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GENETICAL STUDY ON NUCLEO-CYTOPLASMIC HYBRIDS IN WHEAT*

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Chapter I

Introduction

Kihara[14] defined NC-heterosis as "the heterotic effects of cytoplasm over the expression of nuclear gene or genes which could be useful for crop breeding". In accordance with this definition, the hybrids in the combination of nucleus (N) and cytoplasm (C) are designated nucleo-cytoplasmic hybrids (NC-hybrids). The term "alloplasmic line", commonly used in textbooks of plant breeding, is equivalent to NC-hybrid and, therefore, is used as a synonym of NC-hybrid in this paper.

Based on the genome analysis performed by Kihara and his collaborators[5,6,8,9], it became clear that various allopolyploid species of wheats and its relatives were derived from crossings between ten basic diploid species of the genus *Triticum* and *Aegilops*[12]. Combinations between the nuclei of Emmer or Dinkel wheats and the cytoplasms of related species resulted in the production of many alloplasmic lines[10,20,26,28]. Studies on these alloplasmic lines revealed that the diploid species of the genus *Triticum* and *Aegilops* are species-specific in the cytoplasm, and studies on cytoplasmic phylogeny showed that the tetraploid and hexaploid wheats have the cytoplasm derived from a diploid species in the section Sitopsis.

On the other hand, KIHARA[14] tried to use promising alloplasmic lines with the cytoplasm of *Aegilops squarrosa* for wheat breeding, and he demonstrated that some of the NC-hybrids with the nucleus of Dinkel wheat had a superiority over the euplasmic line. However, the Emmer wheats with the *squarrosa* cytoplasm showed that pollen was fertile or the plants grew normally only when the specific chromosome 1D was added[13,23].

There are also studies on the utilization of cytoplasmic male sterility induced by the interaction between the wheat nucleus and an alien cytoplasm[32]. Haploid induction[11] and the utilization of a gametocidal gene[27] by the use of alien cytoplasms have also been proposed.

In this study, several basic problems related to the breeding of NC-hybrids are presented. Chapter II deals with the production of alloplasmic lines with the cytoplasms derived from Einkorn wheat. Hitherto, it has been considered that alloplasmic lines with the cytoplasm of Einkorn wheats can not be used for NC -hybridization due to the retardation of growth and male sterility. This study shows that the fertility-restoring gene is transferable from the nuclear genome of a synthetic amphidiploid, (*boeoticum*) AADD, into Dinkel wheat together with the *boeoticum* cytoplasm. Further investigations are also carried out on the loci and function of the fertility-restoring gene.

In chapter III, the variation of chromosome numbers in the progeny of pentaploid hybrids which have the cytoplasm derived from *Aegilops squarrosa*

were compared with the previous results under euplasm which were obtained by KIHARA and his collaborators.

The NC-hybrid lines used in this study are denoted in parentheses by the species name of the cytoplasmic parent abbreviated in three letters, followed by the nuclear parent name abbreviated. For example, *Triticum aestivum* var. *erythrospermum* with the cytoplasm of *Aegilops caudata* is denoted by "(*cau*) Tve". In sentences, however, the species name of the cytoplasmic parent is not abbreviated, e. g., "*caudata* cytoplasm". When a species is collected from different sites, the collection number is placed after a hyphen as follows : *boe-1*.

Chapter II

Breeding of NC-hybrids with the cytoplasm derived from Einkorn wheat

Objective

It has been reported that alloplasmic lines resulting from the combination of cytoplasms derived from Einkorn wheats and nuclei of Emmer or Dinkel wheats show depression of growth, male sterility and retardation of heading date due to low compatibility between the nucleus and cytoplasm[17,26]. Nevertheless, NC -hybrids with Emmer nuclei can be produced and used for the analysis of differences among the Einkorn cytoplasms. In this study, an investigation was carried out to analyse the variation of Einkorn cytoplasms by the use of the cytoplasms of five lines derived from Einkorn wheats. There have been no detailed reports on the fertility restoring gene (*Rf*) or genes capable with the Einkorn cytoplasm. Only MAAN and LUCKEN[18] introduced the *Rf* gene into Dinkel wheat from (*boeoticum*) AADD, a synthetic amphidiploid which was produced from the cross *T. boeoticum* (AA) × *Ae. squarrosa* (DD). The same synthesized tetraploid plant was used in this study. Furthermore, attempts were made to identify a new fertility restoring gene in Emmer and Dinkel wheats.

Materials and Methods

KU101-2, KU101-3, KU104-2 of *T. boeoticum,* KU103 of *T. boeoticum* var. *thaoudar* and KU199-2 of *T. urartu,* all supplied by Dr. Masatake TANAKA, Plant Germplasm Institute, Kyoto University, were used as cytoplasm donors. Synthesized tetraploid plant of (*boeoticum*) AADD produced by Dr. SEARS in 1954 was given by Yoshihiko FURUTA, Gifu University. The (*boeoticum*) *T. durum* accession 56-1 is the alloplasmic line produced by Dr. MAAN, North Dakota State University, U. S. A. Emmer wheats preserved at the Kihara Institute for Biological Research, Yokohama City University, were used to detect the fertility-restoring gene to the cytoplasm of Einkorn wheat.

Table 1. Nucleus and cytoplasm donors

Species	Genome	Abbreviation & KU number*
Cytoplasm donor		
T. boeoticum	AA	*boe-1* (KU101-2)
T. boeoticum	〃	*boe-2* (KU101-3)
T. boeoticum	〃	*boe-3* (KU102)
T. boeoticum var. *thaoudar*	〃	*tha* (KU103)
T. monococcum var. *vulgare*	〃	*mon-1* (KU104-1)
T. monococcum var. *vulgare* (mutant for early heading)	〃	*mon-2* (KU104-2)
T. urartu	〃	*ura* (KU199-2)
*T. boeoticum***	〃	*boe-4*
Synthesized AADD	AADD	*boe-5* (KU211-1)
Nucleus donor		
T. durum var. *reichenbachii*	AABB	Dur
T. durum var. *melanopus*	〃	Dur m
T. durum accession 56-1	〃	Dur 56-1
T. durum cv. Langdon 1D/1A substitution line	AABB+1D1D −1A1A	Dur 1D/1A
T. polonicum var. *vestitum*	AABB	Pol
T. persicum var. *stramineum*	〃	Per
T. orientale	〃	Ori
T. timopheevi var. *typicum*	AAGG	Tim
Extracted species		
4X Thatcher***	AABB	

* KU number is the accession number of Plant Germplasm Institute, Kyoto University and the abbreviations indicate the names of cytoplasm and nucleus donors.

** The cytoplasm substitution line of *boe-4* was offered by S.S. Maan.

*** 4X Thatcher (AABB) was extracted from *T. aestivum* cv. Thatcher by Dr. E.R. Sears and maintained by Dr. Furuta.

Einkorn wheats were used as female parents and Emmer wheats as male parents to produce alloplasmic lines (Table 1). In addition, the Emmer and Dinkel wheats shown in Tables 2 and 3 were used to detect the fertility-restoring gene.

Experiments were carried out in the field and greenhouse at the Plant Breeding Institute, Faculty of Agriculture, Hokkaido University, Kihara Institute for Biological Research, Yokohama City University and the National Institute of Genetics.

Breaking of dormancy was performed by placing the seeds on moist filter papers in petri dishes, storing them during 48-72 hours in a refrigerator, and thereafter allowing them to stand at room temperature. After four days, the germination rate was recorded and excision of the root tips were excised for

Table 2. Emmer species for screening of *Rfboe* gene

Species	Genome
T. dicoccoides var. *Aaronsohni*	AABB*
fulvo-villosum	〃*
spontaneo-nigrum	〃*
kotschyanum (KU8539)	〃*
〃 (KU8937)	〃*
〃 (KU8808)	〃*
T. dicoccum var. *liguliforme*	〃*
T. durum var. *reichenbachii*	〃*
melanopus	〃*
cv. Dahker	〃*
Langdon	〃*
Langdon 1D/1A	AABB+1D1D −1A1A*
T. polonicum var. *vestitum*	AABB*
T. turgidum SNS	〃*
T. orientale	〃
T. persicum var. fuliginosum	〃
stramineum	〃
T. pyramidale var. *recognitum*	〃*
strain Baladi	〃*
T. palaeocolchicum var. *schwamilicum*	〃*
T. isphahanicum	〃*
T. timopheevi No.1	AAGG*

* These species could be crossed for the screening of *Rfboe* gene.

chromosome counting. Then the seedlings were planted in jiffy pots or wooden boxes in which a mixture of peatmoss and soil at the rate of 4 : 6. was packed. At the 3 or 4-leaf stage, seedlings were transplanted to the field one by one. The transplantation was performed in the spring at Sapporo and fall at Yokohama and Mishima. In the green house, seedlings were planted in plastic pots, 20-25 cm in diameter, and cultivated under long day conditions with artificial light in Yokohama, and under natural day-long conditions in Sapporo and Mishima.

For the observation of somatic cell division (mitosis), root tips excised at 1 cm from the root apex were put in a small 3 cc bottle filled with cold water at about 0°C for 20-24 hours. Thereafter, the root tips were fixed in Farmer's fluid (ethanol : acetic acid=3 : 1 (v/v)) for more than five days. Chromosomes were stained with acetocarmine solution for 24 hours and prepared by the squash method.

For the observation of meiosis, anthers at the first metaphase (M1) were fixed

Table 3. Dinkel species for screening of *Rfboe* gene

Species	Genome	Abbreviation & KU number
From Kihara Inst.		
T. aestivum var. *erythrospermum*	AABBDD	Tve*
cv. Chinese Spring	〃	C.S.
cv. Norin 26	〃	Norin 26*
cv. Thatcher	〃	Thatcher*
strain P168	AABBDD+ 1C1C−1D1D	P168*
T. spelta var. *duhamelianum*	AABBDD	Tsp.d*
strain Rumania	〃	Tsp.R*
T. compactum var. icterium	〃	Tcm*
From Kyoto Univ.		
T. aestivum var.*albidum* Alef.	AABBDD	1613*
milturm Alef.	〃	1527
graecum Körn	〃	1571*
〃 〃	〃	3006*
erythrospermum Körn	〃	3019
ferrugineum Alef.	〃	3034*
T. spelta var.*album* Alef.	〃	3401*
alefeldii Körn	〃	3413*
arduini Körn	〃	3415*
coeruleum Alef.	〃	3417*
duhamelianum Körn	〃	3421
neglectum Körn	〃	3443
rubrovelutinum Körn	〃	3444
T. compactum var. *humboldtii* Körn	〃	151*
fetisowii Körn	〃	152*
creticum Körn	〃	153*
rubrum Körn	〃	302*
wernerianum Körn	〃	306*
splendens Körn	〃	343
wernerianum Körn	〃	364*
T. sphaerococcum var. *rotundatum* Perc.	〃	161*
rubiginosum 〃	〃	162-1*
T. macha var. *sub-letschumicum* Dek.	〃	154*
paleo-imereticum 〃	〃	155*
ibericum 〃	〃	1817*
T. vavilovii var. *vaneum* Jakubz.	〃	192

* These species could be used for the screening of *Rfboe* gene.

and preserved in Farmer's fluid for about two weeks. Thereafter, chromosomes were stained for 24 hours in an acetocarmine solution. Preparations were made by the squash method.

For the estimation of pollen fertility, acetocarmine solution was used for staining. Pollen grains regular in shape and with sound generative and vegetative nuclei were considered to be fertile.

The first and second florets of two spikes per plant were checked for selfed seed set. The average seed setting rate between the two spikes was recorded to be selfed seed set for one plant. Crossed seed set was indicated by the rate of the number of crossed seeds to crossed florets.

For embryo culture, embryos of the F_1 hybrid were extracted on the 14th day after pollination and plated on the culture medium B_5. Till shoots began to develop, they were kept in the dark, and after that, the plants were grown under long-day condition in the growth cabinet kept at 20°C.

