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GENETIC DIFFERENTIATION IN WHEAT NUCLEAR GENOMES IN RELATION TO COMPATIBILITY WITH *AEGILOPS SQUARROSA* CYTOPLASM AND APPLICATION TO PHYLOGENY OF POLYPLOID WHEATS

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CONTENTS

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INTRODUCTION (Historical Review)

Phylogenetic classification of the species of wheat (genus *Triticum*) was first carried out by SCHULZ[94]. The genus was divided into three groups, i. e. Einkorn wheat (Einkorn-Reihe), Emmer wheat (Emmer-Reihe), and Dinkel wheat (Dinkel-Reihe) based mainly on morphological features. It was disclosed by SAKAMURA[90] and SAX[91] that the three groups consisted of diploid, tetraploid, and hexaploid species, whose haploid chromosome numbers are 7, 14, and 21, respectively, as demonstrates in the extensive cytological study carried out by KIHARA[20]. It was also found that the three groups are series of allopolyploids with AA, AABB, and AABBDD genomes, respectively, through the study on triploid and pentaploid hybrids using various combinations among the three groups[21].

Genome analysis was used to study the phylogenetic relationships among wheats[22], with not only the genus *Triticum*, but also the genus *Aegilops*, which consists of wild relatives of wheat. Based on the results of extensive studies conducted by KIHARA and his co-investigators[23,26,29,33,40,41,42,44,45,47,56,57], it was disclosed that ten kinds of genomes (basic genomes) had been differentiated for the diploid species, including Einkorn wheat with the AA genome, and that allopolyploid species (tetraploids and hexaploids) had evolved from the various combinations of the basic genome species. It was thought that nine diploid species of the genus *Aegilops* and Einkorn wheat species (diploid wheat) had differentiated from a common ancestor (U genome species), based on similarities at various levels among the ten basic genomes[24]. Homoeologous relationships among the seven pairs of chromosomes in each genome were partially confirmed by SEARS[95,96,97,98], based on the compensation ability among the chromosomes in the A, B, and D genomes of Dinkel wheat.

Since SCHULZ's taxonomical study, many explorations for wheats and their wild relatives have been carried out in relation to the origin of cultivated wheats and their areas of origin[5,14,48,92,117,122]. Because it was disclosed that *T. timopheevi*, discovered by ZHUKOVSKY[122] in Transcaucasia and initially regarded as an endemic species of Emmer wheat, did not have the AABB genome like Emmer wheat but had the AAGG genome, based on the genome analysis by LILIENFELD and KIHARA[56], Timopheevi wheat (Timopheevi-Reihe) was added to SCHULZ's three groups.

It was disclosed that the A genome, which is common to the four groups of

genus *Triticum,* and the B genome of Emmer and Dinkel wheats are closely related to the S genome of *Ae. speltoides,* which belongs to the section *Sitopsis* in the genus *Aegilops*[41]. It was also observed that the G genome of Timopheevi wheat is related to the S genome[49]. It was suggested that the D genome, which is the third genome of Dinkel wheat, was derived directly from *Ae. squarrosa*[27,72], as had since been proven experimentally[43,73]. BOWDEN[1] proposed that all the species of the genus *Aegilops* should be included in the genus *Triticum,* because it is unlikely that only the series with the A genome can be classified as genus *Triticum* and other series as genus *Aegilops*[100]. This proposal was supported by MORRIS and SEARS[74]. However, despite the fact that this concept is seemed appropriate by many wheat investigators, the two genera are separated, from the practical viewpoint[39,71].

The attempt to reveal the genetic differentiation of the cytoplasm in the *Triticum-Aegilops* group was initiated with KIHARA's study[30] on the nucleo-cytoplasmic hybrid (NC hybrid, alloplasmic line) obtained by crossing *Ae. caudata* (CC genome species) and *T. aestivum* (a species of Dinkel wheat) including reciprocal crossing, and subsequent successive backcrossings. Extensive investigations have since been carried out into the interaction between nuclei and cytoplasms, with the systematic breeding of alloplasmic lines combining nuclei of various wheats (Emmer and Dinkel wheats) and cytoplasms derived from *Aegilops* species, Einkorn wheat, and Timopheevi wheat[7,8,9,10,30,32,34,35,36,37,46,58,65,66,67,77,86,101,111,113,121].

It was thus observed that the genetic differentiation of the cytoplasm is closely related to the genome differentiation[59,60,61,62,63,112,114,115]. In other words, it was disclosed that the genetic differentiation of the cytoplasm corresponded to that of ten kinds of basic genomes for the diploid species in the *Triticum-Aegilops* group, and that the basic genotypes of the genome and cytoplasm of the diploid species were transmitted, even in the evolutionary pathways with subsequent formation of allopolyploids. This finding suggests that the evolution of the polyploid species was more recent than the differentiation of the diploid species (after the end of the last glacial epoch, MAC KEY[70]). Furthermore, the correspondence of the genetic differentiation of the cytoplasm with the genome differentiation suggests that the genetic differentiation of the cytoplasm plays an important role in species differentiation (genome differentiation) in diploid species[39].

The origin of cultivated wheats and their areas of origin have been intensively discussed[16,17,25,27,28,31,33,43,50,68,69,72,73,74,78,79,87,93,102], based on the aforementioned genetic relationships among wheats and their wild relatives, their geographical distributions[3,6,12,13,15,31,53,92,117,118,123], and archaeological evidences[4,11,13,89].

As a result, the phylogenetic relationships between cultivated wheats and their wild relatives have been clarified for the most part (KIHARA[38], SEARS[99]) as follows :

(1) The hexaploid species of Dinkel wheat (AABBDD), to which bread wheat (*T. aestivum,* the most important species of cultivated wheats in modern agriculture) belongs, originated through amphiploidization of the F_1 hybrid produced by crossing cultivated Emmer wheat (AABB) as the female parent (genome and cytoplasm donor) and *Ae. squarrosa* (DD) as the male parent.

(2) One of the tetraploid wheats of cultivated Emmer wheat (true Emmer ; *T. dicoccum,* macaroni wheat ; *T. durum,* etc.) was derived from *T. dicoccoides* (a wild species with the AABB genome), and the other tetraploid wheat of cultivated Timopheevi wheat (AAGG) (zanduri wheat ; *T. timopheevi*) from *T. araraticum* (a wild species with the AAGG genome).

(3) Wild species of Emmer and Timopheevi wheats are amphidiploids resulting from crossings between ancestral species supplying the second genome (B or G) (various species belonging to the section *Sitopsis* in the genus *Aegilops*) as female parents and wild species of Einkorn wheat (AA) (*T. boeoticum* or *T. urartu*) as male parents.

(4) The cultivated species of Einkorn wheat (*T. monococcum*) was derived from *T. boeoticum,* one of the wild species with the AA genome.

These are conclusions with which many wheat investigators agree in relation to the evolution of cultivated wheats.   However, the origin of cultivated wheat is still being debated.   As a whole, the situation remains as described by KIHARA and LILIENFELD[43] in 1949 : "The history of the development of soft wheat (Dinkel wheat) is so far not clear." and "For the ultimate solution we must continue more detailed studies on *Triticum* and *Aegilops* from various stand-points.".   This is mainly because Emmer wheat species as progenitor of Dinkel wheat AABB genome has not yet been identified.

In the history of wheat, Emmer wheat has been the major type of wheat that has been cultivated for many millennia (from the early Neolithic Age to the Roman Republic, HARLAN[11]).   This wheat is widely distributed in Europe, North Africa, Southwest Asia, Central Asia, and Western India[13], an area overlapping the entire zone of distribution of *Ae. squarrosa,* the progenitor of the D genome of Dinkel wheat.   Consequently, the entire zone of distribution of *Ae. squarrosa* can be regarded as the area of possible origin of Dinkel wheat[28] ; VAVILOV[117] however suggested that Afghanistan was the center of origin of Dinkel wheat, on the basis of the genetic diversity in bread wheat (*T. aestivum*) and club wheat (*T. compactum*) of the Dinkel wheat, whereas KIHARA[38] considered that Transcaucasia was the possible center of origin of Dinkel wheat on the basis of the genetic diversity in *Ae. squarrosa.*

Cultivated species of Emmer wheat exhibit a wide genetic diversity ; for example, many endemic species, which are classified as classic species, were cultivated[70].   Consequently, if the genetic relationship between Dinkel wheat and these endemic species of Emmer wheat were to be investigated further, a better understanding of the history of cultivated wheat, especially of the area and time

of origin of Dinkel wheat could be obtained.

