SPECIFIC INTERACTIONS BETWEEN THE D GENOME AND THE THREE ALIEN CYTOPLASMS IN WHEAT

II. SEED INVIABILITY INDUCED BY THE ALIEN CYTOPLASMS¹⁾

SEIICHI TSUJI* AND MINORU MURATA**

Laboratory of Genetics, Faculty of Agriculture
Kyoto University, Kyoto 606

Received August 11, 1976

Common wheat, *Triticum aestivum* L. ($2n=42$), is the allohexaploid which has a genome constitution AABBDD. Emmer wheat contributed the A and B genomes, and the D genome was derived from *Aegilops squarrosa* L. (McFadden and Sears 1944, Kihara 1944). It has been considered that, when an amphidiploid was produced from the cross between Emmer wheat and *Ae. squarrosa* in nature about seven thousand years ago (Helbaek 1965), its cytoplasm might have been inherited from Emmer wheat (Kihara 1966, Suemoto 1968). In 1973, Kihara reported that common wheat whose cytoplasm was substituted into that of *Ae. squarrosa* showed complete fertility and vigor, while Emmer wheat with the *squarrosa* cytoplasm became male sterile and vigorless. These results implied that the D genome of common wheat possesses the genes for restoring male fertility and plant vigor to the *squarrosa* cytoplasm.

Ae. cylindrica Host ($2n=28$) and *Ae. crassa* Boiss. ($2n=42$) have genome constitution, CCDD and DDD²D²M^{cr}M^{cr}, respectively (Kihara and Tanaka 1970), and both species have the D genome in common. Common wheats with these two cytoplasms were completely fertile and vigorous (Maan 1973, Panayotov and Gotsov 1975). By comparing a number of quantitative characters in several cytoplasm substitution lines of common wheat, we found that these cytoplasms were quite similar to that of *Ae. squarrosa* (Murata and Tsunewaki 1975, Tsuji and Tsunewaki 1976). It was concluded, therefore, that the cytoplasms of *Ae. cylindrica* and *Ae. crassa* were derived from *Ae. squarrosa*.

Original purposes of the present work were to localize the supposed genetic factors for the restoration of male fertility and plant vigor to the *squarrosa* and the two *squarrosa*-like cytoplasms on the D genome chromosomes of common wheat, as implied from Kihara's results (1973), and also to confirm the similarity of the *cylindrica* and *crassa* cytoplasms to the *squarrosa* cytoplasm. For identifying particular chromosomal regions in which the genetic factors interacting with the alien cytoplasms are located, the effects of the chromosomal deficiency in the D genome were investigated (rf. Sears

1) Contribution from the Laboratory of Genetics, Faculty of Agriculture, Kyoto University, Japan, No. 405.

* Present address: Department of Agronomy, North Dakota State University, Fargo, North Dakota 58102, U.S.A.

** Present address: Department of Agronomy, Colorado State University, Fort Collins, Colorado 80521, U.S.A.

resulting in the monotrismic plants ($2n=42$, $19''+1'+1'''$) with the three alien cytoplasms. In the spring of 1975, these monotrismic series were backcrossed with disomic and nullitetrasonic CS ($\otimes$). And, in 1975/76, among the offspring of the above crosses, we selected cytologically the monosomic and nullitetrasonic CS with the three alien cytoplasms, and investigated their seed fertilities.

Cytology: Determination of somatic chromosome constitutions was made with root tips pretreated at 0°C for 24 hours, fixed in acetic alcohol (1:3) and squashed in aceto-carmine. Meiotic examination was also made at first metaphase from squashes of anthers fixed in acetic alcohol (1:3) and stained with aceto-carmine.

RESULTS AND DISCUSSION

Table 1 shows the selfed seed fertilities of the ditelosomics with the normal, *squarrosa* and *crassa* cytoplasms. Differences from the normal line were tested statistically by calculating chi-square values, and the results are also presented in Table 1. Every ditelosomic line with the *squarrosa* cytoplasm had fairly high fertility. As with the *crassa* cytoplasm, however, the fertilities of 2D α and 7DS lines were greatly reduced. As previously reported (Tsuji and Tsunewaki 1976), Chinese Spring wheat having the *crassa* cytoplasm manifested slight pistillody, although those with the normal and *squarrosa* cytoplasms did not. Frequencies of pistilloid stamens of the ditelosomic and disomic lines with the *crassa* cytoplasms are shown in Table 2. Ditelo-2D α plants showed almost complete pistillody, and ditelo-7DS showed pistillody in about half of stamens. Since highly negative correlation was found between the fertility and the frequency of pistillody in the ditelosomic lines with the *crassa* cytoplasm ($r=-0.952^{**}$, $d.f.=5$), low fertilities in 2D α and 7DS lines were mainly due to their pistillody. As shown in the male-sterile Emmer wheat with the *squarrosa* cytoplasm, the male sterility and the pistillody were not necessarily related each other. Therefore, the reduc-