Results

1. Progenies of triploid hybrids (Einkorn × Emmer) with Einkorn cytoplasm

In order to produce the alloplasmic lines, the method of nuclear substitution by successive backcrossings was applied. F_1 was produced by crossing three species (four lines) of Einkorn wheat with six Emmer wheats (Table 4). *T. boeoticum* and *T. urartu* of Einkorn wheat are wild species and *T. monococcum*

Table 4. Crossed seed sets and germination rates of triploid hybrids derived from the crosses between Einkorn and Emmer wheats

Female	Male	Florets crossed	Crossed seed set (%)	Germination rate (%)
boe-2	Dur	198	37.4	75.0
	Dur 1D/1A	410	18.0	66.7
tha	Pol	146	39.7	90.0
	Dur	644	42.9	----
mon-2	Per	252	4.0	40.0
	Dur	780	33.3	----
	Dur m	308	15.3	40.0
	Dur 1D/1A	252	4.8	80.0
boe-3	Dur 56-1	---	(8 seeds)	87.5
tha	Tve	240	2.1	0.0
mon-2	Tve	422	10.2	0.0
	C.S.	224	5.8	0.0
Dur	*boe-2*	221	24.9	100.0
	tha	351	8.1	93.3
Ori	*mon-2*	130	6.2	100.0

is a cultivated one. The distribution area of *T. urartu* is localized within that of *T. boeoticum*. However, as a result of the reproductive barriers developed between the two species, the F_1 seeds were abortive with an underdeveloped endosperm. The six kinds of Emmer wheats were used as the female parent for restitution backcrossings, because the flowering date was variable among the Einkorn wheats. Crossed seed set was low irrespective of the cytoplasm, and shrunken seeds were obtained when Einkorn wheat was used as the female parent. The germination rate was high when Emmer wheat was used as the female parent. As reported by WAKAKUWA[30], this phenomenon is due to the imbalance of the chromosome numbers between the embryo and endosperm. Furthermore, F_1 seeds were abortive and failed to germinate in the cross of Einkorn wheat and *T. aestivum* var. *erythrospermum* (abbreviated to Tve) or *T. aestivum* cv. Chinese Spring (abbreviated to C. S.). No significant difference was found between the reciprocal F_1s produced from the crossings between *boe*-2 and *T. durum* (abbreviated to Dur) in the characters of plant type and spikelets. All the pollens were sterile in both euplasm and Einkorn cytoplasm plants. As shown in Table 5, significant differences were recognized in the density of the stomata between eu- and alloplasmic triploid hybrids. It was also observed that cytoplasmic effects were consistent in some characters between (*boe*-4) Dur 56-1 and Dur 56-1, with a remarkable reduction of the stomata density by the effect of

Table 5. Size and density of stomata in the reciprocal triploid hybrids between *boe-2* and *T. durum* 56-1 or its alloplasmic line with *boeoticum* cytoplasm

Line	Length*	Width*	Density***
boe-2 × Dur	47.8	20.0	14.3**
Dur × *boe-2*	46.3	20.0	19.4
(*boe-4*) Dur 56-1	53.7**	18.5**	7.4**
Dur 56-1	44.6	22.4	13.1

* Expressed by the scale of × 0.01 mm.

** Significant difference between the reciprocal crosses showed the effect of *boeoticum* cytoplasm.

*** Number of stomata per 1 mm²

Table 6. Four characters in the four kinds of triploid hybrids with *boeoticum* or *monococcum* cytoplasm

Line	No. of tillers	Culm length (cm)	Spike length (cm)	Spikelets /spike
boe-2 × Dur	18	80 (cm)	10.0 (cm)	19
boe-3 × Dur 56-1	10	122	----*	--
tha × Dur	19	65	9.5	19
mon-2 × Dur	8	66	7.8	17
Dur	14	119	8.5	--

* Not examined.

Table 7. Pairing configurations of reciprocal triploid hybrids between *boe-2* and *T. durum*

3X hybrid	No. of PMC s	I	II			III	Pollen fertility (%)
			Rod	Ring	Total		
boe-2 × Dur	83	9.18 (5-15)	0.64 (0-3)	5.12 (2-8)	5.76 (4-8)	0.11 (0-2)	0.0
Dur × *boe-2*	69	9.48 (7-13)	0.78 (0-3)	4.87 (1-7)	5.65 (4-7)	0.07 (0-1)	0.0

No significant difference was observed between the reciprocal hybrids.

the *boeoticum* cytoplasm. Four characters in four triploid F_1s produced from the crosses of *boe*-2, *boe*-3, *tha* and *mon*-2 with Dur as male parent were compared with those of alloplasmic line, Dur 56-1 (Table 6). No difference in chromosome pairing was recognized between the two reciprocal hybrids (Table 7). The presence of a pair of desynaptic chromosomes between the A genomes of Einkorn and Emmer wheat may be attributed to the mode of chromosome pairing 6II + 9I. The female fertility of these triploid F_1s was extremely low, but they were not completely sterile. As to fertility, YAMASHITA[33] observed some seed sets in open pollination in triploid hybrids, while MATSUMURA[21] reported the absence of selfed seed set after bagging. It is known that the pentaploid hybrids of AABBD produce fertile aneuploid gametes, and that the pollen fertility rate exceeds 80%. However, in the triploid hybrid of AAB type, B genome chromosomes remained univalent and the pollen fertility rate was 0%. This phenomenon was ascribed to the fact that, in the pentaploid hybrids (AABBD), the deficiency of D genome chromosomes was compensated by complete sets of A and B genomes, while in the triploid hybrids of AAB type, the A genome alone was inadequate for the compensation for the lack of B genome chromosomes in pollens as revealed in the present experiment. Triploid F_1s were produced from crosses of *mon*-1 and *ura* with Dur. From the cross of *mon*-1 × Dur, 9 seeds were obtained and 5 seeds germinated. Since, in the case of *ura* × Dur, all the seeds contained a immature endosperm, the embryos at 14 days after crossings were plated on the culture medium and the F_1 plants were successfully grown. However, all of them produced completely sterile pollens. The F_1s with cytoplasm derived from *mon*-1, *mon*-2 or *ura* were backcrossed to the male parent of *T. durum* and SB_1 plants were obtained. Thus the breeding of two lines of (*tha*) Dur and (*mon*-2) Dur with Einkorn cytoplasm and *T. durum* nucleus started from the crosses. *tha* × Dur and *mon*-2 × Dur to Dur. The number of germinated seeds in crossed seeds of SB_1 were 2 in 4 and 1 in 2, respectively (Table 8). The two SB_1 plants were 81 cm and 153 cm tall in height, and had 5 and 14 tillers, respectively (Table 9). By embryo culture, six seeds of SB_1 of (*ura*) Dur were obtained from the cross (*mon*-2 × Dur) × Dur. They all germinated and the number of chromosomes was 2n=28 (1 plant), 29 (2 plants), 30 (1 plant), and 33 (1 plant). All the plants showed complete sterility. Only one SB_1 seed in (*boe*-3) Dur of (KU102 × Dur) × Dur

Table 8. Number of crossed florets and crossed seed sets in SB_1 and RB_1 plants

Female	Male	Crossed florets	Crossed seed sets
boe-2 × Dur	Dur	60	0(0.0%)
boe-3 × Dur 56-1	Pol	84	0(0.0)
boe-3 × Dur 56-1	Dur	36	1(---)
tha × Dur	Dur	661	4(0.6)
mon-2 × Dur	Dur	128	2(1.6)
ura × Dur	Dur	--*	6(---)
Dur × *boe-2*	Dur	680	6(0.8)**
Dur × *tha*	Dur	642	1(0.2)
Ori × *mon-2*	Pol	237	0(0.0)

* Not examined.

** Three seeds showed 2n=28 and others showed 2n=29.

Table 9. Three characters of SB_1 and SB_7 lines

Line	Backcross generation	Germ. No.	Plant height (cm)	No. of tillers
(*tha*) Dur	SB_1	2/4*	81	5
(*mon-2*) Dur	SB_1	1/2	153	14
(*mon-4*) Dur	SB_7	2/2	91	17
Dur		10/10	127	7

* The numerators indicate number of germinated seeds and the denominator indicate number of crossed seed sets.

Table 10. Pairing configuration of SB_1 plant

Line	14 II		13 II+2 I		Total
	Rod*	Ring*	Rod	Ring	
(*mon-2*) Dur	2.11** (0-9)	11.88 (5-14)***	2.08 (0-9)	10.92 (4-13)	
	146		13		159 cells

* "Rod" and "Ring" indicate loosely paired bivalents and tightly paired bivalents, respectively.

** Average pairing number of bivalents.

*** Numbers in parenthesis indicate the range of chromosome pairings.

was obtained by embryo culture. The number of chromosomes in the SB_1 plants was 2n=29, and the plants were completely sterile. Observations on the chromosome pairings in the SB_1 plants of (*mon*-2) Dur revealed the presence of a pair of desynaptic chromosomes in about 10% of the cells. The chromosomes derived from the A genome of Einkorn wheat might be involved in the desynapsis (Table 10).

SB_1 plants of (*mon*-2) Dur were crossed with Dur 56-1 and SB_2F_1 plants were obtained. Their characters were compared with those of the alloplasmic line (*boe*-4) *T. durum* 56-1, which MAAN[18] had formerly produced by backcrossings (SB_8). In the *mon*-2 cytoplasm lines the tillers were more numerous and the

Table 11. Four characters in SB_1F_1, SB_2 and SB_8 plants

Line	Tillers/ plant	Clum length (cm)	Spike length (cm)	Spikelets /spike
(*mon-2*) Dur 56-1 (SB_1F_1)	18	53	5.9	11
(*mon-2*) Dur (SB_2)	11	48	6.9	12
(*boe-4*) Dur 56-1 (SB_8)	5	32	5.1	9
Dur	13	84	7.5	--*

* Not examined.

culms longer than in the *boe*-4 cytoplasm line (Table 11). It remains to be determined whether such a phenomenon was caused by the difference of cytoplasms or due to the heterogeneity of the nucleus. It is also well known that the culm is shorter and the spike thinner than in the euplasmic line. Backcrossings to Dur were further repeated by using SB_2 of (*mon*-2) Dur as female. The SB_4 plants were not vigorous, having the short and thin spike and completely sterile pollen.

As for the cytoplasms derived from *boe*-3, *tha, mon*-1, *mon*-2 and *ura,* all the lines were weak, namely, they showed a reduction in the culm and spike length and were completely sterile. In the SB_1 generation, plants showed a stable chromosome pairing of 14II. It is concluded that all the Emmer wheats lack fertility-restoring genes for the Einkorn cytoplasms regardless of whether they are of the cultivated or the wild type.

2. Nuclear restitution in triploid hybrids (Emmer × Einkorn) with Emmer cytoplasm

In order to obtain restitution lines with Emmer cytoplasm, F_1 hybrids produced from the cross between Emmer wheat (female) and Einkorn wheat (male) were further backcrossed to Emmer wheat (male).

F_1 of Dur × *boe*-2 was backcrossed to Dur. As shown in Table 12, crossed seed set was 0.8% and six seeds were obtained. They all germinated and the number of chromosomes was $2n=28$ for three plants and $2n=29$ for the rest as shown in Table 12. One subterminal chromosome was observed in No. 35 of RB_1 plants. As for the chromosome pairing, 14II which paired closely at both terminal ends was observed in No. 36-2. No significant difference was observed in the height of these plants. However, No. 32 and No. 36-1 were very late in heading date.

Regarding the pollen fertility, the value for No. 36-1 was 0%, while for No. 33, 34 and 35 the values were 55%, 82% and 42%, respectively. Selfed seeds were obtained in each plant except for No. 36-1 and No. 36-2. There were four or less tillers in all the RB_1 plants. The small number of tillers might be due to the green house conditions. The values for plant height and spike length of No. 36

Table 12. Chromosome numbers and seven characters in six RB_1 plants derived from the cross between *boe-2* and *T. durum*

Plant No.	Chrom. number	Heading time	Pollen fert. (%)	No. of tillers	Plant height (cm)	Spike length (mm)	Awn length (mm)	No. of seeds /spike
32	28	E.D.*	--****	4	122	86	108	27/40
33	29	N.***	55	4	110	120	140	50/50
34	29	N.	82	2	114	108	162	42/42
35	28*	S.D.***	42	3	103	80	186	13/36
36-1	29	E.D.	0	4	76	50	126	0/26
36-2	28**	N.	--	3	---	---	---	-----

* Including one sub-terminal chromosome.