The present study was initiated as a part of the Cooperative project on NC (Nucleo-cytoplasmic) Heterosis Breeding directed by Dr. H. KIHARA, Kihara Institute for Biological Research. In the course of this study, it was demonstrated that the *1D* chromosome of bread wheat (AABBDD), the first homoeologous group chromosome of the D genome, was indispensable for the compatibility with the cytoplasm of *Ae. squarrosa*. Furthermore, the tetraploid wheats were classified into three groups, based on the genetic differentiation of the cytoplasm compatibility. All of the Timopheevi wheats (AAGG) were completely compatible with the cytoplasm of *Ae. squarrosa*. On the other hand, the majority of the Emmer wheats (AABB) were totally incompatible, and some endemic species of Emmer wheat (AABB) were incompletely compatible. The AABB genome of Dinkel wheat (AABBDD) including bread wheat was also examined, and classified into two groups. Based on these findings, the phylogenetic relationships among wheat species, especially the evolutionary pathways of polyploid wheats and the area of origin of Dinkel wheat were explored.

CHAPTER I. Compatible relation between wheat nuclear genomes and *Aegilops squarrosa* cytoplasm

Introduction

Since KIHARA's pioneer study[30] on nucleo-cytoplasmic hybrids (NC hybrids, synonym with alloplasmic lines) in wheat (genus *Triticum*) and their wild relatives (genus *Aegilops*), extenive investigations have been conducted with regard to the interaction between genome and cytoplasm[7,32,34,37,51,58,65,66,67,111]. As a result, it has been demonstrated that the genetic differentiation of cytoplasms closely corresponds to the differentiation of genomes in the genus *Triticum* and *Aegilops*[59,112,114,115]. In addition, it has been reported that in the manifestation of genomes in the presence of alien cytoplasm, particular chromosomes or genes are indispensable for the survival of the alloplasmic lines[8,46,59,60,64,80,84,108].

The present study aimed at revealing the compatible relation between the wheat genomes and the cytoplasm from *Ae. squarrosa* (DD) in the alloplasmic lines of Dinkel (AABBDD), Emmer (AABB) or Timopheevi (AAGG) wheats with the *squarrosa* cytoplasm. Furthermore, a genetic analysis was performed to study the function of the D genome chromosome in the compatibility between wheat genomes and the *squarrosa* cytoplasm.

Materials and Methods

This experiment consists of a genetic analysis and cytological observations using a number of strains of *Triticum* and *Aegilops* species maintained at the

Table 1. Materials used in the experiments

Strain	Abbreviation	Genome formula
Cytoplasmic donor		
KU–29*, artificial autotetraploid of *Aegilops squarrosa* var. *typica*	*squarrosa*	DDDD
Nuclear donor		
Dinkel wheat		
Triticum aestivum var. *erythrospermum*	T v e	AABBDD
T. aestivum cv. Chinese Spring	C.S.	"
"　　　" 　Norin 26	Norin 26	"
"　　　" 　Selkirk	Selkirk	"
"　　　" 　Jones Fife	J.F.	"
"　　　" 　Bison	Bison	"
T. compactum var. *Humboldti*	*T. comp. 44*	"
T. spelta var. *duhomelianum*	*T. spelt. duh.*	"
"　strain Rumania	*T. spelt. Rum.*	"
T. macha var. *subletshchumicum*	*T. macha sub.*	"
T. aestivum strain P168	P168	AABBDD *+1C 1C* *−1D 1D*
"　　　" 　Salmon	Salmon	AABBDD *+1Rs 1Rs* *−1Bs 1Bs*
Emmer wheat		
T. turgidum var. *nigro–barbatum*	*T. turgidum*	AABB
T. durum var. *Reichenbachii*	*T. durum*	"
T. polonicum var. *vestitum*	*T. polonicum*	"
T. dicoccum var. *farrum* (Hokudai)	*T. dicoccum*	"
T. dicoccoides var. *spontaneo-nigrum*	*T. dicoccoides*	"
Timopheevi wheat		
T. timopheevi var. *typicum*	*T. timopheevi*	AAGG
T. araraticum var. *Thumaniani*	*T. araraticum*	"
Tester strain		
Nulli–tetrasomic lines of C.S.		
Nullisomic D – tetrasomic A series	Nulli D – tetra A	
Nullisomic D – tetrasomic B series	Nulli D – tetra B	

* Genetic stock number in Plant Germ–plasm Institute, Facul. Agr., Kyoto Univ.

Kihara Institute for Biological Research.

The strains of cytoplasmic and nuclear donors used for the breeding of the alloplasmic lines and the strains to identify the chromosomes are listed in Table 1.

The autotetraploid of *Ae. squarrosa* (KU-29*), used as the cytoplasmic donor in this study, was bred by treating *Ae. squarrosa* var. *typica* (KU 20-2*) with colchicine[52] and has been maintained at the Plant Germ-plasm Institute, Faculty of Agriculture, Kyoto University.

The hexaploid wheats used as the nuclear donors consisted of ten strains of Dinkel wheat and its chromosome substitution lines, P168 and Salmon, twelve strains in total.   P168 is a strain in which the *1D* chromosome in the AABBDD genome of *T. aestivum* var. *erythrospermum* was replaced by the *1C* chromosome (C–Sat–2) of *Ae. caudata* (CC)[30,75].   Salmon is a strain in which the short arm of the *1B* chromosome (*1Bs*) of Dinkel wheat was replaced by the short arm of the first homoeologous group chromosome (*1Rs*) of *Secale cereale* (RR)[109].

The tetraploid wheats used as the nuclear donors consisted of five species of Emmer wheat with the AABB genome and two species of Timopheevi wheat with the AAGG genome, seven strains in total.

The series of nulli-tetrasomics of *T. aestivum* cv. Chinese Spring used for the experiment to identify the chromosomes were bred by Prof. E. R. SEARS[98], the University of Missouri.   In this study, fourteen combinations of lines were used : nullisomics with regard to the D genome chromosomes and tetrasomics with regard to the corresponding homoeologous chromosome of the A or B genome.

The alloplasmic lines of wheats with the *squarrosa* cytoplasm were obtained by successive backcrossings between the autotetraploid of *Ae. squarrosa* as the first female parent (cytoplasmic donor) and Dinkel, Emmer, or Timopheevi wheats as the recurrent male parents (nuclear donors).   Since the nuclear donors were increased to twelve lines in Dinkel wheats, five lines in Emmer wheat, and two lines in Timopheevi wheats during successive backcrossings, the number of backcrossed generations (SB$_n$) differed in each alloplasmic line.   However, the number of backcrossed generations with respective genome components were the same : twelve backcrossings with the ABD genome pollen (hereinafter abbreviated as ×ABD^{12}) in the alloplasmic lines of Dinkel wheat ; ×AB^{13} in the alloplasmic lines of Emmer wheat ; ×AG^8 in the alloplasmic lines of Timopheevi wheat.

In this report the alloplasmic line is indicated by parenthesizing the species name of the cytoplasmic donor and putting the species and variety name (sometimes in abbreviation) of the nuclear donor after the parenthesis[110].   For example, (*squarrosa*) C. S. or (*sq*) C. S. represents the alloplasmic line of *T. aestivum* cv. Chinese Spring, having the cytoplasm of *Ae. squarrosa*.

