GENETIC AND CYTOGENETIC STUDIES ON THE TRISOMIC PLANTS OF RICE, *ORIZA SATIVA* L.

I. On the Autotriploid Plant and its Progenies

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Synopsis. To establish all the possible types of primary simple trisomics of rice, spontaneous autotriploids were collected, pollinated with pollen of diploids, and their progenies were studied cytogenetically.

Introduction

MORINAGA, T. and FUKUSHIMA, E. have collected 139 autotriploids from common rice fields in 1933. Seeds of these autotriploids were sown in 1934 and 231 offsprings (T_1) including a number of variants were grown up to maturity. Seeds obtained from those variants were sown in 1935, and 26 lines were raised (T_2). Most of the T_2 lines contained variants in addition to normal plants, and somatic chromosome numbers of the variants were either 25 or 26. The investigations (on the later generations T_3 and T_4) of these variants were carried out by YUNOKI and MASUYAMA (1945). The variants having 26 somatic chromosomes contained two types, one producing two kinds of trisomic plants in the next generation (double trisomics), and the other producing one kind of trisomic plants and normal diploids in addition to the parental type (tetrasomics). The plants having 25 somatic chromosomes used to show some abnormal or unexpected characters, such as incomplete panicle emergence, erect panicle, late panicle emergence, short panicle, long awn, short culm, small spikelet, notched kernel, and so on. Thus, the trisomic plants were classified into six groups according to the combinations of these characters. In these studies, however, the cytological identification of the extra chromosomes has not been carried out.

The aim of the present studies is to see how many kinds of variants, morphologically and karyologically, will arise in the progenies of autotriploids, and whether these are implicated in the 12 types of primary simple trisomics theoretically expected in rice, and furthermore, to identify the extra chromosomes in respective trisomic types using NISHIMURA's reciprocal translocation testers developed in Nôrin 8.

Material and Methods

Normal diploids, when ripened, bend their panicles as usual, but sterile plants remain to erect their panicles. Among these sterile plants, triploid, if any, can be easily distinguished from the others by its high sterility, tall plant height, awnedness, and gigas nature appearing in grains, stems and leaves. The difference between triploid and tetraploid can clearly be seen in the degree of sterility and plant height.

Thus, in the fall of 1963, a number of spontaneous autotriploids have been collected from common rice fields. The collection was made on several varieties, but stress was placed on the variety, Nôrin 8. The putative autotriploids collected were kept in greenhouse during winter. Before transplanted in the field next spring, number of somatic chromosomes of these plants was determined in RTC's, and the real autotriploids were multiplied vegetatively for crossing.

Since almost all of the seeds set on $3x$

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plants spontaneously or artificially by pollen of $2x$ plants were ill-developed, White's medium was used under sterilized conditions to secure the progeny seedlings from them.

Cytological observations were made on both root-tip cells and anthers, which were fixed with 1:3 acetic-alcohol containing a trace of ferric chloride and preserved at 0°C. In making preparations, aceto-carmin squash method was exclusively used.

Results

I. Frequency of spontaneous autotriploid: In the fall of 1963, 72 autotriploids, in total, containing 51 from Nôrin 8, were collected from common rice fields around Hiratsuka City. Though numbers of $3x$ plants found were different in different fields, varying from null to over 10 plants, the average frequency of occurrence of $3x$ plants per unit area ($10a$) was calculated as 2.3 as shown in Table 1. The difference in frequency of occurrence of $3x$ plants seemed to be due to the varieties examined as well as the fields surveyed.

II. Cytological observations on the autotriploid: Chromosome configurations in MI were examined on 100 PMC's in an autotriploid plant of Nôrin 8, in RTC's of which 36 somatic chromosomes were clearly counted (Fig. 1). The result obtained is given in Table 2. In about 50% of PMC's, twelve trivalents (12_{III}) were exclusively observed, while in the rest, bivalents and univalents ranging between 1 and 5 were formed in addition to some trivalents. The mean chromosome configuration for $3x$ plants observed was $11.11_{III}+0.89_{II}+0.89_{I}$. Most of the trivalent chromosomes observed were shaped as V, except a few as I (Figs. 2-a, -b, and -c).

III. Seed fertility of the autotriploid: Seed fertility of the autotriploid, which appeared in the variety, Nôrin 8, was examined by the following three pollination systems:

- (1) open-pollination under natural conditions,
- (2) self-pollination controlled by covering panicle with paraffin bag,
- (3) cross-pollination between $3x$ and $2x$, taking the former on the female side.

