Cytogenetic Studies on the Artificial Polyploids in the Genus *Oryza*

V. Sterile Amphiploids, *sativa-officinalis* (AACC)

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Synopsis. $4x$ -amphiploids (AACC, $2n=48$) obtained between *O. sativa* (AA, $2n=24$) and *O. officinalis* (CC, $2n=24$) were completely male-sterile because of their lacking anthers or being rudimentary in the development of anthers. The cause of the male-sterility seems to be due to the interaction between A and C genomes.

In the genus *Oryza*, especially among the Section *Sativa*, there are some wild species crossable with cultivated rice, *O. sativa* (AA). Some of them, for example, *O. officinalis* (C C) is overlapped in distribution with A genomic species including cultivated rice. Nevertheless, no amphiploid possessing A genome has ever been found in nature not only at hexaploid level, but also even at tetraploid level. It may be possible to synthesize new types of polyploid rice by combining A genome of the cultivated rice with other genome or genomes.

As already reported in the 3rd paper of this series, two kinds of $6x$ -amphiploids possessing A genome, *sativa-minuta* (AABB-CC) and *sativa-latifolia* (AACCDD), resulted in the complete sterility (WATANABE and Ono 1969). This time, $4x$ -amphiploids having the genomic constitution of AACC were successfully synthesized between *O. sativa* and *O. officinalis*. These were also completely sterile.

Material and Methods

The present $4x$ -amphiploids were synthesized by means of two different procedures, one from the cross between induced tetraploids of *O. sativa* and *O. officinalis*, the other from the chromosome doubling of diploid F_1 hybrid. In the case of the former, crossing was made by using $4x$ -*sativa* in the female side as follows :

$4x$ -*sativa* "Gozen-Mochi" $\times$
 $4x$ *O. officinalis* 8021

$4x$ -*sativa* "Norin 25" $\times$
 $4x$ *O. officinalis* 8021
" " " " 62174
 $4x$ -*sativa* "Sensho" " " "

Two strains of $4x$ *officinalis* used as the pollen parent were of hybrid origin. In the case of the latter, the cross combination was as follows :

$2x$ *O. sativa* "Norin 36" $\times$
 $2x$ *O. officinalis*

Diploid *officinalis* used here was introduced from India. To develop seedlings from the crossed seeds, embryo-culture method using White's medium was employed. The method applied for the chromosome doubling of diploid F_1 hybrid was described in detail in the 4th paper of this series. Cytological observations were made only on RTC's through much the same procedure as in the 1st paper of this series.

Results

I. Crossing and colchicine treatment :

A total of six putative amphiploids were obtained from four cross combinations using induced tetraploids of *O. sativa* and *O. officinalis* (Table 1). By the colchicine treatment of young shoots derived from the dormant buds on the stems of diploid F_1 hybrid, only one putative amphiploid was obtained from 43 shoots treated (Table 2).

II. Cytological observations :

Out of 6 putative amphiploids obtained from the cross between induced tetraploids of *O. sativa* and *O. officinalis*, 1 plant derived from Sensho $\times$ *O. officinalis* 62174 was discarded because of its showing aneuploid nature and

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Table 1. Results of crossing between tetraploids induced

Cross combinations		No. of spikelets pollinated	No. of seeds obtained	No. of amphiploids obtained
♀	♂			
4 <i>x-sativa</i> "Gozen-Mochi"	4 <i>x-officinalis</i> 8021	54	3	1
4 <i>x-sativa</i> "Norin 25"	4 <i>x-officinalis</i> 8021	79	6	2
4 <i>x-sativa</i> "Norin 25"	4 <i>x-officinalis</i> 62174	65	5	2
4 <i>x-sativa</i> "Sensho"	4 <i>x-officinalis</i> 62174	42	2	1

Table 2. Result of colchicine treatment to F_1 shoots derived from the dormant buds on the stems

No. of shoots prepared	No. of shoots treated	No. of shoots survived	No. of amphiploids obtained
52	43	7	1

Fig. 1. Somatic chromosomes in root-tip cell of the amphiploid, *sativa-officinalis* (AACC, $2n=48$), (sketched).

stunted grass type. In other 5 putative amphiploids, 48 chromosomes were clearly observed in RTC's (Fig. 1). Since parental materials themselves used for crossing were tetraploids, it could not be determined simply by counting the somatic number of chromosomes whether these plants were true amphiploids or not. As it will be described later, however, their hybridities were recognized based on their morphological characters.

In RTC's of the putative amphiploid derived from the colchicine treatment of F_1 stems, 48 chromosomes twice as many as the original F_1 hybrid ($2n=24$) were clearly counted. Consequently, this plant was, no doubt, true amphiploid between *O. sativa* and *O. officinalis*, having the genomic constitution of AACC.