** Fourteen bivalents closely paired in meiosis.

*** E. D., S. D. and N mean extremely delayed, slightly delayed and normal, respectively.

**** Not examined.

-1 were 76 cm and 50 cm, respectively, thus lower than in the other plants.

No specific relation was recognized between the seven agronomic characters and the chromosome numbers. Judging from the fact that these RB_1 plants had $2n=28$ or 29 chromosomes, it is evident that the female gametes participating in fertilization had chromosomes of $n=14$ or 15, in which complete sets of A and B genomes were included. In case the triploid F_1 of AAB was used as female parent, the extremely low rate of crossed seed set might be caused by the low production of female gametes with complete sets of both A and B genomes.

No. 33, 35 and 36-2 were further backcrossed respectively to Dur to obtain RB_2 plants, and several sound seeds of RB_2 generation were obtained in each line. Table 13 indicates the mode of chromosome pairings, spike length, and rate of selfed seed set per plant. As No. 4-5-2 showed a 14II+1I pairing ($2n=29$), it was estimated that No. 33 plant of RB_1 had $2n=29$ and that supernumerary chromosomes may have been transmitted through the female gametes. Pairing type of 13II+2I was rarely observed, suggesting that a pair of chromosomes did not form a bivalent. It is considered that desynaptic segment originating from an A genome chromosome of Einkorn wheat partly remained. Spike length was somewhat larger in the three lines than in Dur of the nuclear parent. Selfed seed set rate of No. 4-4-4 was extremely low but the other plants were nearly normal. Based on the above results, it is considered that the nuclear genome of the RB_2 plants was almost the same as that of the nuclear parent. Therefore, it is suggested that the chromosomes derived from Einkorn wheat are eliminated rapidly in restitution backcrosses.

3. Breeding of male fertile Dinkel wheat with *boeoticum* cytoplasm

To produce a fertile line of Dinkel wheat with the cytoplasm of *T. boeoticum,* fertility-restoring gene or genes in the A genome of Einkorn wheat were introduced by backcrossings to the synthetic amphidiploid (*boeoticum*) AADD as initial female parent for C. S.

Table 13. Chromosome numbers, pairing and two characters in RB_2 plants

Plant No.	Chromosome number	Chromosome pairing	Spike length (cm)	Selfed seed set(%)
4-4-1	28	14II (or 13II+2I)	9.9	94
-2	28	----*	9.9	99
-3	28	14II	8.8	97
-4	28	----	9.4	9
-5	28	----	7.9	82
-6	28	14II	9.8	98
4-5-1	28	---	11.2	91
-2	29	14II+1I	9.2	96
-3	28	---	8.0	99
-4	28	14II	8.5	100
-5	28	---	9.5	96
-6	28	14II (13II+2I)	9.2	93
-7	28	----	---	--
4-6-1	28	14II	10.1	95
-2	28	14II	10.0	96
-3	28	----	8.5	97
-4	28	14II (13II+2I)	9.7	97
-5	28	14II	9.5	78
-6	28	14II	8.9	90
-7	28	14II	8.5	100
-8	--	----	11.4	84
Dur	28	14II	8.5	97

* Not examined.

Since the amphidiploid plant lacks B genome, it is relatively easy to introduce B genome by backcrossing with Dinkel wheat. In addition, *boeoticum* cytoplasm was also introduced by crossing with (*boeoticum*) AADD. In this procedure, both pollen fertility and selfed seed set must be restored quickly to attain the genome constitution of AABBDD. (*boeoticum*) AADD was used as the female parent and, after pollination by C. S., nine plants of F_1s were obtained, showing chromosome numbers 2n=34, 35 and 36. Since aneuploids appeared in the F_1s, it was assumed that (*boeoticum*) AADD produced aneuploid gametes. Twelve seeds were obtained in the F_1 by reciprocal crossings. Germination rates of the reciprocal F_1 hybrids were 100%. The chromosome number was 2n=35 in all plants. However, the chromosome pairings were quite irregular in both reciprocal F_1 hybrids. The genome constitution was assumed to be AABDD. A pairing configuration 14II+7I was anticipated. However, average number of bivalent chromosomes was 4.82 and 3.57 in the reciprocal F_1s, and trivalents or tetravalents were observed (Table 14). Furthermore, chromosome bridges were observed in anaphase II. Dyad and tetrad pollens were observed in one anther.

The pollen fertility was almost 0% in (*boeoticum*) AABDD and 4.8% in (*aestivum*) AABDD. Female fertility estimated from the crossed seed set was 9.7% in the former and 8.7% in the latter (Table 14). There were no remarkable differences in the morphological characters between the reciprocal hybrids (Table 15). The pentaploid hybrids with both cytoplasms were repeatedly backcrossed as female

Table 14. Chromosome pairings, pollen fertilities and crossed seed sets in (*aestivum*) AABDD and (*boeoticum*) AABDD hybrids

Line	I	II			III	IV	Pollen fert.(%)	Crossed seed set(%)	No. of PMCs
		Rod	Ring	Total					
(*aes*) AABDD	21.70 (13-33)*	2.86 (0-6)	1.96 (0-6)	4.82 (1-11)	1.04 (0-4)	0.16 (0-1)	4.8	8.7	50
(*boe*) AABDD	26.24 (19-32)	2.43 (0-6)	1.14 (0-3)	3.57 (0-8)	0.48 (0-3)	0.05 (0-1)	0.0	9.7	21

* Pairing range of bivalent.

Table 15. Four characters in (*boeoticum*) AABDD and (*aestivum*) AABDD hybrids

Line	No. of tillers /plant	Culm length (cm)	Spike length (cm)	Spikelet /spike	Observed plants
(*boe*) AABDD	29	64	12.8	19	9
(*aes*) AABDD	36	66	11.4	19	7

Table 16. Chromosome numbers of SB_1 plants and female gametes in F_1 having *boeoticum* cytoplasm

Chromosome number		Observed number
Plant (SB_1)	Female gamete (F_1)	
37	16	1
38	17	4
39	18	0
40	19	1
41	20	2
42	21	1
43	22	0
44	23	1
45	24	1
47	26	1
53	32	1
54	33	1
56	35	2
57	36	1
	Total	17

Table 17. Chromosome numbers of RB_1 plants and female gametes in euplasmic F_1

Chromosome number		Observed number
Plant (RB_1)	Female gamete (F_1)	
30	9	1
35	14	0
36	15	0
37	16	6
38	17	5
39	18	11
40	19	13
41	20	12
42	21	4
43	22	3
51	23	1
54	33	1
60	39	1
	Total	58

by the use of C. S.'s. pollens. As a result, 45 seeds were obtained by backcrossing a 2n=34 plant with *boeoticum* cytoplasm to C. S., and 24 of them germinated. However, two plants died after germination. In 17 out of the 22 plants, the chromosome number was 2n=37 to 57 (Table 16). By the crossing to (*aestivum*) AABDD (2n=35), 58 seeds germinated among 121 seeds obtained (Table 17). Among them, the chromosome number was 2n=30 to 60. Based on observations on the somatic chromosome numbers of the SB_1 and RB_1 plants, the aneuploid female gametes having n=9 to 39 were fertilized with n=21 pollens (Table 16, 17). Some female gametes of eu- and alloplasmic F_1s had n=22 to 39. The gametes over n=22 resulted probably from incomplete unreductional division. Table 18 presents some examples of chromosome pairings in SB_1 plants. There were many plants with indehiscent anthers, and the selfed seed set of those plants were

Table 18. Pairing configurations of SB_1 plants having *boeoticum* cytoplasm

Plant	Chromosome pairing	Plant	Chromosome pairing
55-4	1V+14II+8I	55-6	2III+14II+8I
	1III+14II+10I		15II+12I
	2III+14II+7I		16II+10I
	1III+17II+4I		17II+8I
	17II+7I		(2n=42)
	(2n=41)		
56-1	1IV+2III+18II+10I	57-2	1IV+1III+20II+9I
	1IV+3III+19II+5I		3III+20II+7I
	4III+19II+6I		2III+21II+8I
	(2n=56)		1IV+1III+22II+5I
			1IV+23II+6I
			1III+25II+3I
			(2n=56)

Table 19. Chromosome numbers and four characters of SB_1 plants having *boeoticum* cytoplasm

Plant No.	Chromosome number	Pollen fert.(%)	Anther dehiscence	Open seed set(%)	Spike length(cm)
53-1	44	20	1*	0	12.5
53-3	37	8	1	0	11.0
53-4	45	8	1	0	11.5
55-4	41	2	1	0	10.0
55-6	42	23	2	0	13.0
55-7	?	21	1	0	9.5
56-1	56	44	2	0	13.0
57-2	56	3	2	0	9.5
Others	38-57	0	1-2	0	6.5-14.5
Mean	-	6.5	1.4	0	10.8
C. S.	42	95	3	100	9.0

* 1-indehiscent, 2-partial dehiscent, 3-complete dehiscent.

all 0% (Table 19).

Backcrossings to C. S. were carried out by using No. 55-4 (2n=41), No. 55-6 (2n=42), No. 56-1 (2n=56), No. 57-2 (2n=56) as female parents, and the number of germinated seeds was 9, 9, 2 and 7, respectively (Table 20). The chromosome numbers in the progeny from No. 55-4 and No. 55-6 (SB_1) ranged from 2n=39 to 45 and in those from No. 56-1 and No. 57-2 ranged from 2n=45 to

Table 20. Germination rates of SB_2 plants having *boeoticum* cytoplasm

Line	Backcrossed combination	Germin. rate
2-2	55-4 (2n=41) × C. S.	9/14*
2-4	55-6 (2n=42) × C. S.	9/13
2-6	56-1 (2n=56) × C. S.	2/2
2-9	57-2 (2n=56) × C. S.	7/9

* Germinated seeds/seeds sown.

Table 21. Chromosome numbers and five characters of SB_2 plants having *boeoticum* cytoplasm

Plant No.	Chromosome number	Pollen fert. (%)	Anther dehiscent*	Open seed set (%)	Culm length (cm)	Spike length (cm)
2-2-2	39	50	3	17	59	8.0
-6	42	64	2	18	51	9.0
-9	41	45	1	2	42	7.0
-1	42	0	1	2	31	6.5
-3	42	0	2	0	37	5.5
-4	41	0	3	7	46	10.5
-5	40	0	1	0	47	7.0
-7	42	0	1	0	59	11.0
-8	41	0	1	0	53	8.0
2-4-1	42	61	3	19	55	8.0
-6	44	48	3	11	44	7.0
-2	40	0	-	0	53	8.0
-3	42	-	1	0	42	9.0
-4	42	-	1	0	17	4.0
-5	43	0	1	0	42	7.5
-7	40	0	1	0	53	6.5
-8	45	0	1	0	64	9.5
-9	--	-	1	0	37	8.0
2-6-1	48	43	-	-	49	9.0
-2	49	--	-	7	--	---
2-9-1	48	51	3	18	57	8.5
-2	48	43	2	8	41	8.5
-3	45	10	1	0	23	5.0
-6	--	--	3	45	36	7.0
-7	--	0	1	2	57	8.0
C. S.	42	98	3	100	92	8.5

* 1-indehiscent, 2-partial dehiscent, 3-complete dehiscent.

** The lines of 2-2, 2-4, 2-6 and 2-9 are the progenies of No. 55-4, No. 55-6, No. 56-1 and No. 57-2, respectively.

49 (Table 21). Chromosome pairings were unstable, e. g., trivalents were formed (Table 22). As shown in Table 21, the pollen fertility was 0% in the six plants, while the other three plants showed about 50% in the progeny of No. 2-2 and about 50% in two plants of the progeny of No. 2-4.

Plants with normal pollen fertility showing normal anther dehiscence and seed setting were observed in the SB_2 generation. The spike length reached the level in C. S. and the culm length was 40-50 cm shorter than in C. S.