The results obtained are shown in Table 3. In every pollination system, a small number of plump seeds as well as a lot of ill-developed

Table 1. Frequency of occurrence of autotriploid rice plants in common fields

Variety name	Area examined (a)	No. of triploid plants	No./ $10a$
Nôrin 8	85	16	1.9
Sachi-Watari	10	5	5.0
Kusa-Bue	25	6	2.4
Total	120	27	2.3

Table 2. Chromosome configurations in MI of PMC's of autotriploid rice plant

Configurations	Frequencies
12_{III}	48
$11_{III}+1_{II}+1_{I}$	27
$10_{III}+2_{II}+2_{I}$	16
$9_{III}+3_{II}+3_{I}$	7
$8_{III}+4_{II}+4_{I}$	1
$7_{III}+5_{II}+5_{I}$	1
Total	100

Fig. 1. Somatic chromosomes in the root-tip cell of an autotriploid rice, Nôrin 8 ($2n=36$) ($\times 1,300$)

seeds were obtained. Seed fertility recorded in Table 3 was calculated from the total sum of both plump and ill-developed seeds. From this table it is to be seen that the seed fertility of $3x$ plants was the lowest in self-pollination (0.45%), rather high in open-pollination (5.2%), and the highest in cross-pollination (13.5%).

IV. Progenies of the autotriploid:

(A) Variation of chromosome number

On the progenies of $3x$ plants, which were obtained by open-, self-, and cross-pollination, respectively, the number of chromosomes was determined by means of the observations of PMC's. The results obtained are compiled in

Fig. 2. Microphotographs of PMC's showing 1st metaphasic configurations in an autotriploid rice, Nôrin 8

a: 12_{III} b: $10_{III}+2_{II}+2_{I}$ c: $7_{III}+5_{II}+5_{I}$

Table 3. Seed fertility of autotriploid rice plants (Nôrin 8)

Pollination system	No. of spikelets pollinated	No. of seeds obtained		Fertility (%)
		Perfect	Imperfect	
Open-pollination	12492	143 (1.10)	501 (4.00)	5.20
Self-pollination	26669	21 (0.08)	100 (0.37)	0.45
Cross-pollination*	6422	210 (3.27)	657 (10.23)	13.50

* Artificial crossing between $3x$ and $2x$ using hot water emasculation method.

Table 4. Chromosome numbers in the progenies of autotriploid rice plants

		Chromosome number												Total	
		24	25	26	27	28	29	30	31	32	33	34	35		36
Theoretical frequency*		1	12	66	220	495	792	924	792	495	220	66	12	1	4096
Present authors	No.	6	35	31	12	2		1							87
	%	6.9	40.2	35.6	13.8	2.3		1.1							100
RAMANUJAM (1937)	No.	6	9	8	9	11	4	3							50
	%	12.0	18.0	16.0	18.0	22.0	8.0	6.0							500
KATAYAMA (1963)	No.	3	6	8	1	2	1								21
	%	14.3	28.5	38.1	4.8	9.5	4.8								100
NAKAMORI(unpublished)	No.	4	24	53	49	28	6	3	1	1				1	170
	%	2.4	14.1	31.2	28.7	16.5	3.8	1.8	0.6	0.6				0.6	100
Total	No.	19	74	100	71	43	11	7	1	1				1	328
	%	5.8	22.6	30.6	21.6	13.1	3.4	2.1	0.3	0.3				0.3	100

Note: The percentage values were calculated by the present authors.

* In the case of $3x(\varphi) \times 2x(\delta)$.

Table 5. Relationship between seed fertility and chromosome number in the progenies of autotriploid rice plants

Seed Fertility (%) $2n$	0	10	20	30	40	50	60	70	80	90	100
25				1	2	5		2	4	1	
26	8	2	2			1		1			
27	4		1								
28	1										
28~30	1										
30	1										

Table 6. Relationship between culm-length and chromosome number in the progenies of autotriploid rice plants

Culm-length (cm) $2n$	20	30	40	50	60	70	80	90	100
25						8	2	3	2
26		1	8	1	2	2			
27		2	1						
28		1							
28~30		1							
30		1							

Note: Culm-length of control (Nôrin 8)=96.5 cm

Table 4 together with the available data of several other workers. In the table, the plants of which chromosome number could not be determined because of stunt and poor development were not included. In our material, the chromosome numbers ranging between 24 and 30 were observed, the mode being 25, followed by 26 and then 27. Trisomics ($2n=25$) occupied 40% of the progenies examined, while disomics ($2n=24$) only 7% of the whole.

