Studies on Restoration and Substitution of Nucleus (Genome) in *Aegilotriticum*, IV. Genome exchange between *durum* and *ovata* cytoplasm and its theoretical consideration for male-sterility

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Received October 24, 1956

Introduction

It has been reported in the previous paper of this series (Fukasawa, 1953) that *durum* plants with *ovata* cytoplasm showed complete male sterility. These plants had, however, been obtained by successive backcrosses of *Aegilotriticum* ♀ × *durum* ♂. That is, the female parent used was not pure *ovata*, but *Aegilotriticum* No. 2 which had been synthesized from the cross between *ovata* ♀ and *durum* ♂. Furthermore, only two individuals had been obtained from a second crossing experiment, owing to high sterility in both male and female organs of the hybrid, *Aegilotriticum* ♀ × *durum* ♂. One of them had died before maturing. Consequently, the male-sterile "*durum*" plant had been derived from one individual. It was, therefore, not obvious whether the male-sterile factor came from the *ovata* cytoplasm or occurred spontaneously in a certain individual during the course of successive backcrosses. For that reason, it seemed worth while to repeat the experiment, using *Aegilotriticum* No. 2 as the female parent. In addition, the following two kinds of experiments have been carried out simultaneously, (1) placing of *durum* genomes in *ovata* cytoplasm, using pure *Ae. ovata* as a female parent, (2) restoration of *durum* genomes to amphidiploid (*durum* ♀ + *ovata* ♂) which have possessed *durum* cytoplasm.

Furthermore, the present report deals with reciprocal substitution, i.e. transfer of *ovata* genomes into *durum* cytoplasm. Also, a restoration experiment of *ovata* genomes to male-sterile *durum* plants is reported, which was carried out in search of heritable changes which might have occurred in *ovata* cytoplasm under the influence of *durum* genomes.

Experimental results

EXPERIMENT-I. Placing of *durum* genomes in *ovata* cytoplasm, using *Aegilotriticum* No. 2 as the female parent.

This experiment is a repetition of the experiment described in the first

report of this series. No seed was obtained from the hybrid, *Aegilotriticum* ♀ × *durum* ♂. Further offsprings, therefore, were obtained from the hybrid (*Aegilotriticum* ♀ × *ovata* ♂) ♀ × *durum* ♂. Consequently, as shown in Table 1, *Aegilotriticum* No. 2 plants used here have possessed such chromosome configurations as $1_{IV}+1_{III}+19_{II}+7_I$ and $21_{II}+6_I$ or $18_{II}+12_I$ at meiosis. After four successive backcrosses to *durum* ♂, many 14_{II} -plants consisting of homologous *durum* genomes were obtained in 1955. The chromosomes in root-tips of these individuals were not different from those of normal *durum* plants.

Table 1. Survey of chromosome configurations and pollen fertility following the introduction of *durum* genomes and restoration of *ovata* genomes into *ovata* cytoplasm of *Aegilotriticum* No. 2

1950		1951		1952	
<i>Aegilotriticum</i>					
1) $1_{IV}+1_{III}+19_{II}+7_I$	pollen fertility (%) 82.6	× <i>ovata</i>	$11_{II}+15_I$	pollen fertility (%) 16.4	× <i>durum</i> → $2_{II}+24_I$ 72.3 *
				× <i>ovata</i>	→ $15_{II}+4_I$ 54.4 **
2) $21_{II}+6_I$, $18_{II}+12_I$	pollen fertility (%) 41.9	× <i>ovata</i>	$10_{II}+16_I$	pollen fertility (%) 13.2	× <i>durum</i> → $2_{II}+24_I$ 59.4 ***
1953		1954		1955	
* × <i>durum</i>	$2_{III}+12_{II}+12_I$ 0.7	× <i>durum</i>	$14_{II}+1_I$ 0	× <i>durum</i>	14_{II} 0
	$1_{III}+12_{II}+2_I$, $14_{II}+11_I$	× <i>durum</i>	$14_{II}+3_I$		14_{II} 0
			$14_{II}+1_I$ 3.3	× <i>durum</i>	14_{II} 0
** × <i>ovata</i>	14_{II} 92.9	self	14_{II} 97.0		14_{II} 0
	$14_{II}, 13_{II}+2$		14_{II} 97.8		$14_{II}, 13_{II}+2_I$ 0
			$14_{II}, 13_{II}+2_I$		$14_{II}, 13_{II}+2_I$ 0
*** × <i>durum</i>	$2_{III}+12_{II}+14_I$ 11.7	× <i>durum</i>	$1_{III}+13_{II}+8_I$	self	$14_{II}+7_I$ 46.4
	$1_{III}+13_{II}+13_I$ 7.4	× <i>durum</i>	$14_{II}+6_I$ 37.1	× <i>durum</i>	$14_{II}+6_I$ 35.6
	$14_{II}+14_I$ 10.7	× <i>durum</i>	$14_{II}+7_I, 13_{II}+11_{II}$	× <i>durum</i>	$14_{II}+7_I$ 66.8
					14_{II} 0
					$14_{II}+1_I$