With regard to each alloplasmic line with the *squarrosa* cytoplasm, the chromosome configuration, pollen fertility, selfed seed sets and crossed seed sets were studied in each generation of successive backcrossings.   The chromosome configuration was studied by observing the chromosomes prepared by the aceto-carmine squash method from metaphase I to anaphase II, the meiotic stages of pollen mother cells being fixed with Farmer's solution.   The pollens were observed by staining with a glycerine aceto-carmine solution containing potassium iodide (glycerin 4 : aceto-carmine 1, KI 5%).   A pollen with one vegetative nucleus, two wedge-shaped sperm nuclei (sperm cells) and cytoplasm which turned yellowish-brown through heating treatment was defined as a fertile pollen

(normal pollen) ; a pollen with less than three nuclei or one which did not show such coloration with iodide, even though it contained three nuclei, was defined as sterile.   Selfed seed fertility was expressed as the percentage of seed set of the first and second florets in the middle part of the spike, excluding three spikelets from the tip and two from the base ; two to four spikes of each plant were isolated in bags before flowering.

Results and Discussion

1. The alloplasmic lines with *squarrosa* cytoplasm and their fertility

a) Alloplasmic lines of Dinkel wheat

Using the F_1 offfspring with the genetic constitution of (*squarrosa*) ABDD obtained by crossing autotetraploid *Ae. squarrosa* (DDDD) as female parent and *T. polonicum* (AABB) as male parent, successive backcrossings were carried out with the ABD genome pollens of Dinkel wheats in each generation.   The male parent for the F_1 offspring was Chinese Spring, a cultivar of *T. aestivum*.   By increasing the number of lines of Dinkel wheat as male parents, twelve lines were used as the nuclear donors in the $\times ABD^4$ generation.   Successive backcrossings were continued in the subsequent generations, leading to the alloplasmic lines of Dinkel wheat with the *squarrosa* cytoplasm up to generation $\times ABD^{12}$ in 1980.

The selfed seed fertilities in the early generation of backcrosses are presented in Table 2.   In the $F_1 \times ABD^1$ generation, the anthers did not dehisce, indicating complete male sterility.   However, partial fertility appeared in as early a genera‐ tion as $\times ABD^2$, with all the successively backcrossed lines of Dinkel wheats restoring fertility without exception.   The chromosome configuration became $2n=42$, 21 II in the $\times ABD^6$ generation in all the lines, indicating that the cyto‐ plasm-genome constitution was (*squarrosa*) AABBDD.

Table 2.   Selfed seed fertility of alloplasmic lines of Dinkel wheat with *squarrosa* cytoplasm, in the early generation of backcrosses [1]

Generation	Nuclear donor											
	T v e	C.S.	Norin 26	J.F.	Selkirk	Bison	T.comp. 44	T.splt. duh.	T.splt. Rum.	T.macha	P168	Salmon
	%	%	%	%	%	%	%	%	%	%	%	%
F_1[2] $\times ABD^1$	----	0.0	----	----	----	----	----	----	----	----	----	----
$\times ABD^2$	51.1	72.3	----	60.7	----	----	----	67.1	----	----	----	----
$\times ABD^3$	48.8	75.5	----	92.2	----	93.4	----	68.9	73.1	----	75.0	87.7
$\times ABD^4$	47.5	90.2	95.6	84.3	93.4	85.7	88.5	44.1	65.1	77.0	70.7	76.2
$\times ABD^5$	91.0	98.2	99.2	91.0	83.9	94.9	89.8	33.6	86.7	45.5	91.8	75.8
$\times ABD^6$	94.4	99.0	99.5	93.6	95.0	82.7	88.8	78.1	92.4	78.9	88.1	91.7

1) The first figures in each column indicate the fertility of the first generation crossed with respective nuclear donors.

2) F_1 : *Ae. squarrosa 4x* (DDDD) × *T. polonicum* (AABB).

Table 3. Fertility of alloplasmic lines of Dinkel wheat with *squarrosa* cytoplasm

Line	Pedigree[1]	Seed set in backcross	Selfed seed fertility (mean±sd.)	Selfed seed fertility of last nuclear donor (mean±sd.)
		%	%	%
*(sq)*Tve	F₁[2]/C.S./2*T v e/P168//5*T v e	97.5	88.6±2.8	92.7±2.6
(*"*)C.S.	*"* //9*C.S.	93.9	98.0±1.7	96.0±4.0
(*"*)Norin 26	*"* /2*C.S./Salmon//6*Norin 26	97.5	95.6±5.7	97.5±1.8
(*"*)Selkirk	*"* /C.S./2*J.F.//6*Selkirk	88.9	94.4±4.8	90.1±2.7
(*"*)J.F.	*"* /C.S.//8*J.F.	84.6	94.7±3.0	88.9±6.1
(*"*)Bison	*"* /C.S./J.F.//7*Bison	88.5	85.3±1.5	96.3±0.6
(*"*)T.comp.44	*"* /C.S./2*J.F.//6*T.comp.44	96.3	93.7±7.5	93.7±0.7
(*"*)T.spelta duh.	*"* /C.S.//8*T.spelta duh.	87.3	84.0±5.8	91.8±4.0
(*"*)T.spelta Rum.	*"* /C.S./T.spelta duh.//7*T.spelta Rum.	91.3	91.2±6.5	90.2±4.8
(*"*)T.macha	*"* /C.S./T.spelta/2*T.spelta//5*T.macha duh. Rum.	90.8	89.8±3.7	93.4±3.2
(*"*)P168	*"* /C.S./T v e//7*P168	93.4	96.8±2.8	94.5±4.4
(*"*)Salmon	*"* /2*C.S.//7*Salmon	97.2	94.6±1.9	90.9±1.7

1) Pedigree of each line in the 9th backcross generation with AABBDD genome species.

2) F₁ : *Ae. squarrosa 4x* (DDDD) × *T. polonicum* (AABB).

* Number of backcrossings with respective nuclear donor.

In Table 3, the fertilities of the alloplasmic lines of Dinkel wheat with the *squarrosa* cytoplasm are presented as the means of selfed seed fertilities and crossed seed sets of backcrosses in the $\times ABD^7$, $\times ABD^8$ and $\times ABD^9$ generations. The selfed seed fertility remained high throughout the generations in all the lines, with no significant difference in comparison with the nuclear donors. The crossed seed sets (seed sets in backcrosses) also showed a high percentage, the mean of all the lines being 92.3% (±4.4%). No abnormality was found in female fertility.

The male fertility also was complete in all the alloplasmic lines of Dinkel wheat with the *squarrosa* cytoplasm, including that of the chromosome substitution lines, P168 and Salmon. No noteworthy changes, either morphological or physiological, were observed in any of the combinations between the nucleus of Dinkel wheat and cytoplasm from *Ae. squarrosa*.

b) Alloplasmic lines of Emmer wheat

The breeding process for the alloplasmic lines of five Emmer wheat species (AABB) with the *squarrosa* cytoplasm is shown in Fig. 1. In the successive backcrosses whose first female parent was the autotetraploid of *Ae. squarrosa* and whose recurrent male parents were Emmer wheats, the crossed seed sets were low and plant growth was poor in the early generations. Therefore, it was impossible to continue crossing using the same species of Emmer wheat as recurrent male parent for early generations. In the $\times AB^4$ generation, the chromosome configuration of two plants was 2n=30, 14 II+2 I, showing semi-normal growth and complete male sterility. One of these two was crossed with pollens

Ae. squarrosa 4x   *T. polonicum* (Emmer wheat)

  (squarrosa)-DDDD   ×   *(emmer)*-AABB

                                    |

                          F_1

        (squarrosa)-ABDD   × AB^4

                                |

                                |

        (squarrosa)-AABB +*d' d'*   × AB^8

                $(14_{II} +2_{I})$ (successive backcrossings with
                                             five species of Emmer wheat)