(B) Seed fertility and some characteristics

On the aneuploids which appeared in the progenies of autotriploids of Nôrin 8, seed fertility and some characteristics were investigated. Relationship between seed fertility and chromosome number is shown in Table 5. Generally speaking, the more the chromosome number, the lower the seed fertility, although there is a wide range of variation in each case. Trisomics were divided into two groups, one showing low fertility less than 60%, and the other showing high fertility more than 70%. Most of the plants having 26 chromosomes showed low fertility less than 30%,

though there were two plants showing rather high fertility over 50%. Almost all of the aneuploids possessing more than 27 chromosomes showed considerably low fertility less than 10%.

Some morphological characters such as culm-length, flag-leaf length, number of tillers, number of panicles, panicle-length, number of spikelets and so on, were investigated. Among these characters, a close relation was recognized on culm-length in association with chromosome number. The relation between culm-length and chromosome number is shown in Table 6. In this case also as in the seed fertility, the more the chromosome number, the lower the culm-length. Most of the trisomics were taller than 60 cm, some plants being as tall as normal disomics. On the contrary, most of the aneuploids having 26 to 30 chromosomes were lower than 60 cm. Among them, the plants having 26 chromosomes were variable in plant height, showing a wide range between 30 and 80 cm.

Discussion

Since the pioneer investigation of the primary trisomic types of *Datura* by BLAKESLEE and BELLING (1924), the trisomic method of identifying individual chromosomes with their respective linkage groups as a result of the modification of genetic ratios by extra chromosomes, and also of establishing the independence of the linkage groups has been successfully applied to *Antirrhinum* (RUDOLF-LAURITZEN, 1958), *Hordeum* (TSUCHIYA, 1959), *Lycopersicum* (LESLEY, 1928, 1932; RICK and BARTON, 1954), *Secale* (KAMANOI, 1962; KAMANOI and JENKINS, 1962), *Spinacea* (JANICK *et al.*, 1959), and *Zea* (McCLINTOCK and HILL, 1931; EMERSON *et al.*, 1935). The establishment of all the possible types of primary simple trisomic plants corresponding to the haploid number of chromosomes in the species is desirable for the study of trisomics. In rice, however, such trisomics have not been used as yet for genetic studies owing to the difficulty in inducing the primary simple trisomic types.

In barley, all the seven possible linkage groups were believed to have been established by means of the conventional genetic analysis. However, KRAMER *et al.* (1954) and BURNHAM (1956) using reciprocal translocation and TSUCHIYA (1959) using trisomics, have revealed the fact that the two linkage groups, III and VII, are associated with one and the same chromosome. Although, in rice, twelve possible genetic linkage groups have already been established and linkage maps were to an extent completed through the conventional genetic method by NAGAO and TAKAHASHI (1960, 1963), there is not without some afraid of assigning erroneously genes on one and the same chromosome to two different groups just as in barley. It will be highly desirable, therefore, to establish the theoretically expected 12 types of primary simple trisomic plants of rice, and to ascertain the independence of the linkage groups completed by the conventional genetic analysis.

As the possible sources of simple trisomics, the following eight usually used (*cf.* TSUCHIYA, 1963; SCHERTZ, 1963):

1. Normal diploids (spontaneous occurrence)
2. Asynaptic or desynaptic plants (uneven distribution of chromosomes at meiosis)
3. Progeny of irradiated plants (interchange heterozygotes and other chromosomal variants.)
4. Simple trisomics (one trisomic type occasionally produces some unrelated types)
5. Double-, triple-trisomics and other polysomic plants (two, three, or more different types will be produced)
6. Haploids
7. Triploids
8. Tetraploids

Out of these, the best source is triploid. All or almost all of the expected primary simple trisomics have been obtained in the progenies of autotriploids as have been shown in *Antirrhinum*, *Avena*, *Datura*, *Hordeum*, *Lycopersicum*, *Nicotiana*, *Petunia*, *Secale*, *Sorghum*, *Spinacea*, *Zea*, and so on.

In rice, however, the artificial production of autotriploid plants from the cross between $4x$ and $2x$ is very difficult, although the successes have been reported by MORINAGA

and KURIYAMA (1959) in the cross, $2x(\text{♀}) \times 4x(\text{♂})$, and NAGAMATSU, ÔMURA and KOGA (1964) in the reciprocal cross, $4x(\text{♀}) \times 2x(\text{♂})$, respectively. On the contrary, spontaneous autotriploids frequently occur in common rice fields, more than 70 triploid plants being found by NAKAMORI (1932), and more than 150 by MOINAGA and FUKUSHIMA (1935), respectively, in one season. For the development of trisomics, the present authors thought, it is advisable to use these spontaneous autotriploids which appear in common rice fields. Through what mechanism the triploid originates in nature is not known, but chromosomal variation accompanied by polyembryony such as twin or triplet may be one of the causes of triploid occurrence as pointed out already by MORINAGA and FUKUSHIMA (1935).