All these individuals were fully pollen sterile like those obtained in previous experiments. A complete pairing of 14 bivalents was observed at meiosis of all PMCs inspected. The immediate parent of those plants had $14_{II}+1_I$. Some individuals, however, possessed more cells with $13_{II}+2_I$ than with 14_{II} , although they were derived from the same parent. The occurrence of univalents may be due to about one month delayed sowing.

EXPERIMENT-II. Placing (restoration) of *ovata* genomes into *Aegilotriticum* No. 2.

This experiment is, also, a repetition of the experiment in previous report. Restoration of *ovata* genomes was easily achieved through three successive backcrosses as shown in Table 1. All the plants obtained are typical *ovata* plants, and showed normal pollen fertility, like those of previous experiments.

EXPERIMENT-III. Placing of *durum* genomes in *ovata* cytoplasm, using pure *ovata* plants as a female parent.

In order to find out whether the cytoplasmic male-sterile factor arises as a spontaneous change under the condition of amphidiploid or not, an attempt was made to introduce the nucleus of *durum* into the cytoplasm of pure *ovata* plant, through backcrossing the *ovata* ♀ × *durum* ♂ hybrid *durum* pollen for many generations. F₁ hybrids of *ovata* ♀ × *durum* ♂ have shown 28_I at meiosis and they must have produced many unreduced gametes, because 14_{II}+14_I-plants were obtained in the next backcross generation. Actually, F₁ hybrid possessed giant pollen grains which amounted to 6.25%. As shown in Table 2, complete substitution of *durum* genomes was achieved through four successive backcrosses. These 14_{II}-plants showed a complete pollen sterility, like the those of previous two experiments in which *Aegilotriticum* No. 2 had been used as the female parent. And also they have, in appearance,

Table 2. Survey of chromosome configurations and pollen fertility in the course of introducing *durum* genomes into *ovata* cytoplasm, using pure *ovata* plant as a femal parent.

1951		1952		1953	
<i>Aegilops ovata</i>	pollen fertility (%)		pollen fertility (%)		pollen fertility (%)
14 _{II}	59.7	× <i>durum</i> → 28 _I	6.3	× <i>durum</i> →	14 _{II} +14 _I ..0.3 → *
					14 _{II} +14 _I → **
1954		1955			
* × <i>durum</i> →	1 _{III} +13 _{II} +5 _I .. 7.1	× <i>durum</i> →	14 _{II} +3 _I0		
			14 _{II} +3 _I		
** × <i>durum</i> →	17 _{II} +18 _I 1.5	× <i>durum</i> →	14 _{II} +6 _I3.0		
	14 _{II} +5 _I 0	× <i>durum</i> →	14 _{II}0		
	14 _{II} +5 _I 0.2	× <i>durum</i> →	14 _{II}0		
			14 _{II}0		
			14 _{II}0		
			14 _{II}0		

possessed quite similar morphological and physiological characters as those of male-sterile *durum* plants obtained previously. Chromosome pairing at meiosis of PMCs was complete, showing 14_{II} in every cells inspected. No univalent was observed, even though the individuals were sown one month later than usual.

EXPERIMENT-IV. Placing (restoration) of *durum* genomes into the hybrid, *durum* ♀ × *ovata* ♂, and to its amphidiploid (*durum* ♀ + *ovata* ♂).

From the above experiments, I and III, it was ascertained that the cytoplasmic male-sterile factor does not occur at the amphidiploid condition. However, the following possibility remains. This is, the factor does not come from *ovata* cytoplasm, but it may occur at the hybrid condition of *ovata* ♀ × *durum* ♂ or in certain individuals with special chromosome constituents during the course of further backcrossing. In order to make clear this question, successive backcrosses of (*durum* ♀ × *ovata* ♂) ♀ × *durum*ⁿ ♂ and of amphidiploid (*durum* ♀ + *ovata* ♂) ♀ × *durum*ⁿ ♂ were carry out. The

amphidiploid of *durum* ♀ + *ovata* ♂ was easily obtained from the selfing of F₁ hybrid, *durum* ♀ × *ovata* ♂. As shown in Table 3, restoration of *durum* genomes was achieved after three successive backcrosses. That is, 14_{II}-plants