Table 1. Selfed seed fertilities of the ditelosomics of the D genome chromosomes in Chinese Spring wheat with the normal, *squarrosa* and *crassa* cytoplasms

Line	Cytoplasm								
	normal			<i>squarrosa</i>			<i>crassa</i>		
	No. plants exam.	No. florets exam.	% seed set	No. plants exam.	No. florets exam.	% seed set	No. plants exam.	No. florets exam.	% seed set
Ditelo-1DL	4	210	83.8	4	168	88.7	4	144	84.7
" -2D α	4	174	64.9	3	116	79.8**	4	144	0.0**
" -3D α	4	168	77.4	3	144	71.5	4	168	80.9
" -4DL	4	188	17.1	4	144	86.1*	4	192	56.7**
" -5DL	4	180	89.4	4	192	91.1	2	72	77.8**
" -6D α	4	202	86.6	2	96	81.3	3	120	62.5**
" -7DS	4	156	91.0	4	144	92.4	4	120	22.5**

* and **: Significantly different from the corresponding normal line at the 5% and 1% level, respectively.

Table 2. Frequency of pistilloid stamens of the ditelosomic and disomic lines with the *crassa* cytoplasm

Line	No. of stamens examined	No. of pistilloid stamens	% pistillody	Differences from the disomic
Ditelo-1DL	306	7	2.3	— 1.2
" -2D α	294	285	96.9	+93.4**
" -3D α	324	5	1.5	— 2.0
" -4DL	144	4	2.8	— 0.7
" -5DL	144	0	0.0	— 3.5*
" -6D α	246	34	13.8	+10.3**
" -7DS	390	198	50.8	+47.3**
Disomic	342	12	3.5	—

* and **: Significant at the 5% and 1% level, respectively.

tion of fertility in the ditelosomics with the *crassa* cytoplasm might not be caused by the lack of the restoring genes, but might result from the enhancement of pistillody by the chromosomal aberrations.

In 1975/76, we produced the monosomic series of the D genome chromosomes with the three alien cytoplasm, except mono-5D with the *squarrosa* cytoplasm and mono-2D and -5D with the *crassa* cytoplasm. The F₁ monotrismics for producing these three monosomic lines were found not to show the expected meiotic configuration of 19''+1'+1''', so that we are now producing these lines again. The method described in Fig. 1 was very efficient for producing monosomic series. As a result, 18 out of 21 possible kinds of the monosomics of the D genome chromosomes with the three alien cytoplasm were obtained. Selfed seed fertilities of these monosomics were investigated, and the

Table 3. Selfed seed fertilities of the monosomics of the D genome chromosomes in Chinese Spring wheat with the normal, *squarrosa*, *cylindrica* and *crassa* cytoplasm

Line	Cytoplasm											
	normal			<i>squarrosa</i>			<i>cylindrica</i>			<i>crassa</i>		
	No. plants exam.	No. florets exam.	% seed set	No. plants exam.	No. florets exam.	% seed set	No. plants exam.	No. florets exam.	% seed set	No. plants exam.	No. florets exam.	% seed set
Mono-1D	4	320	96.3	4	120	85.0**	4	217	80.6**	4	160	81.3**
" -2D	4	290	93.4	3	190	97.4	3	176	85.2**	—	—	—
" -3D	3	188	93.1	4	242	85.1*	4	242	84.3**	2	144	87.5
" -4D	4	272	93.4	4	274	93.7	3	124	94.4	3	154	92.9
" -5D	3	188	91.0	—	—	—	2	132	95.5	—	—	—
" -6D	4	244	97.1	4	288	92.0*	1	144	85.4**	4	196	93.9
" -7D	4	176	97.7	3	192	95.8	3	120	85.8**	2	144	80.6**
Disomic	4	252	96.8	4	174	94.3	4	328	97.6	4	226	99.1

* and **: Significantly different from the corresponding normal line at the 5% and 1% level, respectively.