By selfing No. 2-2-2 plants with 2n=39 and No. 2-4-6 with 2n=44 in SB_2, 17 and eight seeds were obtained, respectively. Among them four and eight seeds germinated, respectively. The chromosome numbers were 2n=40 and 41 in the progenies from No. 2-2-2 and 2n=42 to 44 in the progeny from No. 2-4-6 (Table 23). The pollen fertility was 84% in No. 2-2-2-1 plant and 44% in No. 2-4-6-7 plant. Anthers of these plants dehisced and selfed seeds were obtained. By selfing No. 2-2-2-1, 22 seeds were obtained, and all of them germinated. Variation in the chromosome numbers of the progeny was in the range of 2n = 40 to 43. As for the chromosome pairings of 2n=42 plants, only 21II was observed in some plants while 20II+2I in others. The pairing type 20II+2I is presumably due to the structural difference between the two univalents. Pollen fertility segregated into normal (17 plants) and

Table 22. Chromosome pairings of SB_2 plants having *boeoticum* cytoplasm

Plant No.	Chromosome pairing
2-2-4	15II+1I
	16II+9I
	17II+7I
	18II+5I
	(2n=41)
2-4-1	1III+18II+3I
	18II+6I
	19II+4I
	20II+2I
	(2n=42)
2-4-2	18II+4I
	(2n=40)
2-4-6	1III+19II+3I
	1III+20II+1I
	20II+4I
	21II+2I
	(2n=44)
2-9-1	1III+18II+9I
	19II+10I
	1III+21II+3I
	(2n=48)

Table 23. Chromosome numbers, pollen fertilities and anther dehiscence of SB_2F_1 plants having *boeoticum* cytoplasm

Plant No.	Chromosome number	Pollen fert.(%)	Anther dehiscence
2-2-2-1	41	84	3*
-4	40	--**	1
2-4-6-1	42	0	1
-2	42	--	-
-3	43	0	1
-4	42	--	-
-5	42	10	1
-7	44	44	3
-8	43	--	-
C. S.	42	89	3

* 1-indehiscent, 2-partial dehiscent, 3-complete dehiscent.

** Not examined.

Table 24. Chromosome numbers, pairings and fertilities of SB_2F_2 plants having *boeoticum* cytoplasm

Plant No.	Chromosome number	Chromosome pairing	Pollen fert.(%)	Open seed set(%)
2-1-1*	42	20II+2I	97.7	91
-2	--**	--	96.1	100
-3	41	20II+1I	98.2	91
-4	42	20II+2I	98.7	91
-5	--	--	99.2	95
-6	42	20II+2I	93.7	94
-7	42	21II	99.2	100
-8	41	20II+1I	61.5	100
-9	42	20II+2I	93.3	95
-10	41	20II+1I	97.0	99
-11	--	--	61.4	0
-12	42	20II+2I	97.9	85
-13	--	--	96.8	80
-14	42	20II+2I	97.6	96
-15	43	21II+1I	86.5	95
-16	41	20II+1I	80.2	98
-17	43	21II+tI***	89.4	86
-18	41	20II+1I	0.0	93
-19	40	19II+2I	0.0	64
-20	41	20II+1I	0.0	97
-21	42	20II+2I	0.0	85
-22	42	20II+2I	0.0	94
C. S.	42	21II	95.0	95

* Selfed progenies from the plant of No. 2-2-2-1 (abbr. as 2-1).

** Not examined.

*** No. 2-1-17 had one additional telocentric chromosome.

0% (five plants), with a ratio of approximately 3 : 1 ($\chi^2=0.061$, $p=0.80-0.90$). Therefore, it can be considered that fertility restoration may be caused by a single gene. There was no clear relation between the chromosome number and the pollen fertility or selfed seed set. Compared with C. S., the spike length and the spikelet number per spike somewhat increased in the progeny of No. 2-2-2-1 (abbr. as No. 2-1 line) (Table 24, 25).

Table 25. Four characters of SB_2F_2 plants having *boeoticum* cytoplasm

Line	No. of tillers /plant	Culm length (cm)	Spike length (cm)	Spikelets /spike
2-1	26	94	9.7	20
C. S.	26	92	8.5	16

The No. 2-1-7 plant in SB_2F_2 formed 21II ($2n=42$), and the pollen fertility was 99.2% and the selfed seed set was 100%. The germination rate was 95.7%, and plants with only $2n=42$ were produced (SB_2F_3). In this generation, the spike

shape and values for spike length and selfed seed sets were close to normal in all the plants. Therefore, male fertile alloplasmic lines with Dinkel nucleus were established successfully, carrying both the cytoplasm and the fertility restoring gene derived from *T. boeoticum*.

4. Male sterile line of Dinkel wheat with *boeoticum* cytoplasm

As a result of successive backcrossings of No. 2-2-2 plants to C. S., male sterile plants with Dinkel nucleus as well as male fertile plants were produced. Only two plants in the SB_3 generation were used for backcrossing to C. S., and 21 seeds in SB_4 were obtained. Male sterile plants appeared in the SB_4 generation. After a male sterile plant was backcrossed to C. S., two of six plants in SB_5 showed 2n=41 and the rest 2n=42. These plants displayed male sterility, a weak growth, and about eleven days delay of the heading date. Even in the SB_6 generation, all the plants with the chromosome number 2n=41 or 42 appeared to display complete male sterility and degenerated anthers. As the crossed seed set was normal, it was assumed that female fertility was normal. Thus a male sterile line was bred true. Furthermore, the 2n=42 plants in the SB_6 generation were backcrossed to C. S., and the chromosome pairings of the SB_7 plants were all 21II (2n=42). In this generation, the germination rate of the crossed seeds was 50% or less. The reduction in the germination rate was presumably due to the presence of an underdeveloped endosperm in shrunken seeds. In order to confirm the influence of the female parent on embryogenesis, (*boeoticum*) C. S. (sterile) and (*boeoticum*) C. S. (fertile) were crossed to pollen parents of C. S. or (*boeoticum*) C. S. (fertile). Fertility restoration by *Rfboe*-1 derived from (*boeoticum*) C. S. (fertile) was also investigated in these hybrids. When (*boeoticum*) C. S. (sterile) was used as female parent, all the seeds were shrunken. However, when (*boeoticum*) C. S. (fertile) was used as female parent, only normal seeds were obtained. Table 26 indicated the five characters of dominant and recessive homozygous lines with *Rfboe*-1 in (*boeoticum*) C. S. The dominant homozygous line was normal in pollen fertility, tiller number and spike length. However, this line was taller than euplasmic C. S. and showed delayed flowering (+4.0 days). In contrast, the recessive homozygous line showed complete pollen sterility, lower plant height, much delayed flowering (+10.6 days) and increased number of tillers.

Table 26. Five characters of male fertile and male sterile isogenic lines with *boeoticum* cytoplasm

Line	Pollen fert.(%)	Plant height(cm)	Flowering date*	No. of tillers	Spike length(cm)
(*boe*) C. S. (*Rfboe Rfboe*)	96.2	97	+4.0	11	8.5
(*boe*) C. S. (*rfboe rfboe*)	0.0	78	+10.6	27	8.0
C. S. (euplasm)	96.0	87	0.0	11	8.1

* Delay from the flowering date of C. S. (euplasm).

5. Transmission of the fertility restoring gene

At first, the following crosses between (*boeoticum*) C. S. (sterile) and the F_1s produced from the reciprocal crosses of (*boeoticum*) C. S. (fertile) and C. S. were carried out. Segregation ratio of male fertile and sterile plants was 28 : 18 in the first cross, and 9 : 6 in the second as shown in Table 27. Although the united ratio 37 : 24 nearly fitted with 1 : 1 ($\chi^2=2.76$, $p=0.25-0.5$), there was a distinct

Table 27. Transmission rates of *Rfboe-1* from male and female gametes

Cross combination	Germ. rate(%)	Fertile : Sterile**
(*boe*) C. S. (S) × [(*boe*) C. S. (F) × C. S.]	48	28 : 18 ($\chi^2=2.17$)
(*boe*) C. S. (S) × [C. S. × (*boe*) C. S. (F)]	68	9 : 6 ($\chi^2=0.60$)
[(*boe*) C. S. (F) × C. S.] × C. S.	87	79 : 62 ($\chi^2=2.05$)

* S and F in parenthesis mean male sterile and fertile respectively.

** The ratio of male fertile and sterile plants.

tendency that the fertility-restoring dominant gene is inherited with a higher frequency than the recessive gene in the B_1 progenies. Also by using the F_1 of (*boeoticum*) C. S. (fertile) × C. S. as female parent, the B_1 plants were obtained from crossing with C. S. as male parent. Fertile : sterile plants segregated into 79 : 62, thus fitting to the expected ratio of 1 : 1 ($\chi^2=2.05$, $p=0.25-0.1$). Furthermore, the F_2 plants from the cross (*boeoticum*) C. S. (fertile) × C. S. segregated into 148 : 37 (fertile : sterile). This ratio well fitted to 3 : 1 ($\chi^2=2.47$, $p=0.25-0.50$). Thus, pollen fertility was restored by a single dominant gene, *Rfboe*-1, in homozygous and heterozygous condition and the selfed seed set was also normal (Table 29).

6. Monosomic analysis for the location of *Rfboe-1*

The (*boeoticum*) C. S. (fertile) line which was produced from the crossings between the synthesized tetraploid plants of (*boeoticum*) AADD and Chinese Spring had one pair of fertility restoring genes. Using this fertile line, monosomic analysis was carried out as shown in Fig. 1. Firstly, the monosomic series of C. S. was crossed as female with (*boeoticum*) C. S. (fertile). Secondly,

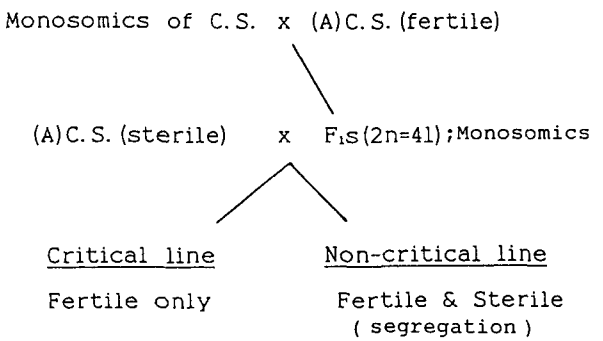

Fig. 1. Procedure of monosomic analysis of *Rfboe-1*

Table 28. Segregation ratios of fertile and sterile plants in monosomic analysis

Line	Germ. rate	Segregation ratio*	Line	Germ. rate	Segregation ratio*
1A	5/26	3 : 2	5B	1/15	-
2A	25/52	18 : 7	1D	23/57	14 : 9
3A	20/33	10 : 9	2D	2/25	2 : 0
4A	22/37	14 : 8	3D	6/11	2 : 3
5A	18/47	11 : 7	4D	20/49	9 : 7
6A	17/47	3 : 9	5D	21/42	8 : 9
7A	9/10	7 : 1	6D	37/48	30 : 6
1B	12/19	8 : 3	7D	16/34	9 : 4
2B	13/23	7 : 4	C. S.	15/22	9 : 6
3B	3/16	2 : 1			

* Fertile : sterile plants.

monosomic plants (2n=41) were selected among F_1s in each chromosome line and were crossed as male parent with (*boeoticum*) C. S. (sterile). All the plants in critical line are expected to show normal fertility, while segregation in fertility occurrs in non-critical lines. Chromosomes from A or D genome may possess *Rfboe-1*, because the tetraploid line, (*boeoticum*) AADD, was male fertile.

As a result (Table 28), 7A line segregated into 7 fertile and one sterile plants. This sterile plant showed an extremely delayed heading and might be a nullisomic plant. Thus all the other plants in 7A line were supposed to have *Rfboe-1*. Therefore, it was considered that *Rfboe-1* located on 7A chromosome.