Table 3. Survey of chromosome configurations and pollen fertility in the course of restoring *durum* genomes to the hybrid, *durum*♀ × *ovata*♂ and to the amphidiploid between them

1950		1951		1952	
		pollen fertility (%)		pollen fertility (%)	
<i>Triticum durum</i> 14 _{II}		96.5	× <i>ovata</i>	28 _I 5.3	Art. self
				28 _I 2.9	× <i>durum</i>
					25 _{II} + 3 _I .. 60.1 *
					15 _{II} + 12 _I .. 3.1 **
					14 _{II} + 14 _I ***
1953		1954		1955	
* × <i>durum</i>		14 _{II} + 14 _I .. 15.9	× <i>durum</i>	14 _{II} + 5 _I .. 6.3	× <i>durum</i>
* × <i>durum</i>		4 _{II} + 4 _I .. 9.6	× <i>durum</i>	14 _{II} + 1 _I .. 29.9	× <i>durum</i>
		4 _{II} + 4 _I .. 13.2	self	14 _{II} (1) + .. 46.0	self
			× <i>durum</i>	14 _{II} 93.6	× <i>durum</i>
				14 _{II} + 1 _I .. 34.8	self
					14 _{II} 96.3
					14 _{II} 96.1
					14 _{II} 94.0
*** × <i>durum</i>		14 _{II} + 3 _I .. 82.4	self	14 _{II} 92.9	
			× <i>durum</i>	14 _{II} 91.3	
				14 _{II} 93.0	self
				14 _{II} 88.0	
				14 _{II} 6.0	
					14 _{II} 97.3

* 14_{II}(1) represents 13 domylete and one loose pairing.

Table 4. Survey of chromosome configurations and pollen fertility in the course of restoring *ovata* genomes to male-sterile *durum* plant

1951		1952		1953	
		pollen fertility (%)		pollen fertility (%)	
male-sterile <i>durum</i> with <i>ovata</i> cytoplasm 14 _{II}		0	× <i>ovata</i>	28 _I 3.7	× <i>ovata</i>
					13 _{II} + 16 _I , 14 _{II} + 14 _I .. 18.2 *
1954		1955			
* × <i>ovata</i>		14 _{II} 96.1	self	14 _{II} 97.6	
			× <i>ovata</i>	14 _{II} 96.3	
				14 _{II} 95.1	

ovata

were obtained in 1955 in the case of amphidiploid, and in 1954 in the lines of F₁ hybrids. Their pollen fertility was very high like that of normal *durum* plants. But, it seems that their plant height was somewhat lower than normal ones.

EXPERIMENT-V. Restoration of *ovata* genomes into the cytoplasm of

male-sterile *durum* plants with *ovata* cytoplasm.

In order to study what heritable changes in the *ovata* cytoplasm might have been induced by the *durum* genomes, restoration of *ovata* genomes was carried out through successive backcrosses of male-sterile *durum* ♀ × *ovata*ⁿ ♂. As shown in Table 4, F₁ hybrid of male-sterile *durum* ♀ × *ovata* ♂ showed 28_I at meiosis, as in the F₁ hybrid between normal *durum* and *ovata* plants.

In the next backcross generation, 14_{II}+14_I and 13_{II}+16_I-configurations were observed. By three successive backcrosses many 14_{II}-plants were easily obtained. They appeared to be typical *ovata* plants, and possessed pollen fertility as high as 96.1%. Offspring by selfing of these plants and the crosses with *ovata* pollen, also, showed no difference in fertility from normal *ovata* plants.

EXPERIMENT-VI. Placing of *ovata* genomes in the *durum* cytoplasm.

Successive backcrosses of (*durum* ♀ × *ovata* ♂) ♀ × *ovata*ⁿ ♂ were carried out since 1950. As seen in Table 5, substitution of *ovata* genomes was achieved through five successive backcrossing. The substitution *ovata* plants showed 14 bivalents at meiosis. Their pollen fertility, however, varied from

Table 5. Survey of chromosome configurations and pollen fertility in the course of introducing of *ovata* genomes into *durum* cytoplasm