Table 4. Chromosome constitution of the offspring from the crosses, (cytoplasm substitution lines of CS×nullitetrasonic CS) F₁×nullitetrasonic CS (♂)

Female parent	squarrosa						cylindrica						crassa									
	No. plants exam.		41	42	43	No. plants exam.	41		42	43	No. plants exam.	41		42	43	No. plants exam.	41		42	43	No. plants exam.	
	NTr*	MTr*	NTr*	MTr*	NTe*	MTe*	NTr	MTr	NTr	MTr	NTe	MTe	NTr	MTr	NTr	MTr	NTr	MTr	NTr	MTr	NTe	MTe
Mono-1D tri-1A	6	0	5	0	1	4	0	2	0	2	0	2	0	5	0	5	0	5	0	0	0	0
" -2D " -2A	9	5	1	2	1	4	3	0	0	0	1	—	—	—	—	—	—	—	—	—	—	—
" -3D " -3B	8	5	1	1	1	9	4	1	2	2	2	5	2	0	3	0	3	0	3	0	0	0
" -4D " -4A	7	3	2	1	1	8	3	1	1	1	3	6	4	1	1	0	4	1	1	0	1	0
" -5D " -5A	—	—	—	—	—	8	2	0	4	2	—	—	—	—	—	—	—	—	—	—	—	—
" -6D " -6B	4	2	0	2	0	6	3	2	1	0	0	9	6	3	0	0	6	3	0	0	0	0
" -7D " -7A	10	6	2	1	1	6	0	0	0	5	1	6	2	2	0	2	2	2	0	0	2	2

*NTr, MTr, NTe and MTe: nullitri-, monotri-, nullitetra- and monotetrasomics, respectively.
 (Note) In each line, ten seeds were sown in the fall of 1975. Only the plants which were cytologically checked were included in this table. The 42-chromosome plants screened by root-tip observation were grown in the experimental field, and their chromosome constitution was determined by PMC's observation, classifying them into monotrisomics (19''+1'+1''') and nullitetrasonics (19''+1'').

results are given in Table 3. All monosomic plants showed high fertilities and normal growth. Seed fertilities, however, were slightly reduced in mono-1D, -3D and -6D with the *squarrosa* cytoplasm, mono-1D, -2D, -3D, -6D and -7D with the *cylindrica* cytoplasm, and mono-1D and -7D with the *crassa* cytoplasm.

Next, it was attempted to produce the nullitetrasonic series with the three alien cytoplasm by backcrossing each cytoplasm substitution line with nullitetrasonics. Table 4 shows the chromosome constitutions of the offspring obtained from these backcrosses. As shown in Fig. 1, when the F₁ monotrisomics were crossed with nullitetrasonics, the following four types of segregants were expected; nullitri-, monotri-, nullitetra- and monotetrasonics, which have 41, 42, 42 and 43 chromosomes, respectively. Sears (1944, 1954) reported that the female transmission rates of the univalent in the monosomics and of the extrachromosomes in the trisomics are about 25% and 40%, respectively, although these are slightly different from line to line. Using Sears' data, we can expect the ratio of the four segregants to be 45:15:30:10. Because of a very small population size and, probably, of the cytoplasmic influences, the segregation ratios obtained in some lines did not fit to the expected one. Nevertheless, nullitetrasonics with the alien cytoplasm were obtained in twelve lines. Their selfed seed fertilities are presented in Table 5. All nullitetrasonic plants obtained in 1975/76 had fairly high fertilities.

The fertility restoration controlled by a simple Mendelian factor to the *T. timopheevi* and *Ae. ovata* cytoplasm have been reported by several workers (Tahir and Tsunewaki 1969, 1971, and others). If the restoration to the *squarrosa* (or *squarrosa*-like) cytoplasm

Table 5. Selfed seed fertilities of the normal nullitetrasonics and those with the three alien cytoplasm obtained from the crosses, cytoplasm substitution lines × nullitetrasonic CS²

Line	Cytoplasm											
	normal			<i>squarrosa</i>			<i>cylindrica</i>			<i>crassa</i>		
	No. plants exam.	No. florets exam.	% seed set	No. plants exam.	No. florets exam.	% seed set	No. plants exam.	No. florets exam.	% seed set	No. plants exam.	No. florets exam.	% seed set
Nulli-1D tetra-1A	3	94	86.2	—	—	—	—	—	—	—	—	—
Nulli-2D tetra-2A	6	156	98.1	2	104	95.2	—	—	—	—	—	—
Nulli-3D tetra-3B	5	144	88.2	1	48	92.3	2	74	79.7	2	96	74.0**
Nulli-4D tetra-4A	5	240	88.8	1	24	75.0	1	48	93.8	1	26	88.5
Nulli-5D tetra-5A	3	84	38.1	—	—	—	3	120	68.3**	—	—	—
Nulli-6D tetra-6B	2	40	85.0	2	106	94.3	1	48	81.3	—	—	—
Nulli-7D tetra-7A	7	246	98.0	1	68	79.4**	5	264	96.2	—	—	—