7. Detection of the fertility-restoring gene for *boeoticum* cytoplasm

The fertility-restoring gene for the cytoplasm of *T. boeoticum* and *T. monococcum* has not yet been detected in Emmer and Dinkel wheats[19),26)]. In this study, attempts were made to detect the fertility-restoring gene for the cytoplasm of *boeoticum* among seven strains of wild Emmer wheats, 15 strains of cultivated Emmer wheats and 34 strains of Dinkel wheats (Tables 2 and 3). F_1s between (*boeoticum*) *T. durum* accession 56-1 (male sterile) as female and Emmer wheats as male were examined. Also to detect the fertility-restoring gene in Dinkel wheats, (*boeoticum*) C. S. (male sterile) was crossed with Dinkel wheats. Actually, six strains of wild and cultivated Emmer wheats were examined to detect *Rf* genes. Since pollen fertility and the seed set of these F_1s were both 0%, it was assumed that all the Emmer wheats used lacked the *Rf* gene for *boeoticum* cytoplasm. Heading was not observed in 27 strains of F_1s of Dinkel wheats with *boeoticum* cytoplasm by winter killing. However, *T. aestivum* var. *graecum* Körn accession No. 1571 and No. 3006 showed anthers with partial dehiscent, partial pollen fertility and selfed seeds when crossed with (*boeoticum*) C. S. (male sterile)

(Table 29). The above F_1 plants showed partial pollen fertility, and the selfed seed set was 68% and 71%, respectively. The fertility restoration of this F_1 was weaker than that of the F_1 with heterozygous state of *Rfboe*-1. The Dinkel wheats of accession No. 1571 and 3006 (*T. aestivum* var. *graecum* körn) were collected in the Islamic Republic of Pakistan and in Armenia, CIS. Since this variety is distributed in Asia, Europe and Africa, the fertility restoring gene must be detected in those areas by examining a larger number of strains.

Table 29. Pollen fertilities and selfed seed sets in the three F_1s

Line	Pollen fertility (%)	Selfed seed set (%)	Plants observed
(*boe*) C. S. (S) × No. 1571	41	68	4
× No. 3006	63	71	1
× (*boe*) C. S. (F)	98.6	100	10
(*boe*) C. S. (F)	95.7	100	10
C. S. (euplasm)	98.7	100	20

* These data were examined at Mishima.

** No, 1571 and No. 3006 are *T. aestivum* var. *graecum* collected in the Republic of Armenia, CIS and in the Isramic Republic of Pakistan, respectively.

8. Desynapsis of A genome chromosomes

In this section, the letters A^I, A^E and A^D are used to distinguish the A genomes of Einkorn, Emmer and Dinkel wheats. D^D and D^S refer to the D genomes of Dinkel and *Ae. squarrosa.*

As for the chromosome pairings of the F_1 plants of (*boeoticum*) AADD × C. S., the average number of bivalent chromosomes ranged from 2 to 3 in agreement with the results obtained by OKAMOTO[25]. However, OKAMOTO gave no reason for the low pairings. KIHARA and LILIENFELD[7] indicated that there was some chromosomal differentiation between A^I of Einkorn and A^D of Dinkel wheat. The 7II was observed between A^E and A^D and also between the B genome from Emmer wheat (Abbr. B^E) and Dinkel wheat (Abbr. B^D). However, the frequency of pairings between A^I and A^E and between A^I and A^D was lower than 7II. In this study, the affinity among the three A genomes was investigated. To estimate the pairing affinity between A^I and A^E, F_1s with the genomic constitutions, $A^IA^EB^ED^S$ and $A^IA^EB^E$ were produced from crosses between synthesized amphidiploid ($A^IA^ID^S D^S$) × *T. durum* ($A^EA^EB^EB^E$) and between *T. boeoticum* (A^IA^I) × *T. durum* (Table 30). The average number of bivalent chromosomes in the hybrid was 2.5 and 5.8, respectively. As to the chromosome pairing between B^E and D^S in the former F_1, it is considered that *Ph* located on 5B may

Table 30. Average chromosome pairings between A^I and A^E genomes in two kinds of hybrids

Genome constitution	I	II	III	No. of PMCs
$A^IA^EB^ED^S$*	23.5	2.5	0.3	151
$A^IA^EB^E$**	9.2	5.8	0.1	83

* F_1 hybrid between synthesized AADD and *T. durum.*

** F_1 hybrid between *T. boeoticum* and *T. durum.*

completely depress the pairing of homoeologous chromosomes between B^E and D^S genomes. Therefore, the presence of 2.5 II in the hybrid $A^IA^EB^ED^S$ may only be limited to the bivalents between A^I and A^E. According to DRISCOLL[2], another *Ph* is located on 3D. Therefore, it is assumed that the homeologous pairings are partly restored by the loss of the suprresson located on 3D. The number of bivalent chromosomes observed between A^I and A^E in the hybrid of $A^IA^EB^E$ was 5.8 (mode was 6). Therefore, the pairing affinity between A^I and A^D should be estimated in F_1 hybrids lacking the D genome ($A^IA^DB^D$) produced from the cross between Einkorn wheat (A^IA^I) × 4X Thatcher ($A^DA^DB^DB^D$, 2n=28). Mode of pairings in F_1s was 6II+9I as shown in Table 31. The number of bivalents was similar to that in $A^IA^EB^E$. Based on the above results, the pairing affinity between A^I and A^D was almost the same as that between A^I and A^E and there was only a pair of desynaptic chromosomes in A^I genomes so far as inferred from the pairing affinity of 6II+9I in the hybrid $A^IA^EB^E$.

Table 31. Chromosome pairings between A^I and A^E or between A^I and A^D genomes in the two kinds of hybrids

Chromosome configuration	$A^IA^DB^D$*	$A^IA^EB^E$**
3II + 15I	0	1
4II + 13I	0	4
5II + 11I	7	24
6II + 9I	55	48
7II + 7I	22	14
8II + 5I	0	1
1III + 4II + 10I	0	3
1III + 5II + 8I	17	1
1III + 6II + 6I	0	3
2III + 4II + 7I	0	1
	100(%)	100(%)
Total	108 cells	161 cells

* F_1 hybrid from the cross between 4X Thatcher which is extracted from 6X cultivar Thatcher and *T. monococcum*.

** F_1 hybrid from the cross, between *T. durum* and *T. monococcum*.

According to JOPPA and MAAN[4], one desynaptic chromosome was compensated for the loss of 4B except for the function of *GA* gene. However, in their study, they did not identify the homoeologous group of these desynaptic chromosomes. Einkorn wheat (A^IA^I) was crossed with the chromosome substitution lines of *T. durum* cv. Langdon (AABB) in which the A and B genome chromosomes were substituted for D genome chromosomes (chromosome substitution lines). Then the homoeologous group of the desynaptic chromosomes was identified on the basis of the pairing type of their F_1s. The F_1 between the line Dur 4A (4D) with D genome chromosome substitution and Einkorn wheat showed the chromosome pairings shifted from 5II+11I to 6II+9I (Table 32) thus differing from the pairings shown in the other crosses. Then the desynaptic chromosome was determined as $4A^I$. DVORAK[3] also reported the presence of mispairing between $4A^I$ and $4A^D$. In conclusion, differentiation between A^I and A^E or A^D genomes, in terms of pairing, may be associated with structural changes of the 4A chromosomes. It may be safely be assumed that in

Table 32. Chromosome pairings in the crosses between disomic chromosome substitution lines A(D) of *T. durum* cv. Langdon and *T. monococcum* var. *vulgare* (KU104-1)

Cross combination	3II+15I	4II+13I	5II+11I	6II+9I	7II+7I	Others	No. of cells
Dur 2A(2D) × 104-1	0.0(%)	3.5	67.8	24.5	2.8	1.4	143
Dur 3A(3D) × 〃	0.0	17.0	69.1	13.3	0.0	0.6	165
Dur 4A(4D) × 〃	0.1	28.1	35.6	30.0	0.0	6.2	160
Dur 5A(5D) × 〃	0.0	7.6	55.7	31.6	5.1	0.0	79
Dur 6A(6D) × 〃	6.0	31.0	53.4	8.6	0.0	1.0	116
Dur 7A(7D) × 〃	27.4	62.9	0.1	0.0	0.0	9.6	62

the course of evolution, after the transmission of $4A^{l}$ to Emmer wheat, $4A^{E}$ or $4A^{D}$ chromosomes were established by chromosomal structural changes.

The plants in the SB_2F_3 generation derived from (*boeoticum*) AADD × C. S. showed a stable pairing of 21II, but the pairings 20II+2I were observed in three plants when crossed with C. S. In order to analyse these univalent chromosomes, C. S. was backcrossed to the plant with the 20II+2I pairing, and nine plants out of 28 showed the pairing type of 20II+2I. There was no clear relationship between the pairing type and pollen fertility (Table 33).

Table 33. Relationship between chromosome pairings and pollen fertilities of F_1 plants from the cross, (*boe*) C. S. (20II+2I) × C. S.

Pairing	Fertile	Sterile
20II + 2I	7	2
21II	8	11

Discussion

To analyse the genetic diversity of the cytoplasms in Einkorn wheats, PANAYOTOV[26] examined seven kinds of Einkorn cytoplasms, and found that the cytoplasm derived from *T. aegilopoides* indicated a normal fertility when combined with Dinkel nucleus, and that the cytoplasm of *T. monococcum* var. *hornemanii* caused extreme depression of growth. In the present study, however, all the alloplasmic lines with Emmer nucleus with the cytoplasms derived from three species of Einkorn wheats showed complete male sterility. Also the "fertile cytoplasm" reported by PANAYOTOV was not detected. Therefore, it is concluded that genetic diversity is absent among the cytoplasms of Einkorn wheats in regard to fertility. Generally, growth depression manifested by a reduction of plant height and thin culm was recognized in the SB_1 generation. Since the homo- and heterozygous states of *Rfboe*-1 derived from (*boeoticum*) AADD showed complete restoration of pollen fertility, it was assumed that fertility-restoring lines with Einkorn cytoplasm (R-line) could be established in Dinkel wheats. Also, male sterile lines with the nucleus of Emmer or Dinkel wheats and the *boeoticum* cytoplasm were produced.

In this study, male sterile C. S. (SB_8) with *boeoticum* cytoplasm showed a low

vigor and delayed heading, while in the male fertile counterpart with *boeoticum* cytoplasm (SB_2F_6), the plant growth and heading date were normal both in the homo- and the heterozygotes of *Rfboe*-1. However, plants obtained from the crosses between (*boeoticum*) C. S. (male sterile) × [(*boeoticum*) C. S. (male fertile) × C. S.] and between (*boeoticum*) C. S. (male sterile) × [C. S. × (*boeoticum*) C. S. (male fertile)] segregated into male fertile with normal vigor and male sterile with low vigor. Therefore, pleiotropic effects or a close linkage between the genes for plant vigor and fertility are suggested.

In the male sterile (*boeoticum*) C. S. with AABBDD genomes, the number of tillers was generally larger than in the male sterile (*boeoticum*) *T. durum* with AABB genomes. Therefore, the compensatory effects of the D genome for the A and B genomes may be related to the tiller number in the presence of *boeoticum* cytoplasm. Development of fertile NC-hybrid lines with Einkorn cytoplasm and Dinkel nucleus may be promising in wheat breeding.

It was confirmed that *Rfboe*-1 was transmitted somewhat preferentially from male gametes. A similar phenomenon is observed for the certation between the pollens with 1C and 1D.

There are many reports on the chromosome pairings of A genomes derived from Einkorn, Emmer and Dinkel wheats. For example, the pairing mode of A genome in the F_1 between Einkorn and Dinkel wheats was as low as 3II-4II[8], while the mode of 6II+9I was common in the F_1 between Einkorn and Emmer wheats. KIHARA and LILIENFELD[7] reported that the difference on the pairing affinity of the A genome in the F_1s between Einkorn and Emmer or Dinkel wheats was due to the chromosome differentiation among the A genomes of these three kinds of wheats. However, when the D genome was absent as in the case of the triploid hybrids between *T. monococcum* (A^1A^1) and extracted 4X Thatcher ($A^DA^DB^DB^D$), the mode of pairing reached to 6II+9I due to the absence of *Ph* located on 3D, which indicated the direct pairing affinity between the A^1 genome of Einkorn and the A^1 genome of Dinkel wheat. Accordingly, there was no significant difference between A^1 and A^E or A^D except for a desynaptic chromosome $4A^1$.

Chapter III

Transmission of D genome chromosomes in alloplasmic pentaploid hybrids with the cytoplasm derived from *Aegilops squarrosa*

Objective

The transmission pattern of the D genome chromosomes in pentaploid hybrids of the AABBD type with the cytoplasm of Emmer or Dinkel wheats has been studied by many investigators since KIHARA[5]. He demonstrated that the

chromosome pairings and viability of the plants became stabilized when the progenies converged to either AABB (2n=28) or AABBDD (2n=42) chromosome constitution. Plants with the number of chromosomes intermediate between AABB (2n=28) and AABBDD (2n=42) were unstable for chromosome pairing and for viability of plants.