1950	1951	1952
<p><i>Triticum durum</i> 14_{II} 96.5</p>	<p>pollen fertility (%)</p>	<p>pollen fertility (%)</p>
<p>× <i>ovata</i> →</p>	<p>28_I 5.3 28_I 2.9</p>	<p>× <i>ovata</i> →</p>
		<p>14_{II}+14_I 30.5 * 14_{II}+14_I</p>
1953	1954	1955
<p>* × <i>ovata</i> → 14_{II}+6_I .. 42.6</p>	<p>pollen fertility (%)</p>	<p>pollen fertility (%)</p>
	<p>14_{II}+3_I .. 67.3 14_{II}+2_I .. 78.7 14_{II}+2_I .. 85.2 14_{II}+1_I .. 93.0 14_{II}+2_I .. 56.6</p>	<p>× <i>ovata</i> →</p>
		<p>14_{II}(1) 17.2 14_{II} 49.2 14_{II} 90.3 14_{II}+1_I 97.7 14_{II} 92.3 14_{II}+1_I 91.7 14_{II} 94.6 14_{II}+1_I 94.3 14_{II} 0.5 14_{II} 95.6 14_{II}+2_I 41.8</p>

0.45 to 95.58%. Some of individuals with low pollen-fertility have shown 13 complete and one loose pairing bivalents. Therefore, it may be considered that the chromosomal substitution in such plants is not complete as yet. Furthermore, some of individuals with complete 14_{II}-chromosomes possessed slender leaf and culm and lower plant height than normal *ovata* plants. Therefore, the genic substitution in such 14_{II}-plants may be not fully accomplished. This phenomenon may come from a consequence of rare crossing over between *ovata* and *durum* chromosomes. Most of the other individuals

Fig. 1. Diagrams showing the interrelationships of genome exchange between *Triticum*

with complete 14 bivalents showed a normal growth habit and nearly normal pollen-fertility. The male-sterile condition did not appear in these individuals. None of the plants showed as much chlorophyll variegation as in male-sterile *durum* plants. Thus, it may be said safely that *ovata* genomes may manifest their genic expression in the *durum* cytoplasm without giving rise to male-sterility. It has, however, been observed that the heading of "substituted" *ovata* plants was delayed for 6-7 days as compared to normal *ovata* plants. This problem will be described in a separate paper.

Discussion

It has been reported by several authors that cytoplasmic male-sterility was occasionally observed in species hybrids or in their offspring, particularly in hybrids between distantly related species. According to Michaelis (1954) pollen sterile plants always segregated in successive backcrosses of *Epilobium luteum* ♀ × *hirsutum* ♂ hybrid by *hirsutum* ♂. The genome of *E. hirsutum* together with *luteum* plasmon produces pollen sterility. However, pollen fertile plants or sectors sometimes occur in lines that have been pollen-sterile for many generations. He reported (1955) a theory for the alteration of the cytoplasmic factor which is probably produced by recombination, by transfer of male cytoplasm, by mutation of the cytoplasmic units, by nuclear-produced plasmagenes and so forth. In my material, however, cytoplasmic alterations did not occur during six generations, at least as to the male-sterile condition.

In maize (Rhoades, 1933, 1943 and Schwartze, 1951) cytoplasmic male-sterility appears to be relatively common, and also in beet (Owen, 1946) and in onion (Jones and Clarke, 1943). Rhoades (1950) has shown that the recessive gene *iojap* (*ij*) in maize induces not only an effect on the plastid but also produces mutation of a second cytoplasmic factor concerned with male-sterility. Therefore, it could not be concluded easily that the occurrence of cytoplasmic male-sterility in species hybrids is attributed to only the disharmony between nucleus and constant foreign cytoplasm. It might be considered that cytoplasmic male-sterile factor may be induced, *de novo*, in a certain individual in the course of successive backcrosses, like a case of maize. Kihara (1948, 1951) postulated methods of nucleus substitution in order to study the influence of alloplasm on genome manifestations. He has reported that genomes of *Triticum vulgare* were introduced into the cytoplasm of *Aegilops caudata*. The plant is sterile, if the substitution is complete (Kihara, 1951). Clayton (1950) reported male-sterile tobacco which was produced in the backcrosses of *Nicotiana debneyi* ♀ and *N. tabacum* ♂. In this case male-sterility appeared in BC¹, increased in BC² and was completed in BC³. The author stated that the gradual development was based on progressive incompatibility between cytoplasm and nuclear constituents. How-

durum and *Aegilops ovata*, concerning male-sterility. Inner circles represent nucleus (genome) and outer zone cytoplasm: *Ovata* cytoplasm is dotted

ever, reciprocal substitution has not been described, as well as in the case of *T. vulgare* $\times$ *Ae. caudata*. In the present study, however, reciprocal substitution and restoration between *durum* and *ovata* have been completed. Consequently, the results might contribute to making clear the problem of male-sterility caused by alloplasm. The conclusive results obtained in the present experiment are schematically summarized in Fig. 1.