                                            |

                                            |

                                            |

                (squarrosa)-AABB +*dx*

                    $(14_{II} +1_{I})$

                (five lines of alloplasmic Emmer wheat
                having *squarrosa* cytoplasm
                and one chromosome of D genome)
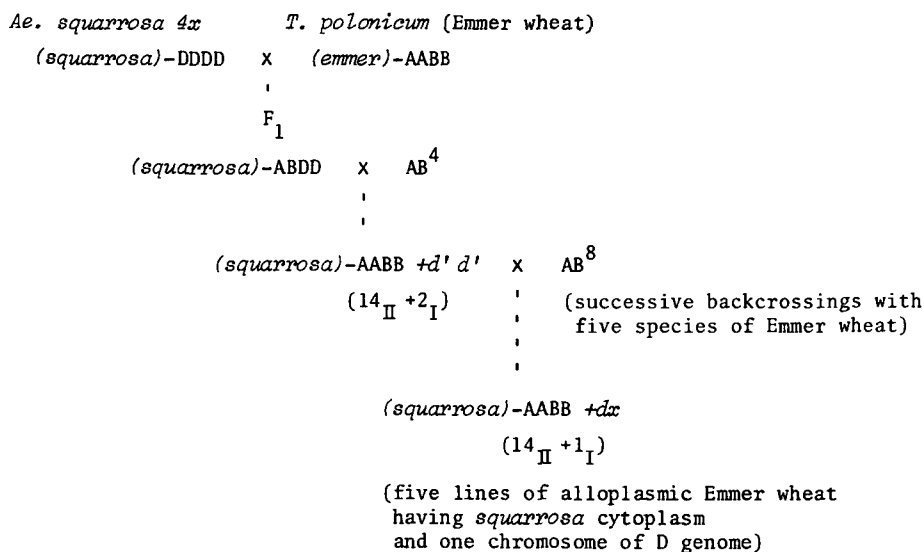
Fig. 1. Breeding of alloplasmic lines of Emmer wheat.
       (Note) Figures at upper right of genome formula indicate the number of
               backcrosses with each male parent.

of five species of Emmer wheat.   This procedure permitted the continuation of
the successive backcrossings of each line.   In the $\times AB^5$ generation, forty-eight
plants were produced out of all the backcrossed lines.   Ten out of the forty-eight
showed the same chromosome configuration : 2n=29, 14 II+1 I.   In the $\times AB^6$
generation, all forty plants investigated also retained an additional chromosome
in the configuration of 2n=29, 14 II+1 I.   Since all the backcrossings were
continued with the pollens of Emmer wheat having the AB genome, the 14
bivalents were considered to have resulted from the pairing with the chromo-
somes of the AABB genome, and the univalent was estimated to be a D genome
chromosome derived from the *Ae. squarrosa* used as the female parent in the
initial cross, the cytoplasmic donor.   This additional chromosome is denoted as
dx chromosome in this paper.

    Table 4 presents the results of the investigations of the chromosome configu-
ration in the generations from $\times AB^8$ to $\times AB^{13}$ in the alloplasmic lines of Emmer
wheat with the *squarrosa* cytoplasm.   Despite successive backcrossings with the
AB genome pollens of Emmer wheat in every generation, all the plants retained
the chromosome configuration of 14 II+1 I with *dx* chromosome.

    The pollen fertility and selfed seed fertility of each alloplasmic line of Emmer
wheat with the *squarrosa* cytoplasm and the seed sets obtained by backcrossings
are shown in Table 5.   It should be noted that a large number of abortive seeds
were produced by crossing with the AB genome pollens of Emmer wheat in all the
lines.   Although all the lines developed anthers that dehisced normally, the pollen
fertility was partial and varied with the lines, the mean being about 25%.   The

Table 4. Selective transmission of an additional chromosome (*dx*) in backcross generations of alloplasmic lines of Emmer wheat with *squarrosa* cytoplasm

Nuclear donor	Chromosome configuration in each generation					
	×AB⁸ ('75)		×AB⁹ ('76)		×AB¹⁰ – ×AB¹³	
	14 II	14 II + 1 I	14 II	14 II + 1 I	14 II	14 II + 1 I
T. turgidum var. *nigro–barbatum*	0	43	0	50	0	37
T. durum var. *Reichenbachii*	0	67	0	70	0	28
T. polonicum var. *vestitum*	0	17	0	38	0	27
T. dicoccum var. *farrum* (Hokudai)	0	17	0	20	0	3
T. dicoccoides var. *spontaneo–nigrum*	0	22	0	3	0	5

Table 5. Male and female fertilities of alloplasmic lines of Emmer wheat[1] with *squarrosa* cytoplasm

Nuclear donor	Control		*squarrosa* cytoplasm			
	Pollen fertility	Selfed seed fertility (mean±sd.)	SB generation[2]	Pollen fertility	Seed set in backcross[3] (mean±sd.)	Selfed seed fertility (mean±sd.)
	%	%		%	%	%
T. turgidum var. *nigro–barbatum*	98.6	98.3±2.3	SB₇	39.1	21.9±4.6	25.2±35.1
T. durum var. *Reichenbachii*	95.0	98.5±2.9	SB₇	23.7	10.5±1.5	3.8±4.0
T. polonicum var. *vestitum*	97.2	90.5±5.9	SB₆	28.9	24.3±6.8	0.0±0.0
T. dicoccum var. *farrum* (Hokudai)	96.6	86.5±5.3	SB₆	17.5	16.6±4.6	0.1±0.1
T. dicoccoides var. *spontaneo–nigrum*	95.7	81.0±8.6	SB₇	13.6	8.7±1.1	1.7±1.1

1) *(squarrosa)*AABB + *dx* lines.

2) Substitution backcross generation of each line in the ×AB¹³ generation.

3) Normal seeds were counted in the calculation of seed set percentage.

percentage of normal seeds produced by backcrossings also varied with the lines, the mean being 24% at maximum in any case. The chromosome configuration of the immediate offspring of the normal seeds was 2n=29, 14 II+1 I in all the alloplasmic lines of Emmer wheat, indicating a genetic constitution of (*squarrosa*) AABB+ *dx*. The selfed seed fertility was almost completely lost in all the lines except for that whose nuclear donor was *T. turgidum*. In the (*squarrosa*) *T. turgidum* + *dx* line, the selfed seed set (selfed seed fertility) largely fluctuated among individuals, spikes and years investigated (0~60%). All the lines were free of distinctive morphological abnormalities, except that the plant height was slightly reduced compared with that of the nuclear donor plants.

Successive backcrossings using five species of Emmer wheat as pollen donors resulted in the same genetic constitution of (*squarrosa*) AABB+ *dx,* indicating that the continuation of backcrossing was not capable of producing the (*squarrosa*)

AABB.   The fact that the pollen fertility of these backcrossed lines was incomplete and that a large number of abortive seeds were segregated by backcrossing in all the generations suggests that there was no genetic compatibility between the AB genome of those five species of Emmer wheat and the cytoplasm of *Ae. squarrosa*.

c) Alloplasmic lines of Timopheevi wheat

The breeding scheme of the alloplasmic lines with the *squarrosa* cytoplasm whose nuclear donors were two species (*T. timopheevi* and *T. araraticum*) of Timopheevi wheat (AAGG) is shown in Fig. 2.   In this scheme, in contrast to the alloplasmic lines of Emmer wheat (AABB), the preferential retention of the dx chromosome was not observed and all the plants had the chromosome configuration $2n=28$, 14 II in the $\times AG^4$ generation ; i. e., the alloplasmic lines with the genetic constitution (*squarrosa*) AAGG were established.