Observing the chromosome configurations such as $5\text{III}+10\text{II}+1\text{I}$, $10\text{III}+3\text{II}$ and $8\text{III}+6\text{II}$ in autotriploid rice plants, ICHIJIMA (1934) and Hu *et al.* (1963) suggested the association between non-homologous chromosomes. In our material, however, such configurations were not met with. Hu *et al.* (1963) observed even the formation of a hexavalent chromosome in their material, while in our material, no multivalent chromosome except trivalent could be seen.

Autotriploid plants of rice showed very low seed fertility in self-pollination, while in cross-pollination by pollen of the normal diploids they showed somewhat high fertility. This means that a fairly large proportion of the egg-cells have the fertilizing-ability. Accordingly, it may be concluded that the seed fertility of 5.2% obtained in open-pollination resulted from the outcross by pollen of the diploid plants growing adjacent to triploids.

The variation of chromosome number in the progenies of autotriploid plants of rice is shown in Table 4 with the data of three different workers. In every case, more hyponeuploids were observed than hyperaneuploids. Comparing the data obtained by those four workers with the theoretical frequency of the plants to be formed in the case of $3x \times 2x$, the plants of 29 to 31 chromosomes, which should be formed abundantly, were considerably fewer. On the contrary, the plants of 28 to 25 chromosomes, which

should decrease gradually in number, were formed abundantly. In other words, the female gametes possessing 13 to 16 chromosomes play better function than those having over 17 chromosomes. On the other hand, the disomic plant was very few, perhaps not because of the poor function of 12 chromosome female gametes which were formed in $3x$ plants, but because of the scantiness of the absolute number of 12 chromosome female gametes to be formed. In our material, the mode of chromosome number was observed on 25 (trisomics), which occupied 40.2% of the whole examined, while in the other three worker's materials the mode was seen on 26 or 28.

There were considerably wide ranges of variation on culm-length and seed-fertility of 25 and 26 chromosome plants. This means that kinds and numbers of the extra chromosomes will give the different influences on the expression of the morphological characters. Since the possibility is so high to obtain trisomics in the progenies of aneuploids as described before, the utilization of autotriploid and its progenies will be of great value for the establishment of all the possible types of trisomics.

Summary

1. To establish all the possible types of trisomic plants of rice, spontaneous autotriploids collected from common rice fields were used as the source.

2. The frequency of occurrence of spontaneous autotriploids was calculated 2.3 per 10a.

3. The mean configuration of $11.11_{III} + 0.98_{II} + 0.89_I$ was observed in MI of PMC's of autotriploid ($2n=36$).

4. The seed fertility of autotriploid was almost 0% in self-pollination, while in cross-pollination of $3x \times 2x$ rather high fertility of 13.5% was obtained. The seed fertility of about 5% obtained by open-pollination was assumed to be resulted from the outcross with pollen of the normal diploids growing adjacent to it.

5. Various chromosomal variants as well as disomics ranging between 24 and 30 chromosomes were formed in the progenies of auto-

triploid plants. Thirty-five plants (about 40%) out of 87 examined were trisomics.

6. In the progenies of autotriploid plants examined, the higher the chromosome numbers, the lower the seed fertility and the shorter the culm-length.

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イネのトリゾミック植物に関する遺伝学的 ならびに細胞遺伝学的研究

第 I 報 同質 3 倍体とその子孫について

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栽培イネ (*Oryza sativa* L.) に理論的に期待される 12 型のトリゾミック植物 (Primary simple trisomics) をそろえる目的で、主として農林 8 号について同質 3 倍体を普通水田よりさがし求めた。その出現頻度は圃場により異なつたが、おおよそ 10 アール当り 2 株の割合であつた。採集された同質 3 倍体は根端で $2n=36$ が確認され、PMC の MI では平均接合型 $11.11_{III}+0.89_{II}+$

0.89_I が観察された。同質 3 倍体に正常の 2 倍体の花粉を交配して得た雑種種子、および自家受粉あるいは自然受粉によつて着生した種子より次代を育て、それらの染色体数を算定したところ、 $2n$ で 24 から 30 にわたる染色体数の変異がみられ、モードは 25 にあり、観察個体数の 40% がトリゾミック植物であつた。