From the results obtained by re-investigating the placing of *durum* genomes into *Aegilotriticum* No. 2, it was ascertained that even if any individuals of *Aegilotriticum* No. 2 are used as a female parent the resulting plants become male-sterile (Fig. 1 A). It is not obvious, however, whether the male-sterile factor came from *ovata* cytoplasm or was produced spontaneously under the condition of amphidiploidy (*Aegilotriticum* No. 2) for many years. The answer may be found in the results of the experiment-III, using pure *ovata* species as a female parent (Fig. 2 C). Namely, all resulting offspring became male-sterile, as in the case of using *Aegilotriticum* No. 2. Therefore, it is evident that the male-sterile factor does not occur in the amphidiploid condition. Yet, the following possibility exists. That is, the male-sterile factor may have occurred in a certain individual with a special chromosome constituent in the course of further backcrossing. The question may be resolved by the experiment-IV (Fig. 2 D). Namely, when the hybrids of *durum* $\varphi \times$ *ovata* σ^7 are successively backcrossed with *durum* pollen, all resulting offspring become to normal *durum* plants. Accordingly, it may be decided that male-sterile factor could not originate during the course of backcrossing.

Moreover, if a special male-sterile factor would occur, *de novo*, during the course of backcrossing, the cytoplasm of male-sterile *durum* plants might express the male-sterile condition also when *ovata* genomes are restored in the cytoplasm again. Experiment-V, male-sterile *durum* $\varphi \times$ *ovata*ⁿ σ^7 , has given a negative result for such a possibility (Fig. 2 E). Therefore, as already pointed out in the second report in this series (Fukasawa, 1955), it may be concluded reasonably that the male-sterile factor has really come from *ovata* cytoplasm. It is noteworthy that no alteration of the *ovata* cytoplasm has, at least concerning the male-sterility, been recognized for a long time. This phenomenon differs from the case of bud variation in *Phaseolus* (Sirks, 1938) which is caused by a transitory plasmic change, and in a few generations it was entirely restored. From the present date, at any rate, it was found that Kihara's hypothesis (Kihara, 1948) about the substitution of nucleus is applicable.

Reciprocal substitution, i.e., transfer of *ovata* genomes into *durum* cytoplasm (experiment-IV), has shown that *durum* cytoplasm does not effect the manifestation of *ovata* genomes concerning pollen production (Fig. 2 F). As found in experiment-V, even if *ovata* genomes were replaced in the cytoplasm of male-sterile *durum* plants no change appeared. Therefore, it may be considered that *ovata* genomes contain some nuclear genes which make

a male-sterile factor inactive. The male-sterile factor of *ovata* cytoplasm may be thought of as virus-like particle as in a case of CO₂-sensitivity in *Drosophila* (L'Heritier, 1951) or kappa particles in *Paramecium* (Sonneborn, 1946, 1951). However, no infection is seen on the neighboring *durum* plants. A few inoculation experiments showed a negative transmission of the factor. Therefore, it seems to be more like a normal constituent of *ovata* cytoplasm than a virus-like one. Of course, however, it is hopeless to draw the line between plasmagene and viruses, as shown by Darlington (1949) and others.

Summary

1) Transfer of *durum* genomes into *ovata* cytoplasm of *Aegilotricum* No. 2 was made in order to ascertain if male-sterility might always occur even if any lines of *Aegilotrium* No. 2 are used. All resulting offspring became completely male-sterile as in previous experiments.

2) Using pure *ovata* plants as a female parent, *durum* genomes were substituted into *ovata* cytoplasm. Resulting 14_{II}-plants become male-sterile without exception.

3) Using the new synthesized amphidiploid (*durum* ♀ + *ovata* ♂), restoration of *durum* genomes was made. All 14_{II}-offspring showed normal pollen-fertility.

4) *Ovata* genomes were replaced again in the cytoplasm of male-sterile *durum* plants. All resulting 14_{II}-individuals were normal with high pollen-fertility.

5) Placing the *ovata* genomes into *durum* cytoplasm was carried out. Most of 14_{II}-plants showed normal pollen-fertility.

6) It has been discussed that the male-sterile factor came from the *ovata* cytoplasm and that *ovata* plants contain a certain nuclear gene which makes the male-sterile factor inactive.

Acknowledgements

The present author wishes to express his cordial thanks to Dr. H. Kihara and Dr. J. Nishiyama who gave him valuable advices and encouragement. The author's thanks are also due to Dr. H. Gabelman for kindness in revision of the manuscript.

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