The pollen fertility, selfed seed fertility and seed sets in backcrosses of the alloplasmic lines of two species of Timopheevi wheat with the *squarrosa* cytoplasm are presented in Table 6.   In the backcrossed seed sets, no abortive seeds appeared in any generation of these lines.   In the line with *T. timopheevi* as nuclear donor the fertilities were practically normal in males and females ; however, in the line whose nuclear donor was *T. araraticum* the mean selfed seed fertility was 38.7% ($\pm 21.2\%$), which was incomplete and unstable, presumably because the anthers were partially degenerated.   The pollen fertility of anthers that normally developed and had dehisced was 93.7% in the line whose nuclear donor was *T. araraticum*.

```
Ae. squarrosa 4x        T. polonicum (Emmer wheat)

  (squarrosa)-DDDD    x    (emmer)-AABB
                      ¦
                      F
                       1
       (squarrosa)-ABDD    x    AB⁴
                          ¦
                          ¦
             (squarrosa)-AABB +d' d'   x    AG⁸
                    (14    +2  )        ¦    (successive backcrossings with
                        II    I         ¦      two species of Timopheevi wheat)
                                        ¦
                                        ¦
                  (squarrosa)-AAGG
                        (14   )
                           II
               (two lines of alloplasmic Timopheevi wheat
                having squarrosa cytoplasm)
```
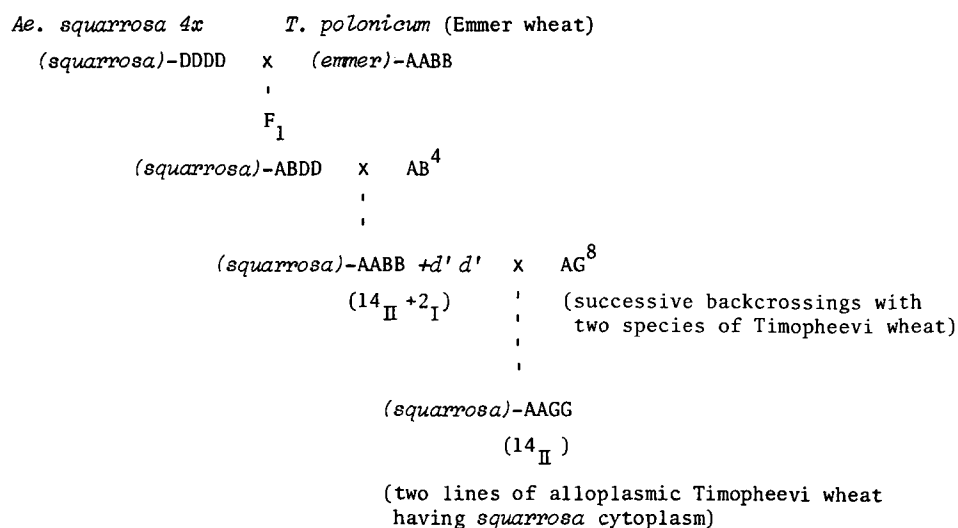
Fig. 2.   Breeding of alloplasmic lines of Timopheevi wheat.
        (Note)   Figures at upper right of genome formula indicate the number of
                backcrosses with each male parent.

Table 6. Male and female fertilities of alloplasmic lines of Timopheevi wheat
with *squarrosa* cytoplasm

Nuclear donor	Control		*squarrosa* cytoplasm			
	Pollen fertility	Selfed seed fertility (mean ± sd.)	SB generation[1]	Pollen fertility	Seed set in backcross (mean ± sd.)	Selfed seed fertility (mean ± sd.)
	%	%		%	%	%
T. timopheevi var. *typicum*	97.5	96.0±4.2	SB₃	86.5	84.1±1.1	83.3±9.7
T. araraticum var. *Thumaniani*	98.7	87.0±9.7	SB₇	93.7	63.8±5.5	38.7±21.2

1) Substitution backcross generation of each line in the ×AG[8] generation.

From these findings it can be deduced that the AG genome of Timopheevi
wheat is compatible with the *squarrosa* cytoplasm.

2. Selective transmission of an additional chromosome (dx)
in the alloplasmic lines of Emmer wheat with *squarrosa* cytoplasm

a) Zygotic lethality caused by the absence of dx chromosome

In the alloplasmic lines of five species of Emmer wheat with the cytoplasm of
Ae. squarrosa, all the plants obtained by successive backcrossings displayed the
same genetic constitution of (*squarrosa*) AABB+ *dx*. Therefore the presence of
the extra chromosome (*dx*),
derived from *Ae. squarrosa*,
was thought to be essential
for the survival of the allo-
plasmic lines.

The resulting seed sets
when lines with the above
genetic constitution were
crossed with the AB
genome pollens of Emmer
wheats are presented in
Table 7. In comparison
with the results of self-pol-
lination of the control
plants, i. e. the nuclear
donors, the alloplasmic
lines with the *squarrosa*
cytoplasm markedly differ-
ed in that they produced a
large number of abortive
seeds as shown in Fig. 3.
In such abortive seeds, the
seed coats were fully de-

normal   abortive   normal   abortive
seed   seed   seed   seed

AABB +1D   AABB   AABB +1D   AABB
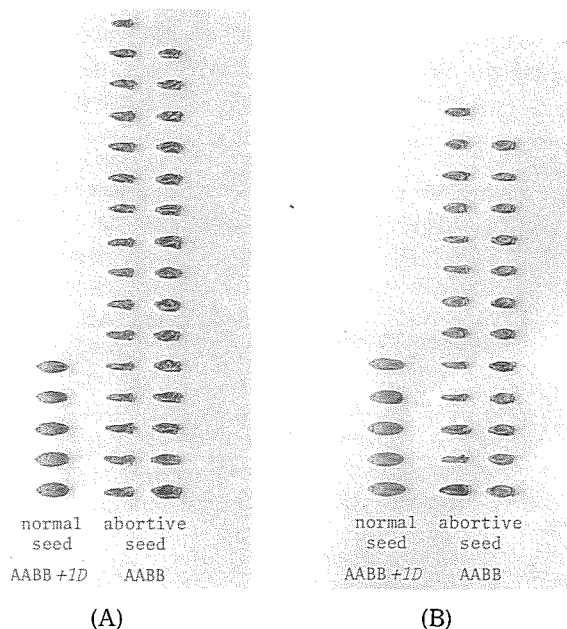
(A)   (B)

Fig. 3. Abortive seeds in (*squarrosa*)AABB+ *dx* lines
crossed with AB genome pollen of Emmer
wheat.
(A) : (*squarrosa*) *T. turgidum* + *dx* × *T. turgidum*.
(B) : (*squarrosa*) *T. durum* + *dx* × *T. durum*.

Table 7. Abortive seed sets in alloplasmic lines of Emmer wheat with *squarrosa* cytoplasm backcrossed with AB genome pollen

Line	No.of crossed florets	Seed morphology		Percentage of abortive seeds
		Normal	Abortive	
(*squarrosa*)*T. turgidum* + *dx*	1,002	233	535	69.7%
(*"*)*T. durum* + *dx*	1,580	181	1,046	85.2
(*"*)*T. polonicum* + *dx*	928	227	351	60.7
(*"*)*T. dicoccum* + *dx*	248	41	66	61.7
(*"*)*T. dicoccoides* + *dx*	424	35	220	86.3

veloped, whereas the embryos and endosperms degenerated and completely lost their germination potency.   The mean rate of abortive seeds in the backcrossed seed sets was about 73%, though it varied with the lines.   Plants from normal seeds showed the chromosome configuration of 14 II+1 I at metaphase I in the meiosis.   Therefore, all these plants, which were obtained in the generation following backcrosses, exhibited the genetic constitution (*squarrosa*) AABB+ *dx*, no individual displaying the 14 II chromosome configuration, i. e. the genetic constitution of (*squarrosa*) AABB.

