{II} + 4_I$	1 } 2
60	$2_{III} + 26_{II} + 2_I$	1
	$2_{IV} + 26_{II}$	1 } 3
	$4_{IV} + 22_{II}$	1
99	$1_{IV} + 3_{IV} + 8_{III} + 24_{II} + 10_I$	1
Total		30

modal number of these aneuploid cells, though their chromosome configurations were different in different cells. It is interesting to note that out of these 4 cells, 2 showed the extreme configurations, one the most regular pairing of 28_{II} , the other the most irregular pairing of $4_V + 3_{IV} + 5_{III} + 4_{II} + 1_I$, respectively. As a result of such meiotic irregularities, the pentaploid plant showed low pollen fertility of 8.5%. Even these pollen grains stained red with aceto-carmines did not seem to possess the functioning ability.

This plant showed anthesis similar to cleistogamy, but fertilization has never taken place, being characterized by extreme parthenocarpy. The number of anthers

Fig. 3. Pollen grains with 2 germ-pores.

in a floret varied from 3 to 6, the mode being 6, though 1 to 5 of them showed rudimentary development (Table 2). The most conspicuous feature of this plant was the formation of multi-germ-pores on pollen grains, which has never been found in rice. That is to say, the pollen grains with 2 to 4 germ-pores were frequently observed in addition to the normal grains with one germ-pore. The modal number of germ-pores observed was 2 (Table 3, Fig. 3).

Morphologically, pentaploid rice plant showed similar grass type to that of tetraploid in having coarser and thicker leaves, longer panicles, larger spikelets with awns, larger size of stomata and pollen grains than diploid (Table 4, Fig. 4). Pentaploid plant, however, was somewhat more dwarf, produced more tillers and panicles than tetraploid, and had a peculiar type of root system as

Fig. 4. Grass-types of $2X$ (right) and $5X$ (left) plants.

Table 2. Variation of the number of anthers in a floret of 5X plant

No. of anthers in a floret	1	2	3	4	5	6	2+(4)*3+(3)	4+(2)	5+(1)	0+(5)	1+(4)	3+(2)	Total	
Frequency	0	0	2	1	2	8	1	8	12	11	1	1	3	50
(%)	0	0	4	2	4	16	2	16	24	22	2	2	6	100

* Numeral in brackets denotes the number of deformed anther.

Table 3. Variation of the number of pollen-germ-pores in 5X plant

No. of germ-pore	1	2	3	4	Total
Frequency observed	135	152	22	1	310
%	43.6	49.0	7.1	0.3	100

pointed out already by Nishi and Mitsuoka (1969). Even in open conditions, pentaploid plant was completely sterile. When crossed with the pollens of diploid plants, pentaploid set only imperfect seeds having no germinating ability.

Discussion

Tissue culture in plant has revealed a brilliant prospect for the practical breeding by inducing haploid plants through the culture of pollen grains or anthers containing them. Recently, however, it has been generally accepted that the callus contains a various number of chromosomes in its constituting cells, and therefore, among the recovered plants derived from tissue culture, various types of polyploids and aneuploids can be found.

Nishi and Mitsuoka (1969) succeeded to secure a series of polyploids from haploid to pentaploid by rice tissue culture. Out of these, pentaploid plant obtained from anther culture is the first case of auto-pentaploid in rice. As a result of the meiotic observations carried out on this plant, it was found that its PMCs reveal the extreme chromosome-mosaicism in spite of its RTCs possessing normal chromosome numbers ($2n=60$) as the pentaploid.

Somatic polyploidy in root-tips or chromosome-mosaicism showing a lot of aneuploid cells in one and the same anther-locule has been reported by many workers in normal varieties, hybrid progenies or amphidiploids (Hollingshead 1932, Kattermann 1933, Love 1938, O'Mara 1942, Mochizuki 1943, Li and Tu 1947, Sachs 1952, Watanabe *et al.* 1955, '56 a, b, '58, '60 a, b, Watanabe, 1961, '62 a, b, Morinaga *et al.* 1965, Lange 1971). Such an example, however, has never been known up to date as the plant itself recovered from callus showed the meiotic instabilities.

The chromosome-mosaicism observed in the present material does not seem to be the property of pentaploid rice plant. The material used here is the plant designated A4 by Nishi and Mitsuoka (1969). Whether the remaining 6 plants except A4 out of 7 pentaploid plants raised by them show such chromosome-mosaicism or not is an interesting problem.

Table 4. Measurements of some characters of 5X plant

Character	Plant	Plant height (cm)	Panicle length (cm)	No. of panicles	No. of tillers	Flag leaf		No. of spikelets per panicle	Panicle density*	Awn length (cm)	Stoma length**	Pollen grain		Spikelet		Fertility	
						length (cm)	width (cm)					long diameter meter	short diameter meter	length (mm)	width (mm)	pollen (%)	seed (%)
	2X	98.5	16.5	21	24	28.0	1.5	98	5.9	0	9.1	16.2	15.8	7.4	3.7	94.7	78.6
	5X	60.5	19.5	27	34	32.9	1.4	42	2.2	1.0	12.0	19.8	18.8	8.8	3.7	8.5	0

* number of spikelets ÷ length of panicle

** readings of the ocular micrometer (one score = 2.58 μ).

In addition to the chromosome-mosaicism described above, one more interesting feature has been observed in this pentaploid plant. It is the formation of multi-germ-pores on the pollen grain. In rice, it has been determined that one pollen grain possesses one germ-pore. In the present pentaploid rice plant, however, the number of germ-pores per pollen grain varied between 1 and 4, 2 being the mode. In other crops, for example, *Beta vulgaris* (Nagao and Takahashi 1963), *Solanum schreiteri* (Kirsanova 1968) or *Trifolium* spp. (Najcevska and Speckmann 1968), it has been reported that the numbers of germ-pores on the pollen grain varied together with the multiplication of chromosome numbers, while, in rice it has been thought that there is no variation of the numbers of germ-pores accompanied with polyploidy. Perhaps, not only the chromosome-mosaicism but also the variation of the numbers of germ-pores on the pollen grain, may have some associations with the fact that the present pentaploid plant was redifferentiated from the callus.

Summary

A pentaploid rice plant designated A4 by Nishi and Mitsuoka (1969) showed chromosome-mosaicism in MI of PMCs. When reckoned 2n numbers from the meiotic chromosome configurations, various aneuploid cells ranging from 33 to 99 were observed in one and the same anther-locule.

Morphologically it resembles tetraploid, but anthesis is quite different. As it is similar to cleistogamy, fertilization has never taken place, resulting in the parthenocarpy. When crossed with the pollens of normal diploids, it set only imperfect seeds having no germinating ability. This plant was characterized by the formation of multi-germ-pores on the pollen grain.

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