III. Morphology :

Under the controlled short-day condition in the field at Hiratsuka, most of the amphiploids were unable to shoot the panicles out

Fig. 2. Comparison of anthers in F_1 and amphiploid between *O. sativa* and *O. officinalis*.

Upper : F_1 , Low : Amphiploid

of the sheath. The panicles occasionally shot out carried frequently white withered spikelets completely deformed. The amphiploids grown up all through in the green house shot out the panicles normally as the natural day-length became enough short in the fall. However, the spikelets were completely male-sterile because of lacking anthers or being rudimentary in the development of anthers, while the ovaries appeared to be normal in shape with sizable feather-like stigmata, though somewhat smaller (Fig. 2). By pollinating them with pollens of $2x$ or $4x$ varieties of *O. sativa*, a small number of seeds were obtained (Table 3). This clearly shows that the ovaries play their function well as

Table 3. Cross-ability of amphiploid

Cross combinations		No. of spikelets pollinated	No. of seeds obtained	%
♀	♂			
Amphiploid, <i>sativa-officinalis</i>	2x- <i>sativa</i> "Shinriki"	280	2	0.7
"	4x- <i>sativa</i> "Takara"	120	3	1.5
"	4x- <i>sativa</i> "Norin 29"	79	2	2.5

Fig. 3. Comparison of grass type in F_1 and amphiploid between *O. sativa* and *O. officinalis*.
Left : F_1 , Right : Amphiploid

the female sex organ. In these respects, the amphiploid *sativa-officinalis* was very similar to the amphiploids, *sativa-minuta* and *sativa-latifolia* previously reported. The grass type of the amphiploid grown up all through in the green house is shown in Fig. 3 and the spikelets in Fig. 4.

Discussion

In the genus *Oryza*, several kinds of amphiploids have been synthesized up to date by the present authors as follows :

minuta-australiensis (BBCCEE)

sativa-minuta (AABBCC)

sativa-latifolia (AACCCDD)

sativa-officinalis (AACC)

Among these, the last three combinations which involve A genome were found to be completely sterile in spite of the first one being fertile. The sterile amphiploid, *sativa-minuta* (AABBCC) differs from the fertile amphiploid, *minuta-australiensis* (BBCCEE) only in the point that it involves A genome instead of E. Accordingly, it is certain that

Fig. 4. Comparison of spikelets in F_1 and amphiploid between *O. sativa* and *O. officinalis*.
Left : F_1 , Right : Amphiploid

A genome itself plays an important role in the expression of sterility. However, the cultivated rice, *O. sativa* L. possessing only A genome is fertile not only at diploid level but also at tetraploid level. Therefore, the sterility in question may be due to the interaction between A and other genome or genomes. Judging from the fact that the present amphiploid, *sativa-officinalis* is also sterile, the sterility of the amphiploids synthesized in the genus *Oryza* seems to be due to the interaction between A and C genomes which are involved in common to these three combinations.

It is said that 2x-*punctata* possesses B genome (KATAYAMA 1967), and the amphiploid (AABB) synthesized between *O. sativa* and *O. punctata* is fertile (OGAWA and KATAYAMA 1971). This presents an additional evidence to our supposition.

Summary

4x-amphiploids (AACC, $2n=48$) were successfully obtained by treating dormant buds with colchicine on the stems of F_1 hybrids between *O. sativa* and *O. officinalis* or by making a cross between auto-tetraploids induced of respective species. These amphiploids showed complete male-sterility because of their lacking anthers or being rudimentary in anther development. The ovaries were proved to play their function well as the female sex organ, since a small number of seeds were obtained by pollinating them with pollens of *O. sativa*. The cause of sterility of these amphiploids including *sativa-minuta*, *sativa-latifolia* as well as *sativa-officinalis* seems to be due to the interaction between A and C genomes existing in common to these three combinations.

Literature Cited

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イネ属の人為倍数体に関する細胞遺伝学的研究

V. 不稔性複2倍体 *sativa-officinalis* (AACC) について

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Oryza sativa L. (AA, $2n=24$) と *O. officinalis* Wall. ex Watt (CC, $2n=24$) の F_1 の茎のコルヒチン処理, あるいはコルヒチンであらかじめ倍加しておいた両種の同質4倍体同志の交配から4倍性の複2倍体 *sativa-officinalis* (AACC, $2n=48$) を得た。小穂は葯の発達不良または欠除を示し花粉を作らないが, 子房は小形ながら *sativa* の花粉で若干の雑種種子を着けた。

本複2倍体が, さきに報告した6倍性の複2倍体, *sativa-minuta* (AABBCC, $2n=72$) および *sativa-latifolia* (AACCDD, $2n=72$) と同様な雄性不稔を示したことから, その不稔の原因はこれら3種の複2倍体に共通して存在するAゲノムとCゲノム間の相互作用によるものであろうと推定した。