On the other hand, it was reported that the presence of the 1D chromosome was essential for the viability of the seeds of Emmer wheat with *squarrosa* cytoplasm (2n=29, AABB+1D)[23]. Furthermore, OHTSUKA[24]. demonstrated that microspores lacking the 1D chromosome did not develop to fertile pollens in the presence of *squarrosa* cytoplasm. Based on these findings, KIHARA planned to examine the specificity of the chromosomal distribution divergence in the progenies of pentaploid hybrids with *squarrosa* cytoplasm.

Thus the transmission pattern of the D genome chromosomes from pentaploids with *squarrosa* cytoplasm was compared with that of the pentaploids with the cytoplasm from Dinkel wheat.

Materials and Methods

The pentaploids with *squarrosa* cytoplasm were produced by crossings between *Triticum aestivum* var. *erythrospermum* (Tve) with *squarrosa* cytoplasm as female parent and *T. durum* var. *reichenbachii* (Dur) or *T. turgidum* var. *nigro-barbatum* (Tur) as male parent. For comparison, euplasmic pentaploids were also produced in this study. After random selection of the plants with 2n=28 to 42 in the progenies, ten selfed seeds from the indivdual plant were examined in each generation and the chromosome numbers were checked. These materials are shown in Table 34.

Table 34. Tetraploid and hexaploid lines used as parents

Line	Abbrev.	Nuclear genome	Backcrossed generation
-Male parent-			
T. durum var. *reichenbachii*	Dur	AABB	
T. turgidum var. *nigro-barbatum*	Tur	〃	
-Female parent-			
T. aestivum var. *erythrospermum* with *squarrosa* cytoplasm	(*sqr*) Tve	AABBDD	SB_{12}
T. aestivum var. *erythrospermum*	Tve	〃	

Results

Germination rates, pollen fertilities and selfed seed sets of the parental lines used are shown in Table 35. By using these data, four kinds of pentaploids, Tve × Dur, Tve × Tur, (*sqr*) Tve × Dur and (*sqr*) Tve × Tur were produced.

Table 35. Germination rates, pollen fertilities and selfed seed sets of pentaploids with eu- and *squarrosa* cytoplasms

Line	Germ. rate(%)	Pollen fert.(%)	Selfed seed set(%)
Dur	92	98	97
Tur	90	99	97
Tve	100	99	95
(*sqr*) Tve	96	98	90
Tve × Dur	80	86	39
Tve × Tur	97	92	60
(*sqr*) Tve × Dur	93	39	7
(*sqr*) Tve × Tur	100	46	33

Germination rates of the pentaploids were normal in all the combinations. However, the pollen fertility of the pentaploids with *squarrosa* cytoplasm decreased markedly. Selfed seed set of (*sqr*) Tve × Dur was extremely low as 7%.

It is reasonable to assume that the low selfed seed set was caused by the sterility of pollens lacking 1D in the presence of *squarrosa* cytoplasm and by the zygotic lethality due to the imbalanced ratio of chromosome numbers between embryo and endosperm similar to that of euplasmic pentaploids[5]. In (*sqr*) Tve × Dur, the anther dehisced partially and the fertilization of pollens was prevented resulting in an extremely low selfed seed set.

At first, the transmission pattern of the D genome chromosomes from the male gametes of the pentaploids was examined in the presence of the cytoplasm from euplasmic lines. Certation among aneuploid pollens was estimated by such crossings as Tve × (Tve × Dur) and Tve × (Tve × Tur). Also, similar certation crossings were carried out in the presence of *squarrosa* cytoplasm. Since hexaploids were used as the female parent, the crossed seed sets of certation crosses were low, while the germination rates exceeded 70% (Table 36). The influence of *squarrosa* cytoplasm was not pronounced in the crossed seed set and germination rate. Chromosome numbers of the male gametes of the pentaploids which participated in fertilization were estimated from the somatic chromosome number of B_1 by pollinating Tve as female. It was shown that n=14 pollens did not participate in the fertilization in both crosses of Tve × ((*sqr*) Tve × Dur) and

Table 36. Crossed seed sets and germination rates in the certation crossings

Cross combination	Crossed florets	Crossed seed set(%)	Germination rate(%)
Tve × (Tve × Tur)	366	12.6	77.8
〃 × ((*sqr*) Tve × Tur)	1080	13.9	74.8
Tve × (Tve × Dur)	259	30.5	88.6
〃 × ((*sqr*) Tve × Dur)	578	10.2	96.6

Tve × ((*sqr*) Tve × Tur) in the presence of *squarrosa* cytoplasm. In contrast, the pollens near n=21 were fertilized at the highest rate in (*sqr*) Tve × Dur and (*sqr*) Tve × Tur (Table 37, Fig. 2). Based on the compatible relation between 1D and the *squarrosa* cytoplasm[23,24], the pollens (n=15) of AB+2D~7D chromosome constitution were sterile, and only the AB+1D (n=15) pollens became fertile in the pentaploids with *squarrosa* cytoplasm. Accordingly, it was assumed that fertile pollens were produced at the probability of 1/7 among all the n=15 pollens. Similarly, the frequency of fertile pollens with n=16 to 21 were expected to be 2/7, 3/7, 4/7, 5/7, 6/7 and 7/7 for n=16, 17, 18, 19, 20 and 21 respectively in the presence of *squarrosa* cytoplasm. By multiplying the above ratios with the observed frequencies of pollens participated in fertilization in certation crosses in the presence of euplasm, expected frequencies of fertilized pollens with n=15 to

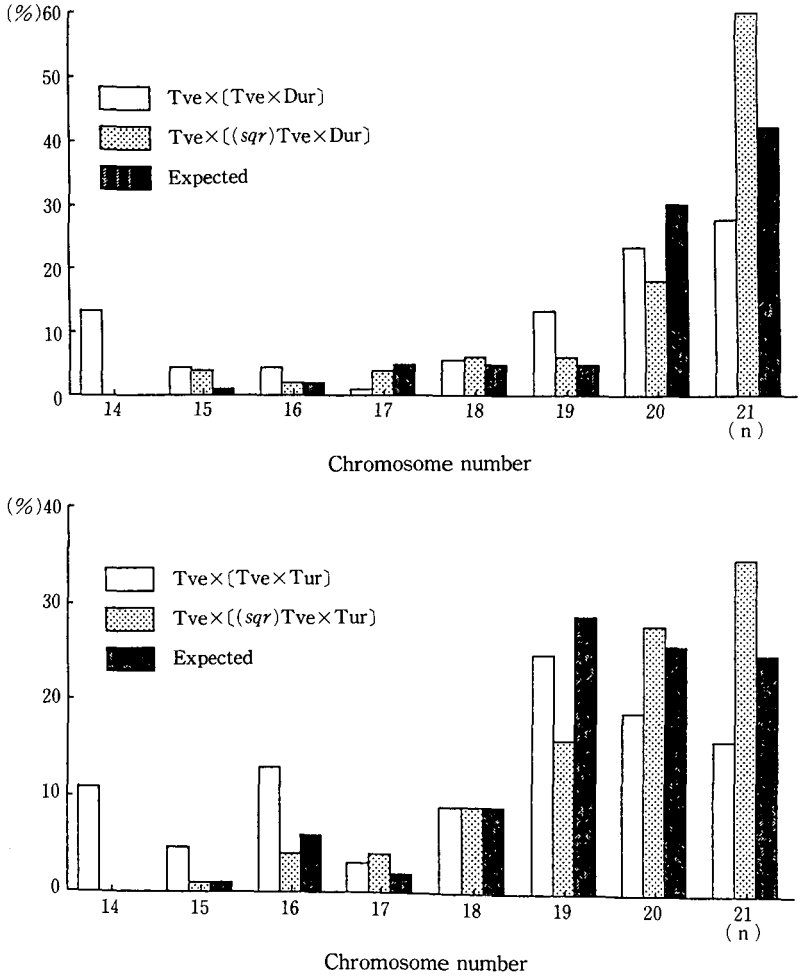

Fig. 2. Chromosomal distributions of male gametes fertilized in the crosses Tve × 5X and Tve × (*sqr*) 5X

Table 37. Observed and expected numbers of male gametes fertilized in the pentaploids with eu- and *squarrosa* cytoplasm

Chromosome number of male gamete	Observed		Expected	Observed		Expected
	Tve × 5X-1*	Tve × (*sqr*) 5X-1*	Tve × (*sqr*) 5X-1	Tve × 5X-2*	Tve × (*sqr*) 5X-2*	Tve × (*sqr*) 5X-2
14	12	0	0.0	7	0	0.0
15	4	2	0.5	3	1	1.0
16	4	1	1.0	8	4	5.8
17	7	2	2.5	2	4	2.3
18	5	3	2.4	6	9	8.5
19	12	3	7.3	16	16	28.6
20	21	9	15.2	12	28	25.8
21	25	30	21.1	10	35	25.1
Total	90	50	50.0	64	97	97.1
Chi-square value		$\chi^2=13.6$ $(0.05>p>0.025)$ d. f.=6			$\chi^2=11.5$ $(0.1>p>0.05)$ d. f.=6	

* 5X-1 is F_1 hybrid between Tve and *T. durum*. 5X-2 is F_1 hybrid between Tve and *T. turgidum*.

21 with *squarrosa* cytoplasm were calculated. The expected frequencies of fertilized aneuploid pollens were calculated by the following formula.

$$F_p=\left[\frac{{}_6C_{p-15}}{{}_7C_{p-14}}\times ON \Big/ \sum_{p=14}^{21}\frac{{}_6C_{p-15}}{{}_7C_{p-14}}\times ON\right]\times 100\ (\%)$$

* $F_{14}=0$

** p : Chromosome number of fertilized pollens.

*** F_P : Frequency of fertilized pollens with chromosome number p in the presence of *squarrosa* cytoplasm.

**** ON : Observed number of fertilized pollens with n=15~21, estimated from the certation crosses in the presence of euplasm.

The observed values approached to the expected ones calculated from the above formula in two certation crossings in the presence of *squarrosa* cytoplasm (Table 37 and Fig. 2). It was noted that the increment of the chromosome numbers toward n=21 and complete absence of n=14 were observed only in the presence of *squarrosa* cytoplasm. Thus, in comparison with euplasmic certation, the distribution of chromosome numbers was remarkably biased in the presence of *squarrosa* cytoplasm. As for the transmission of the D genome chromosomes from female gametes, equational crossings were also carried out in the presence of both *aestivum* and *squarrosa* cytoplasms (Table 38). The hexaploid variety of Tve was used as pollen parent to avoid a reduction of the seed set caused by the chromosomal imbalance between embryo and endosperm[29,30,31]. In these equational crossings, female gametes with n=14 participated in the fertilization irrespective of *squarrosa* cytoplasm. However, there was a significant difference in the chromosomal distribution between the eu- and *squarrosa* cytoplasm lines. Table 39 show a decrease of the chromosome numbers, down to 2n=14 and 15 in

Table 38. Crossed seed sets and germination rates in the equational crossings

Cross combination	Crossed florets	Crossed seed set(%)	Germination rate(%)
(Tve × Tur) × Tve	180	43.9	51.9
((*sqr*) Tve × Tur) × 〃	865	37.8	34.7
(Tve × Dur) × 〃	231	48.5	53.6
((*sqr*) Tve × Dur) × 〃	326	38.0	50.0

Table 39. Observed numbers of female gametes which participated in fertilization in the pentaploids with eu- and *squarrosa* cytoplasms

Chromosome number of female gamete	Observed		Observed	
	5X−1 × Tve	(*sqr*) 5X−1 × Tve	5X−2 × Tve	(*sqr*) 5X−2 × Tve
14	11	2	4	2
15	18	3	15	7
16	16	12	25	14
17	22	17	38	28
18	15	13	30	27
19	14	8	25	21
20	3	4	4	12
21	1	2	3	7
Total	100	61	144	118
Chi-square value	$\chi^2=60.3$ (0.01> p)* d. f.=7		$\chi^2=21.6$ (0.01> p)* d. f.=7	

* Significant difference was recognized.