** : Significantly different from the corresponding normal line at the 1% level.

is also controlled by a single dominant factor, we should find a single nullitetrasomic and/or ditelosomic line which showed complete male sterility or highly reduced fertility (Tables 1 and 5). Unfortunately, such line could not be found among the nullitetrasomics and the ditelosomics obtained. For this, complete nullitetrasomic series of the D genome chromosomes with the three alien cytoplasms should be established. However, we cannot discard the possibility that the restoration is controlled by multiple factors or polygenes. Since, in fact, several monosomic lines showed the slightly reduced fertilities, the restoring genes might be scattered in their monosomic chromosomes.

In the data of Table 4, it is noted that the offspring from the crosses, mono-1D tri-1A's with the three alien cytoplasms (♀) × normal nulli-1D tetra-1A's (♂), showed a peculiar segregation. These cross combinations produced none of both nulli-1D tri-1A's and nulli-1D tetra-1A's. In other combinations, except those between mono-7D tri-7A with the *cylindrica* cytoplasm and the corresponding nullitetrasomics, about a half offspring was 41-chromosome plants (nullitrisomics), as expected from the Sears' data. Although only small populations were investigated, this result suggests whether the seeds with these alien cytoplasms could not germinate when they totally lack 1D chromosomes, or those deficient for 1D chromosomes could not be produced in the above crosses. As described below, of these two possibilities, the latter will be verified partly by the crossed seed fertilities of these combinations. In the spring of 1975, we investigated the seed fertilities of the crosses between F₁ monotrisomics (♀) and nullitetrasomic and disomic CS (♂); the results are shown in Table 6. The data of the *Ae. juvenalis* (2n=42, genome constitution DDMⁱMⁱC^uC^u) cytoplasm, which also has the *squarrosa*-like cytoplasm (Tsuji and Tsunewaki 1976), were included in this table. When mono-1D tri-1A's with the four cytoplasms were crossed with normal nulli-1D tetra-1A's, the fertilities were greatly reduced (in the *squarrosa*, *cylindrica*, *crassa* and *juvenalis* cytoplasms, 26.8%, 29.9%, 15.1% and 7.7%, respectively). As previously mentioned, the female transmission rate of the univalents is about 25% (Sears 1944), which is alike

Table 6. Seed fertilities of the crosses between the monotrisomics (♀) and nullitetrasomic and disomic Chinese Spring (♂) for producing nullitetrasomic and monosomic series with the four alien cytoplasms

Cytoplasm		<i>squarrosa</i>		<i>cylindrica</i>		<i>crassa</i>		<i>juvenalis</i> *	
Female	Male	NT**	DI**	NT	DI	NT	DI	NT	DI
	Mono-1D tri-1A	26.8	81.9	29.9	78.3	15.5	80.4	7.7	95.8
	" -2D " -2A	80.7	86.2	86.9	92.9	—	—	94.1	83.3
	" -3D " -3B	86.0	75.0	82.5	83.2	75.9	85.7	85.1	83.3
	" -4D " -4A	81.7	84.5	76.9	85.7	86.3	89.9	65.0	81.8
	" -5D " -5A	—	—	55.3	68.6	—	—	34.2	81.8
	" -6D " -6B	56.1	79.7	75.0	93.9	76.5	75.0	65.6	84.6
	" -7D " -7A	90.3	98.0	87.8	88.7	91.0	91.6	95.6	91.7

* Data in the spring of 1976.