The chromosomes at metaphase I and anaphase I in the meiosis of the alloplasmic lines with the *squarrosa* cytoplasm whose nuclear donors were *T.*

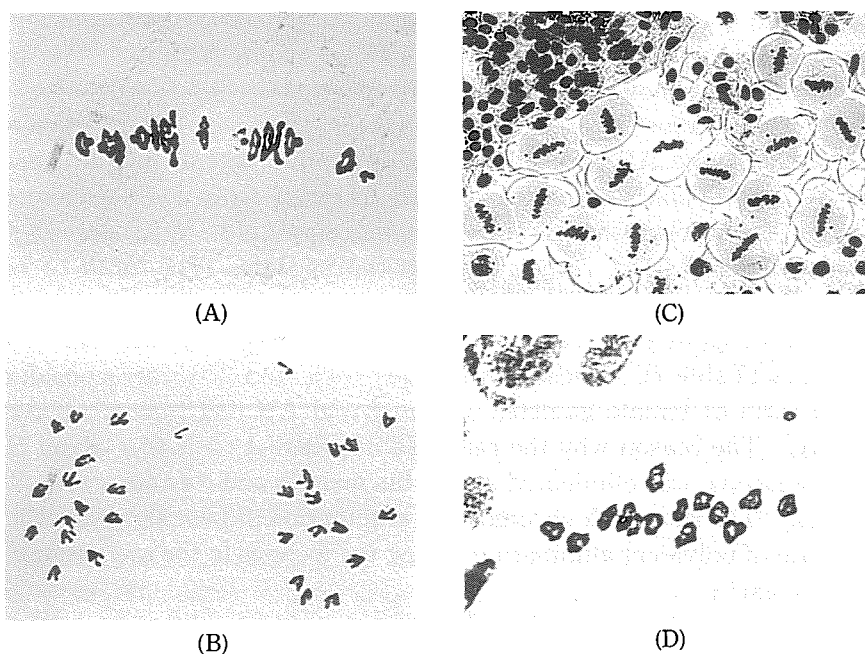(A)

(C)

(B)

(D)

Fig. 4. The *dx* chromosome observed in PMC's of (*squarrosa*)AABB+ *dx* lines.
(A) metaphase I and (B) anaphase I of (*squarrosa*) *T. turgidum* + *dx* line.
(C) and (D) metaphase I of (*squarrosa*) *T. durum* + *dx* line.

Table 8. Seed-sets of *(squarrosa)*AABB + *dx* lines, when crossed with AG genome pollens of Timopheevi wheat

Female line	Crossed with *T. timopheevi*			Crossed with *T. araraticum*		
	No. of crossed florets	Normal seeds	Abortive seeds	No. of crossed florets	Normal seeds	Abortive seeds
(squarrosa)T. turgidum + dx	50	46	0	182	144	0
(")*T. durum + dx*	30	24	0	150	125	1
(")*T. polonicum + dx*	--	--	--	56	45	0
Total	80	70	0	388	314	1

turgidum and *T. durum* are shown in Fig. 4. Although the extra chromosome (*dx* chromosome) occurring in the alloplasmic line of *T. turgidum* was a usual univalent, that of the line whose nuclear donor was *T. durum* showed a small ring-shape at metaphase I (and split lengthwise into two bi-arm sister chromatids at anaphase I). Therefore it is possible that the *dx* chromosome in the alloplasmic line of *T. durum* become an isochromosome.

Table 8 shows the seed sets obtained when *(squarrosa)* AABB + *dx* lines were crossed with the AG genome pollens of two species of Timopheevi wheat. In this case, in contrast to the crossing with the AB genome pollens of Emmer wheat (Table 7), abortive seeds rarely occurred, and the mean of the crossed seed sets was 81%. It was assumed that the genetic constitution of the female gametes in the alloplasmic lines of Emmer wheat used as the female parents consisted of a majority of *(squarrosa)* AB and minority of *(squarrosa)* AB + *dx*. The present crossing experiment showed that both were functional.

Therefore, the abortive seeds, accounting for a mean of 73% of the seed sets backcrossed with the AB genome pollens of Emmer wheat, in the *(squarrosa)* AABB + *dx* lines (Table 7) were considered to result from the degeneration of embryos and endosperms that took the genetic constitution of *(squarrosa)* AABB and *(squarrosa)* AAABBB, respectively, in the zygotes. The ratio of normal seeds in the backcrosses represents the rate of transmission of the *dx* chromosome from the female gamete to the zygote. The mean was 27%, although it varied with the lines (Table 7). Those percentages (73% and 27%) are considered to reflect the ratio of female gametes of *(squarrosa)* AB and *(squarrosa)* AB + *dx*, respectively. The reason why the ratio did not fit to 1 : 1 is that about 50% of the *dx* chromosome was eliminated during the meiosis and was not transmitted to the gametes. The rate of *dx* chromosome elimination did not significantly differ from the rate of univalent elimination during the meiosis in the monosomic series of Dinkel wheat[97].

These results indicate that the selective transmission of the *dx* chromosome during successive backcrossings in the alloplasmic lines of Emmer wheat having the *squarrosa* cytoplasm was not caused by the selective sterility of the female gametes but by the lethality of the zygotes into which the *dx* chromosome was not

transmitted through fertilization.

b) Gametophytic sterility in pollen grains without *dx* chromosome

In any alloplasmic line of Emmer wheat with the genetic constitution (*squarrosa*) AABB+ *dx,* the anthers normally dehisced except in terminal florets. As for the pollen fertility, all lines showed partial sterility (Table 5).

The pollen grains of the alloplasmic lines of Emmer wheat with the *squarrosa* cytoplasm and control lines, euplasmic Emmer wheat, are shown in Fig. 5. A normal mature pollen grain of wheat has one vegetative nucleus and two wedge-shaped sperm nuclei, filled with starch grains. Therefore, staining with an aceto-carmine solution containing 5% potassium iodide enables to reveal the three nuclei in a dense hue if the pollen grain is normal, along with the coloration of the cytoplasm due to iodine. Although sterile pollen grains occurred in euplasmic Emmer wheat as well, the occurrence rate was very low. Since the diameter of the sterile pollen grains was small in most cases, the cytoplasm degenerated with only one or two nuclei. On the other hand, most of the sterile pollen grains of the alloplasmic lines of Emmer wheat with the genetic constitution (*squarrosa*) AABB+ *dx* showed three developed nuclei, apparently differing from normal pollen grains in that the cytoplasm did not show the iodo-starch reaction.
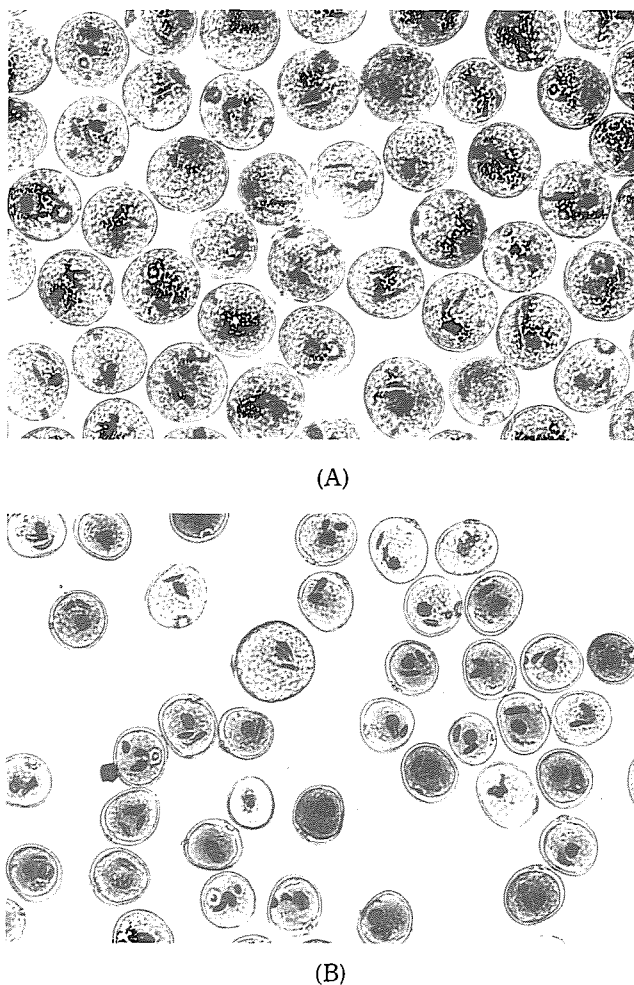
Table 9 presents the results of observations of the normal and sterile pollen

(A)

(B)

Fig. 5. Pollen grains of *T. durum* var. *Reichenbachii* (A) and (*squarrosa*) *T. durum* var. *Reichenbachii*+ *dx* (B) lines.