the equational crosses in the presence of *aestivum* cytoplasm. Therefore, a reduction of fertile female gametes with lesser chromosome numbers in pentaploids with the *aestivum* cytoplasm was prominent (Table 39). The germination rates of the F_2 seeds derived from Tve × Dur or Tve × Tur were 66% and 76%, respectively. Also, in the cases of (*sqr*) Tve × Dur and (*sqr*) Tve × Tur, germination rates were 36% and 52%, respectively. The low germination rates of the F_2 seeds in the presence of *squarrosa* cytoplasm were attributed to chromosomal imbalance between embryo and endosperm in the zygote[29),30)]. In the presence of euplasm, the chromosome number of the F_2 plants ranged from 2n=28 to 42 and the distribution was almost symmetrical in (Tve × Dur) × Tve (Fig. 3). On the other hand, the F_2 populations in the presence of *squarrosa* cytoplasm had 2n=31 to 42 chromosomes and the number of plants with chromosomes less than 2n=33, designated as the "diminishing group" plants by KIHARA[5)], decreased markedly (Fig. 4). Especially, 2n=28 plant did not appear due to the complete sterility of the pollens with n=14 chromosome. As for the selfed seed sets, there was a remarkable difference associated with the effects of

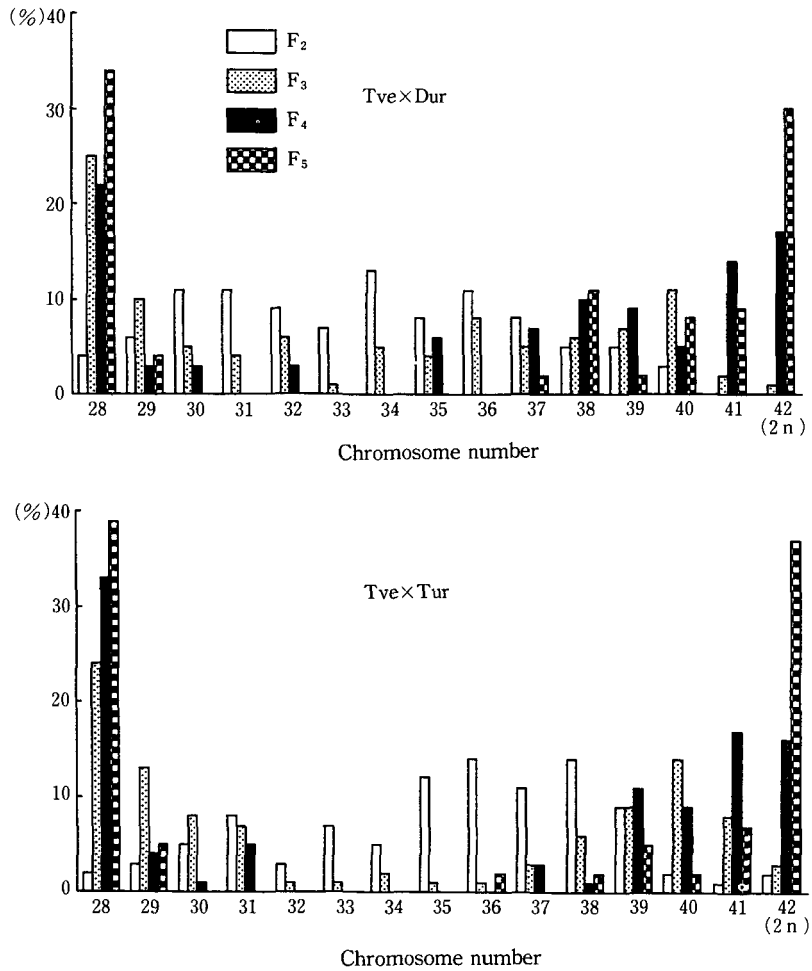

Fig. 3. Chromosome numbers in progenies of pentaploids (from F_2 to F_5) with *aestivum* cytoplasm.

the cytoplasms, namely, plants in "diminishing group" showed a low seed set in the presence of *squarrosa* cytoplasm, while plants in "increasing group" (over 2n= 36) showed rather higher seed sets even in imbalanced combinations. In contrast, the variation of the selfed seed set in the presence of euplasm was symmetrical showing a low frequency of seed set of the plants with intermediate number of chromosomes. KIHARA[5] classified the progenies into "balanced" and "imbalanced" ones on the basis of the D genome chromosome pairing type. However, in the presence of *squarrosa* cytoplasm, only a small number of plants in the "diminishing group" was obtained. Even the plants with balanced chromosome combinations failed to set selfed seeds (Table 40), presumably due to

Table 40. Pollen fertilities, selfed seed sets and plant heights of F_2 plants with eu- and *squarrosa* cytoplasms in balanced or imbalanced combinations in diminishing and increasing groups

Cytoplasm			Pollen fertility(%)	Selfed seed set(%)	Plant height(cm)
Euplasm	Diminishing group	Balanced	83.2±10.1*	45.3±17.0*	87.7±7.6*
		Imbalanced	70.6±23.3	30.7±19.0	82.9±13.7
	Increasing group	Balanced	91.3±7.3	56.9±19.1	101.1±8.2
		Imbalanced	83.3±11.7	18.9±21.4	91.2±7.0
squarrosa cytoplasm	Diminishing group	Balanced	21.1±19.8	0.0±0.0	89.5±2.1
		Imbalanced	78.3	4	83.0
	Increasing group	Balanced	67.9±27.6	48.3±23.5	94.4±10.5
		Imbalanced	86.8±3.0	45.3±14.2	97.0±8.9

* ±S. D. was designated.

the high rate of pollen abortion associated with the lack of 1D chromosome in those plants. Due to the small number of F_2 plants within the "diminishing group", there appeared few F_3 plants in this group.

The germination rates of F_3 seeds were normal regardless of the chromosome numbers in the presence of cytoplasm from either euplasmic or *squarrosa* cytoplasm line. A small number of plants were obtained in the "diminishing group", and 2n=28 (tetraploid) plants in F_3 were not observed in the presence of *squarrosa* cytoplasm. The number of plants in the "diminishing group" further decreased in the F_4 generation. KIHARA[9] postulated that in the presence of euplasm, the chromosome numbers of the plants in the "diminishing group" returned to the euploid state (2n=28) faster than those in the "increasing group" owing to (1) the elimination of univalent chromosomes in the 2nd meiotic division of meiosis, and (2) high rate of fertilization of n=14 pollens. The relationship of the chromosome numbers between F_2 plants and F_3 progenies is shown in Table 41. Generally, the chromosome numbers of the F_3 progenies shifted to higher numbers in the presence of *squarrosa* cytoplasm than the euplasmic lines ; this is due to the fact that n=14 pollens are sterile in F_2 in the presence of *squarrosa* cytoplasm. In the F_3 of (*sqr*) Tve × Dur, two plants with 2n=30 were obtained ; they showed normal pollen fertility, although no selfed seed set was observed. Plants with a chromosome number close to 2n=42 in the F_3 showed a high rate of selfed seed set. Selfed seeds were hardly obtained from plants with chromosome numbers under 2n=36 in the F_3 in the presence of *squarrosa* cytoplasm, while plants with 2n=28 showed normal selfed seed set in the presence of euplasm.

In the F_4, the germination rate was not related to the chromosome numbers. As for the chromosome numbers, only the plants in "increased group" remained in the presence of *squarrosa* cytoplasm (Fig. 4). On the other hand, the chromosome numbers approached to 2n=28 or 2n=42 in the presence of euplasm (Fig. 3). In the presence of either eu- or *squarrosa* cytoplasm, plants with intermediate chromosome numbers (2n=31 to 37) showed a reduction in the rate of selfed seed

Table 41. Relationship of the chromosome numbers between F_2 plants and F_3 progenies in the pentaploids, Tve × Dur and (*sqr*) Tve × Dur

	Chromosome number of F_2 plant*	Mean chromosome No. of F_3s	
		Tve × Dur	(*sqr*) Tve × Dur
Diminishing group	28	28.0	-
	29	28.4	-
	30	28.3	-
	31	29.0	30.3
	32	29.8	30.8
	33	31.0	32.0
	34	32.4	-
Increasing group	36	36.9	38.0
	37	35.3	38.9
	38	38.9	40.0
	39	39.4	39.4
	40	39.8	40.0
	41	-**	40.1
	42	-	42.1

* 2n=35 plants were excluded.

** Plants were not obtained.

sets. In the presence of *squarrosa* cytoplasm, only plants with a chromosome number larger than 2n=39 showed a high selfed seed set, while plants with chromosomes less than 2n=38 were rapidly eliminated. The mode of chromosome numbers in the F_5 reached 2n=42 in the progeny of (*sqr*)Tve × Tur and 2n=38 in (*sqr*)Tve × Dur, while no plants were included in the "dimnishing group" in both the derivatives. On the other hand, two modes, 2n=28 and 2n=42, appeared in the presence of euplasm (Fig. 3). The "increased group" in the presence of cytoplasm from either the eu- or *squarrosa* cytoplasm lines contained a larger number of aneuploids than the "diminishing group". A chromosome number of 2n=38 tended to occur in (*sqr*)Tve × Dur (Fig. 4) and there was a remarkable difference in the chromosomal distribution between these cytoplasmic progenies of pentaploids. In the case of the *squarrosa* cytoplasm progenies, many plants with larger chromosome numbers were observed in F_2. Selfed seeds were obtained only in the "increased group" in the presence of *squarrosa* cytoplasm. On the other hand, chromosomal distributions in advanced generations from euplasmic pentaploids followed those reported by KIHARA[5]. There was no difference between the progenies from (*sqr*)Tve × Tur and the corresponding euplasmic progenies of Tve × Tur having a trend towards 2n=42 through F_3~F_5, while there was a remarkable delay in the progenies of (*sqr*) Tve × Dur showing the mode on 2n=38 in F_5. The transmission of the D genome chromosomes in the presence of *squarrosa* cytoplasm was characterized by a

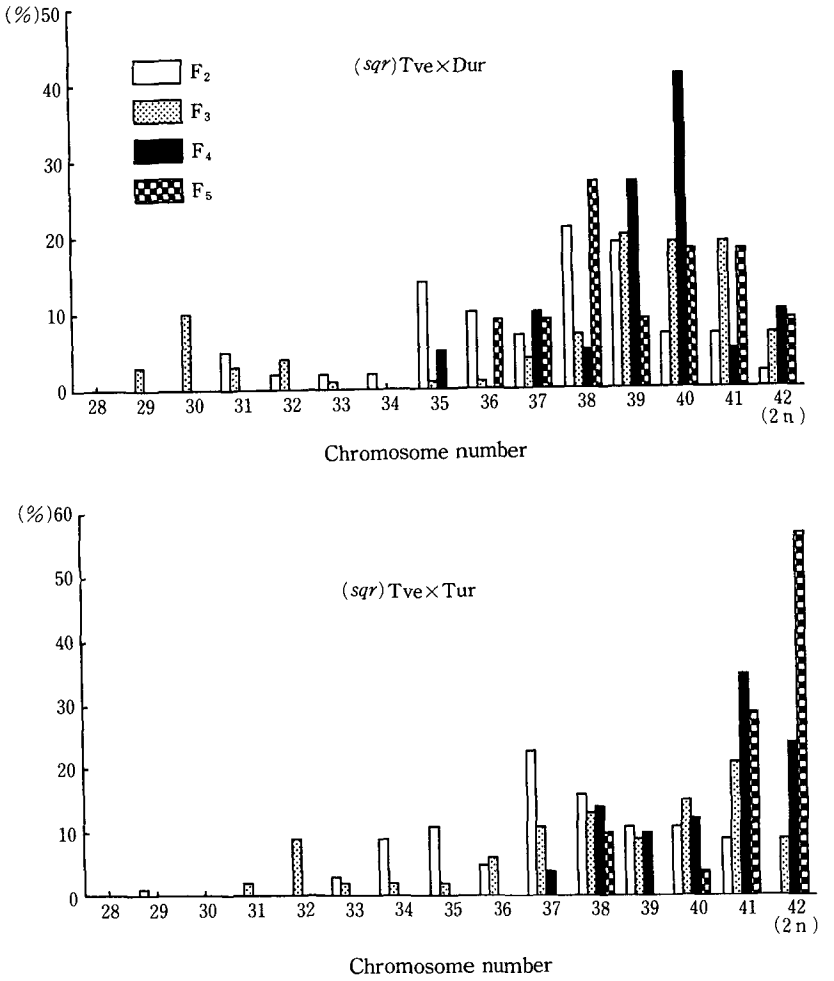

Fig. 4. Chromosome numbers in progenies of pentaploids (from F_2 to F_5) with *squarrosa* cytoplasm.

reduction of the "diminishing group" and low pollen fertility in the balanced combinations in F_2. As a result, no plants belonging to "diminishing group" were observed after F_3.