** NT and DI: nullitetrasomic and disomic Chinese Spring, respectively.

to the percent seed fertilities observed in these four crosses. However, when they were crossed with disomic CS, the resulted fertilities became fairly high. The difference clearly resulted from the deficiency of the 1D chromosome. Therefore, it seemed that, after fertilization, the seeds nullisomic for the 1D chromosome could not develop, and only those having this chromosome developed normally. From these considerations, it may be concluded that the gene(s) for zygotic viability in the three alien cytoplasms is located on the 1D chromosome of common wheat. Moreover, ditelo-1DL plants with, at least, two alien cytoplasms were highly fertile (Table 1). This fact indicates that the gene(s) is located on the long arm of the 1D chromosome. Murata and Tsuji (1976) reported that the selfed seed fertilities of pentaploid hybrids (AABB) with the cytoplasms of the *squarrosa*, *cylindrica*, *crassa*, Emmer wheat and common wheat were 32.3%, 30.0%, 20.0%, 64.1% and 80.0%, respectively. These reductions of fertility is also explicable by the inviability of seeds which are deficient for the 1D chromosome.

Maan (1973) reported that complete substitution of the *T. durum* genome (AABB) into the *Ae. squarrosa* cytoplasm resulted in non-germinating seeds, and that all seedlings from the fifth backcross with the *T. durum* pollen had 29 chromosomes ($2n=14''+a$ complete or telocentric D-genome chromosome). Same results were obtained by Kihara and Otsuka (1975). They stated that the genomically complete AABB nucleus ($2n=28$) with the *Ae. squarrosa* cytoplasm did not result in normal plant development, for which one chromosome belonging to the D genome was indispensable. Also in the present work, several very small and shrivelled seeds which did not germinate were found among the offspring of the crosses between the mono-1D tri-1A plants with the *squarrosa*, *cylindrica*, *crassa* and *juvenalis* cytoplasms (♀) and the corresponding nullitetrasomics and Emmer wheat (♂). In calculating the crossed seed fertility presented in Table 6, however, these seeds were excluded. Probably, they are deficient for the 1D chromosome. We infer that the D genome chromosome reported by Maan (1973) and Kihara and Otsuka (1975) to be indispensable for normal development of Emmer wheat with the *squarrosa* cytoplasm should be the 1D chromosome.

Finally, we could not identify the chromosome in which the fertility restoring genes to the *squarrosa* (or *squarrosa*-like) cytoplasm are located. These genes might be linked to the seed viability genes discussed above. For this, further studies are needed. However, we could demonstrate cytogenetically from the data of Tables 4 and 6 that the cytoplasms of *Ae. squarrosa*, *Ae. cylindrica*, *Ae. crassa* and *Ae. juvenalis* are quite similar to one another, although they show some degree of differentiation (cf. Table 1).

SUMMARY

In order to clarify the specific nucleo-cytoplasmic interactions between the D genome chromosomes in common wheat and the *Ae. squarrosa*, *Ae. cylindrica* and *Ae. crassa* cytoplasms, we produced three aneuploid series of the D genome chromosomes, *i.e.*, ditelosomics, monosomics and nullitetrasomics, of *Triticum aestivum* L. cv. Chinese Spring with the above three cytoplasms. And, the seed fertilities of these lines were investigated.

Seven ditelosomic lines with the *squarrosa* cytoplasm had high fertilities. However, ditelo-2D α and -7DS with the *crassa* cytoplasm showed highly reduced fertilities. Their fertility reduction was mainly due to pistillody.

We produced 18 monosomics of the D genome chromosomes with the three alien cytoplasms, except mono-5D with the *squarrosa* cytoplasm and mono-2D and -5D with the *crassa* cytoplasm. Every monosomic line had high seed fertility, although several lines showed slight reduction when compared with the corresponding normal monosomic lines.

By backcrossing the three cytoplasm substitution lines with nullitetrasomics, we attempted to obtain the nullitetrasomics with the alien cytoplasm. As a result, twelve lines, which were all fertile, were obtained. However, chromosomal segregations were biased particularly in the crosses between mono-1D tri-1A plants with the three alien cytoplasms (♀) and the normal nulli-1D tetra-1A's (♂), and none of nulli-1D tri-1A's and nulli-1D tetra-1A's was obtained. Furthermore, mono-1D tri-1A's with these cytoplasms showed extreme reduction of seed fertility when pollinated by nulli-1D, while normal seed fertility by the disomic. Ditelo-1DL plants with those alien cytoplasms were normally fertile. Taking all these results in consideration, we concluded that the viability gene(s) against the three alien cytoplasms is located on the long arm of the 1D chromosome of common wheat.

ACKNOWLEDGMENTS

We are grateful to Mr. Tokuhiko Makino, Natl. Inst. Agr. Sci., for his valuable suggestions to the present works. We are also grateful to Prof. Koichiro Tsunewaki for his valuable suggestions and reading a manuscript of this paper.

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