Table 9. Types of pollen grains in alloplasmic lines of Emmer wheat*
with *squarrosa* cytoplasm

Nuclear donor	Control				*squarrosa* cytoplasm			
	Normal grains	Abortive grains		Percentage of abortive grains (mean±sd.)	Normal grains	Abortive grains		Percentage of abortive grains (mean±sd.)
		3 nuclei	2 nuclei or less			3 nuclei	2 nuclei or less	
				%				%
T. turgidum var. nigro-barbatum	3,589	10	66	2.0±0.2	1,549	1,864	543	60.9±0.7
T. durum var. Reichenbachii	3,973	60	152	5.1±1.1	2,492	7,541	440	76.4±1.0
T. polonicum var. vestitum	2,682	5	74	2.8±1.6	1,393	2,379	1,107	71.1±1.4
T. dicoccum var. farrum (Hokudai)	2,773	25	72	3.4±0.3	798	3,148	607	82.5±0.6
T. dicoccoides var. spontaneo-nigrum	2,737	14	108	4.6±1.3	1,187	6,681	807	86.4±1.2

* *(squarrosa)*AABB + *dx* lines

Table 10. Transmission of an additional chromosome (*dx*) from pollen grains of
(squarrosa) AABB + *dx* lines

Crossing	Chromosome configuration of F_1 plants			
	14 II	14 II + 1 I	15 II	dwarf and dead before heading
T. turgidum × (squarrosa)T. turgidum + dx	0	26	0	0
(squarrosa)T. turgidum + dx × (squarrosa)T. turgidum + dx	0	39	7	18
T. durum × (squarrosa)T. durum + dx	0	46	0	0
(squarrosa)T. durum + dx × (squarrosa)T. durum + dx	0	41	3	4

grains in the alloplasmic lines of (*squarrosa*) AABB+ *dx* and control euplasmic Emmer wheat. In the latter the pollen fertilities were constantly high, sterile pollen grains occurring at a rate of 2~5%. The occurrence of sterile pollen grains in the alloplasmic lines was comparatively high, about 75% as a mean, though the differences between lines were not negligible. The pollen fertility within each alloplasmic line was constant, and free of large variation among individuals and spikes. This fact suggests that the sterile pollen grains in the alloplasmic lines resulted from gametophytic sterility due to a certain genetic constitution of the male gametophytes (pollen grains) themselves.

Table 10 presents the results of the investigation of *dx* chromosome transmission from the pollens of the alloplasmic lines of (*squarrosa*) AABB+ *dx*. In the crossings in which the euplasmic lines of Emmer wheat, (*emmer*) AABB, were used as the female parents, all the 72 offsprings in the subsequent generation, when the two lines were joined, exhibited the chromosome configuration of 14 II+ 1 I. This result indicates that all the pollen grains involved in the fertilization of

the alloplasmic lines of Emmer wheat had a genetic constitution of (*squarrosa*) AB + *dx*, i. e., that the pollen grains selectively transmitted the *dx* chromosome. When the alloplasmic lines of Emmer wheat were selfed, i. e., the (*squarrosa*) AABB + *dx* plants crossed with the pollens of (*squarrosa*) AABB + *dx* plants, no abortive seed was produced.   This is additional evidence which suggests that the *dx* chromosome was transmitted selectively from the pollens of (*squarrosa*) AABB + *dx* plants to all the zygotes.   In the generation following such selfing, 22 individuals out of the 112 offsprings, puting the two lines together, were dwarf. Furthermore, most of the 22 dwarf plants died without heading, thus making it impossible to investigate the meiosis in their pollen mother cells.   Nevertheless, the investigation of ten dwarf plants revealed the 15 II chromosome configuration.   However, since they were sterile, no subsequent generation was obtained. This phenomenon is considered to be related to dwarfism and the rosette character caused by the 15 II plants in wheat, the product of KIHARA's "sterile combination"[21].   The other normal 80 individuals exhibited the 14 II + 1 I chromosome configuration.   These results support the conclusion that no male gametophytes except those having the genetic constitution (*squarrosa*) AB + *dx* possessed the fertilization potency and gave rise to 14 II + 1 I plants through fertilization with (*squarrosa*) AB female gametes.   And dwarf offsprings were produced by the 15 II chromosome configuration as a result of fertilization with (*squarrosa*) AB + *dx* female gametes.

These data suggest that the gametophytic sterility in the males was selective, in that the microspores of (*squarrosa*) AB + *dx* alone developed into normal pollens in (*squarrosa*) AABB + *dx* lines, and those of (*squarrosa*) AB became sterile pollens. The rate of transmission of the *dx* chromosome to microspores of the (*squarrosa*) AABB + *dx* lines was directly reflected in the pollen fertility of their alloplasmic lines, i. e. about 25% as a mean, though it varied with the lines (Table 9).

c) Selective transmission of *dx* chromosome indispensable for the compatibility with *squarrosa* cytoplasm

In the lines of (*squarrosa*) AABB + *dx*, sterility or lethality occurred in the male gametophytes or in the zygotes to which the *dx* chromosome was not transmitted.   Such sterility or lethality resulted in the selective transmission of the *dx* chromosome to subsequent progenies through male or female gametes. Hence a further experiment was conducted to confirm whether such selective sterility or lethality occurred only in the cytoplasm of *Ae. squarrosa.*

Table 11 shows the pollen fertility, selfed seed fertility and crossed seed sets with AB genome pollens in the F_1 line of (*emmer*) AABB + *dx* obtained by crossing euplasmic *T. durum* with the pollens of the (*squarrosa*) *T. durum* + *dx* line, together with the data of the (*squarrosa*) AABB + *dx* line of *T. durum* and of the (*emmer*) AABB line of euplasmic *T. durum* (control).   As seen in the table, the selfed seed fertility of the *T. durum* + *dx*, (*emmer*) AABB + *dx* line, was

Table 11. Pollen fertility and selfed and crossed seed sets of *T. durum* with an additional chromosome (*dx*) with either its own or *squarrosa* cytoplasm

Line	Pollen fertility	Percentage of selfed seed sets (mean±sd.)	Crossed seed sets with *T. durum*			
			No. of crossed florets	Normal seeds	Abortive seeds	Percentage of normal seeds
	%	%				%
T. durum (pure line)	95.7	98.1±1.6	480	471	0	100.0
T. durum + *dx*	92.3	81.6±11.7	222	193	3	98.5
(*squarrosa*)*T. durum* + *dx*	20.9	0.7±1.9	224	23	159	12.6

slightly lower than that of the control *T. durum,* i. e. (*emmer*) AABB ; but no difference was found in the pollen fertility. Furthermore, crossing with the AB genome pollen produced only three abortive seeds (about 1.5% in crossed seed sets) in the (*emmer*) AABB+*dx* line, the seed sets being normal. These results indicate that the gametophytic sterility in males and the zygotic lethality observed in the (*squarrosa*) AABB+*dx* lines did not occur in the (*emmer*) AABB+ *dx* line.

As a whole this experiment demonstrated that the selective transmission of the *dx* chromosome in the (*squarrosa*) AABB+*dx* lines was a phenomenon specific to the *squarrosa* cytoplasm, due to the essential function of the gene(s) of the *dx* chromosome in the development of the compatibility of the AB genome with the *squarrosa* cytoplasm.