Discussion

A detailed study on pentaploid hybrids (AABBD) between Emmer and Dinkel wheats has been carried out following the report of KIHARA[5]. In the meiosis of the pentaploid hybrids, D genome chromosomes remained univalent (7I) and were distributed randomly at the anaphase of the second division. Theoretically, the chromosome number of the gametes followed the binomial distribution of $(0.5+0.5)^7$, but there were chromosome eliminations at the formation of male and

female gametes. In addition, the germination rate, pollen fertility and selfed seed set affected the chromosomal distribution in the progenies of the pentaploids. As for the chromosomal analysis in the progenies of pentaploid hybrids produced in this study, both certation and equational crossings were carried out to examine the transmission of the D genome chromosomes through both male and female gametes of pentaploid hybrids.

As for the production of alloplasmic wheat with the cytoplasm of *Ae. squarrosa,* KIHARA and OHTSUKA[13] first produced hybrids between a synthesized tetraploid plant of *Ae. squarrosa* (DDDD) and tetraploid Emmer wheat, and the hybrids were backcrossed into Dinkel wheats successively. Later, it was found that one specific chromosome of the D genome was indispensable for producing the fertile pollens and for the viability of seeds. This chromosome was identified as the 1D chromosome of *Ae. squarrosa* by chromosomal analysis using the nulli-tetra series of Chinese Spring[23].

In this study, two kinds of pentaploid hybrids from different cross combinations were produced in the presence of eu- or *squarrosa* cytoplasm. They were used for both certation and equational crossings.

The frequencies of fertilized male gametes were estimated from certation crosses. "J" type curves were obtained in all the crosses with the highest peak at $n=21$ irrespective of the difference of cytoplasm. However, $n=14$ gametes did not appear in the pentaploid hybrids with *squarrosa* cytoplasm and higher frequencies of $n=21$ gametes were observed instead.

In the equational crosses, there were some differences in the chromosomal distribution in the progenies between the pentaploids with euplasm or *squarrosa* cytoplasm. However, the chromosomal distribution under (*squarrosa*) cytoplasm rather fitted to the binomial distribution of $(0.5+0.5)^7$. Thus the results supported the hypothesis that pollen lacking 1D was sterile, while fertility of eggs was not affected by the *squarrosa* cytoplasm.

The relationship between the pairing types, so-called "balanced" and "imbalanced" chromosome combinations, and selfed seed sets should be shown on the progenies from pentaploids with euplasm or *squarrosa* cytoplasm. However, F_3 plants of the "balanced combination" in the "diminishing group" were not obtained in the presence of *squarrosa* cytoplasm. It is suggested that the pollen fertility is always below 50% due to the presence of 1D in the "balanced combination" of the "diminishing group" with *squarrosa* cytoplasm, which resulted in no seed sets. According to KIHARA[5], the D genome chromosome combinations of male gametes ($n=14\sim21$) in pentaploids were 1, 7, 21, 35, 35, 21, 7 and 1 (128 in total) in the presence of euplasm. In contrast, the numbers of chromosome combinations of the fertile male gametes including the 1D chromosome were reduced as described before in the presence of *squarrosa* cytoplasm. Due to the decrease of fertile pollens with 1D in the presence of *squarrosa* cytoplasm or the absence of pollens having $n=14$ without 1D, the

pollens of n=21 were more frequently fertilized in the progenies. Accordingly, the number of plants belonging to the "increasing group" increased markedly after the F_2 generation in the presence of *squarrosa* cytoplasm. Plants belonging to the "diminishing group" (under 2n=34) disappeared before the F_4 or F_5 generation. In the "increasing group", there was no clear difference in the chromosomal increment between the pentaploid hybrids with eu- and *squarrosa* cytoplasm. As reported previously[5], the plants in the "increasing group" reached a stable chromosome number (2n=42) less rapidly than those (2n=28) of the "diminishing group". In the pentaploid hybrid with *squarrosa* cytoplasm, it was demonstrated that the 2n=28 plants did not appeare throughout the F_2 to F_5 generations and skewness to higher chromosome numbers was prominent in the later generations.

It is suggested that all this can be ascribed to the selective transmission of 1D in Dinkel wheat (Tve) in the presence of *squarrosa* cytoplasm. As already described, Emmer wheat with 1D chromosome derived from *Ae. squarrosa* (2n=28+1D) is viable and only the pollens with 1D are fertile in the presence of *squarrosa* cytoplasm. Therefore, the genes on 1D from *Ae. squarrosa* controlling the development of pollen, endosperm and viability of plants were the same as the genes on 1D of *T. aestivum* var. *erythrospermum*.

Chapter IV

General Discussion

According to KIHARA[8], Dinkel wheat (AABBDD) has appeared by the diploidization of the hybrids between cultivated Emmer wheat (AABB) as female and *Ae. squarrosa* (DD) as male. Therefore, it was disclosed that the cytoplasm of common wheat originated from Emmer wheat. KIHARA[14] developed nucleo-cytoplasmic hybrids with *squarrosa* cytoplasm and indicated the possibility to utilize it for wheat breeding. Thereafter, various kinds of nucleo-cytoplsmic hybrids were produced by combining several nuclear genotypes of common wheat with cytoplasms derived from various related species[20,28]. It was important to note that the nucleo-cytoplasmic hybrids could become sources of materials for the broadening of the genetic variability associated with the interaction between the nuclear genome and the cytoplasm[15,16].

As for the cytoplasm derived from Einkorn wheat, male sterile lines with the cytoplasm from *T. boeoticum* showed a markedly reduced plant vigor and complete pollen sterility. Since the A genome is involved in the nuclear constitution of common wheat, the cytoplasm from Einkorn wheat may be compatible with the nuclear genotype of common wheat. However, all the alloplasmic lines with Einkorn cytoplasm produced in this experiment showed complete male sterility, depression of growth and delayed heading.

Subsequently, the synthetic amphidiploid with the cytoplasm from *T. boeoticum* was crossed with Chinese Spring. In the progenies of the pentaploid hybrids with *boeoticum* cytoplasm, the chromosome numbers converged to hexaploidy (2n=42) in the SB_2F_2 plants. The hexaploid plants showed a regular chromosome pairing of 21II and normal pollen and seed setting. In addition, plant vigor was completely restored and there was a remarkable difference in the plant characters compared with the male sterile counterpart. In the course of investigations for the detection of fertility-restoring gene(s) among the Dinkel wheats, it was observed that two detection of *T. aestivum* var. *graecum* Körn showed a weak fertility restoration in the heterozygous genotype with *boeoticum* cytoplasm.

It is possible that in the course of evolution, the A genome chromosomes underwent a structural changes during the transfer of the genome from A^l (for Einkorn) to A^E or A^D (for Emmer or Dinkel wheat)[1,4,22]. In this study, test crosses by using a series of homoeologous chromosome substitution lines between A and D genomes revealed that there was a desynaptic 4A chromosome between A^l genome and A^E or A^D genome.

NC-hybrids with *squarrosa* cytoplasm show normal fertility and vigor if the D genome is present. Subsequently, it was demonstrated that one of the D genome chromosome (1D) had the fertility-restoring gene for the *squarrosa* cytoplasm. Therefore, the extra-chromosome 1D is essential for the viability of *T. durum* with *squarrosa* cytoplasm. Based on these relations, it is anticipated that the pentaploids from the crossings between *T. durum* and *T. aestivum* with *squarrosa* cytoplasm may show a different chromosomal distribution in their progenies in comparison with the corresponding euplasmic pentaploids. Assuming that the male gametes lacking 1D are not viable, it is likely that the transmission pattern of the D genome chromosomes in the male gametes from certation crosses could correspond to the calculated frequencies expected from the above mentioned assumption. Furthermore, there was a marked difference in the chromosomal convergence between the progenies derived from the pentaploids with euplasm and those with *squarrosa* cytoplasm. In the F_5 generation from the pentaploid hybrids with *squarrosa* cytoplasm, there were no plants in the "diminishing group", and a bias toward the chromosome numbers above 2n=38 was observed. It is concluded that NC-hybrids from various sources can be extensively used for cytoplasmic engineering of wheat.

Summary

1. Five kinds of cytoplasmic substitution lines were produced by recurrent backcrosses with Emmer wheat by using *T. boeoticum, T. monococcum* and *T. urartu* as initial female parents. All the five cytoplasmic substitution lines showed male sterility, depression of growth and delayed heading. In this experiment, a cytoplasmic substitution line with *T. urartu* cytoplasm was produced

for the first time and this line showed male sterility, depression of growth and delayed heading as well.

2. The cytoplasm of *T. boeoticum* was introduced into *T. aestivum* cv. Chinese Spring by successive backcrossings by using synthetic amphidiploid, (*boeoticum*) AADD and Chinese Spring. At the same time, the dominant fertility-restoring gene of *Rfboe*-1 was introduced into the cytoplasmic substitution line.

3. *Rfboe*-1 introduced from the synthetic amphidiploid was transmitted 1.5 times more frequently through male gametes in the heterozygotes. The hetero- and homozygotes for *Rfboe*-1 showed normal growth and fertility, while the homozygotes for *rfboe*-1 showed depressed growth and male sterility.

4. By monosomic analysis, it was determined that the fertility restoring gene of *Rfboe-1* for *boeoticum* cytoplasm was located on 7A chromosome derived from the synthesized tetraploid plants of (*boeoticum*)AADD.

5. Attempts were made to identify the fertility-restoring gene compatible with the *boeoticum* cytoplasm in 17 lines of Emmer wheats and 27 lines of Dinkel wheats. It was found that two lines of *T. aestivum* var. *graecum* had the weak fertility-restoring gene.

6. Desynapsis of A genome chromosomes was investigated among three different A genomes, namely A^I (Einkorn), A^E (Emmer) and A^D (Dinkel). The mode of chromosome pairings between A^I and A^D was 6II+9I in the hybrids with the genome constitution $A^IA^DB^D$, which was obtained by the crossing between a tetraploid plant ($A^DA^DB^DB^D$) extracted from the cv. Thatcher and Einkorn wheat (A^IA^I). It is well known that 2 to 3 bivalents are formed in the hybrid of $A^IA^DB^DD^D$. A pairing inhibitor, *Ph,* derived from the D genome may be responsible for the chromosome pairing between A^I and A^D. Further, a desynaptic pair of chromosomes between A^I and A^D genomes were identified as 4A by examining the pairing type in crossings with a series of homoeologous chromosome substitution lines between the A and D genomes.

7. The chromosome numbers in the derivatives of pentaploid hybrids with euplasm or *squarrosa* cytoplasm were investigated. The pollen fertility of the pentaploid hybrids with *squarrosa* cytoplasm was lower than 50%. A few plants belonging to the "diminishing group" (2n=34 or less) appeared and the rate of selfed seed sets was very low. These plants exibited a low pollen fertility due to the elimination of 1D chromosme. In the "increasing group", most of the plants in F_5 had high chromosome numbers near 2n=38. Based on the relation between the 1D chromosome and *squarrosa* cytoplasm, expected frequencies were calculated in the certation crosses. Although there were some discrepancies in the frequency of the n=21 gametes, the observed frequencies corresponded fairly well to the expected values by calculation. Thus the intimate relationships between *squarrosa* cytoplasm and 1D chromosome was demonstrated in the derivatives of pentaploid hybrids with *squarrosa* cytoplasm.

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Explanation of Plate

1. Spikes of synthesized AADD (KU211-1) (left) and Chinese Spring (right).
2. Plant types of male sterile (*boeoticum*) C. S. (left) and male fertile (*boeoticum*) C. S. (right).
3. Spikes of Chinese Spring (left), male fertile (*boeoticum*) C. S. (middle) and male sterile (*boeoticum*) C. S. (right).