3. Identification of *dx* chromosome responsible for the compatibility with *squarrosa* cytoplasm

a) Function of *dx* chromosome in the compatibility of AB genome with *squarrosa* cytoplasm

A number of experiments conducted thus far have revealed that the presence of the additional chromosome (*dx* chromosome) is indispensable for the development of the compatible relation between the AB genome of Emmer wheat and the cytoplasm of *Ae. squarrosa.*

Fig. 6 shows the behaviour of the *dx* chromosome in the alloplasmic lines of (*squarrosa*) AABB+*dx,* as indicated in the previous sections. In the (*squarrosa*) AABB+*dx* lines, all the female gametes were functional, irrespective of their genetic constitution, whether (*squarrosa*) AB+*dx* or (*squarrosa*) AB. However, selective lethality occurred in the zygotes to which the *dx* chromosome was not transmitted from female gametes, when the (*squarrosa*) AABB+*dx* lines were crossed with the AB genome pollens of Emmer wheat. This zygotic lethality was manifested in the formation of the abortive seeds of (*squarrosa*) AABB. Selective sterility was also observed in male gametophytes of the (*squarrosa*) AABB+*dx* lines. The microspores of (*squarrosa*) AB+*dx* alone developed into normal
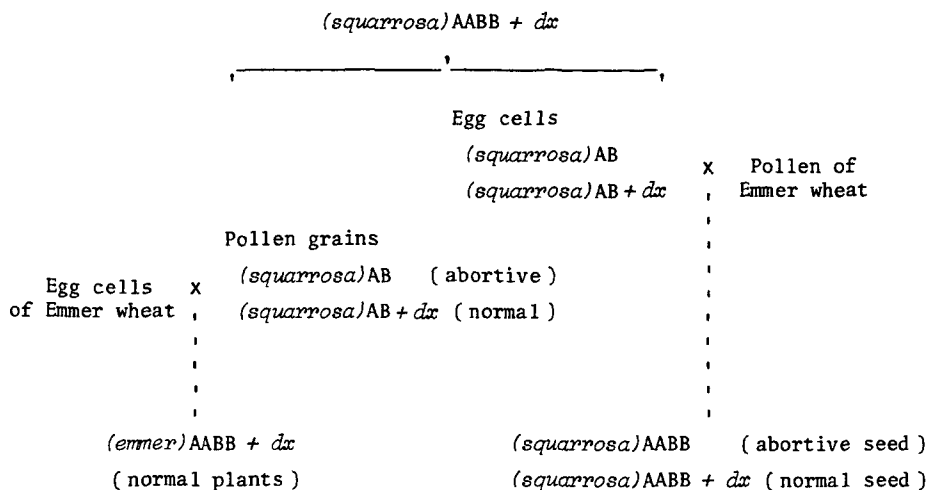
(squarrosa) AABB + *dx*

Egg cells

(squarrosa) AB

(squarrosa) AB + *dx*   X   Pollen of
,   Emmer wheat

Pollen grains

Egg cells   X   *(squarrosa)* AB   (abortive)
of Emmer wheat   ,   *(squarrosa)* AB + *dx*   (normal)

(emmer) AABB + *dx*   *(squarrosa)* AABB   (abortive seed)

(normal plants)   *(squarrosa)* AABB + *dx* (normal seed)

Fig. 6. Diagram showing the behaviour of the additional chromosome (*dx*) in (*squarrosa*)AABB + *dx* plants.

pollens ; while the other microspores lacking the *dx* chromosome became sterile pollens. On the other hand, gametophytic sterility and zygotic lethality did not appear in the monosomic-addition lines of (*emmer*) AABB + *dx* obtained by crossing euplasmic lines of Emmer wheat with pollens of the (*squarrosa*) AABB + *dx* lines. This result demonstrated that the selective transmission of the *dx* chromosome is indispensable for the compatibility of the AB genome with the cytoplasm of *Ae. squarrosa*.

b) Monosomic-*iD*-trisomic-*iA* or -*iB* series in the alloplasmic lines of Dinkel wheat with *squarrosa* cytoplasm

The alloplasmic line of *T. aestivum* cv. Chinese Spring with the genetic constitution (*squarrosa*) AABBDD was crossed with the pollens of fourteen lines of nulli-tetrasomics of cv. Chinese Spring lacking one pair of each D genome chromosome but having four chromosomes of the A or B genome in the corresponding homoeologous groups (Table 12). This crossing produced fourteen kinds of mono-trisomics lacking one chromosome of each D genome chromosome in the alloplasmic Dinkel wheat with the *squarrosa* cytoplasm but having three chromosomes of the A or B genome in the homoeologous relation {hereinafter represented as (*squarrosa*) monosomic-*iD*-trisomic-*iA* or -*iB* lines, i=1~7}.

If the *dx* chromosome, which was selectively maintained in the alloplasmic lines of Emmer wheat with the *squarrosa* cytoplasm and considered to carry the gene(s) for compatibility with the *squarrosa* cytoplasm, is a definite chromosome of the D genome, gametophytic sterility in males and zygotic lethality without the definite D genome chromosome should appear in a certain line of (*squarrosa*) monosomic-*iD*-trisomic-*iA* lines and in a certain line of (*squarrosa*) monosomic-

Table 12. Cross breeding of monosomic–D – trisomic–A or –B lines of alloplasmic line of Dinkel wheat with *squarrosa* cytoplasm

Female parent [*(sq)*AABBDD]	Male parent (nulli–tetrasomics of Chinese Spring)			No. of crossed florets	Seed sets	*(sq)*mono-trisomics obtained				
(sq) C.S.	nullisomic– *1D* – tetrasomic–*1A*			120	115	*(sq)*monosomic –*1D* – trisomic–*1A*				
"	*"*	– *2D*	*"*	–*2A*	146	121	*"*	*"*	–*2D*	*"*	–*2A*
"	*"*	– *3D*	*"*	–*3A*	128	111	*"*	*"*	–*3D*	*"*	–*3A*
"	*"*	– *4D*	*"*	–*4A*	120	106	*"*	*"*	–*4D*	*"*	–*4A*
"	*"*	– *5D*	*"*	–*5A*	171	115	*"*	*"*	–*5D*	*"*	–*5A*
"	*"*	– *6D*	*"*	–*6A*	86	70	*"*	*"*	–*6D*	*"*	–*6A*
"	*"*	– *7D*	*"*	–*7A*	140	127	*"*	*"*	–*7D*	*"*	–*7A*
"	*"*	– *1D*	*"*	–*1B*	148	134	*"*	*"*	–*1D*	*"*	–*1B*
"	*"*	– *2D*	*"*	–*2B*	120	102	*"*	*"*	–*2D*	*"*	–*2B*
"	*"*	– *3D*	*"*	–*3B*	143	130	*"*	*"*	–*3D*	*"*	–*3B*
"	*"*	– *4D*	*"*	–*4B*	144	93	*"*	*"*	–*4D*	*"*	–*4B*
"	*"*	– *5D*	*"*	–*5B*	116	101	*"*	*"*	–*5D*	*"*	–*5B*
"	*"*	– *6D*	*"*	–*6B*	116	105	*"*	*"*	–*6D*	*"*	–*6B*
"	*"*	– *7D*	*"*	–*7B*	116	114	*"*	*"*	–*7D*	*"*	–*7B*

iD-trisomic-*iB* lines, as in the (*squarrosa*) AABB+ *dx* lines.

c) Gametophytic sterility and zygotic lethality in the monosomic-*iD*-trisomic-*iA* or -*iB* series of Dinkel wheat with *squarrosa* cytoplasm

Investigations on the pollen fertility, selfed seed fertility, and crossed seed sets via the pollens of nullisomic-*iD*-tetrasomic-*iA* or -*iB* lines were conducted in the lines of (*squarrosa*) monosomic-*iD*-trisomic-*iA* and (*squarrosa*) monosomic-*iD*-trisomic-*iB*, by analysing the gametophytic sterility and zygotic lethality.

Table 13 shows the pollen fertility and selfed seed fertility of these (*squarrosa*) mono-trisomic lines. The selfed seed fertility of the (*squarrosa*) mono-trisomics series of *T. aestivum* cv. Chinese Spring tended to be slightly lower than that of the euplasmic Chinese Spring and (*squarrosa*) Chinese Spring, but no lines showed as low a fertility as the (*squarrosa*) AABB+ *dx* lines. However, a noticeable decrease in pollen fertility was found in the lines whose *1D* chromosomes were monosomic ; i. e., the pollen fertility of the (*squarrosa*) monosomic-*1D*-trisomic-*1A* line was 31.7% and that of the (*squarrosa*) monosomic-*1D*-trisomic-*1B* line was 34.6%. None of the monosomic lines with the rest of the D genome chromosome showed such a low pollen fertility as those two.

Table 14 shows the seed sets obtained when fourteen lines of (*squarrosa*) monosomic-*iD*-trisomic-*iA* or -*iB* series were crossed with the pollens of nullisomic-*iD*-tetrasomic-*iA* or -*iB* lines corresponding to the fourteen lines of (*squarrosa*) mono-trisomics and the ABD genome pollens of Chinese Spring. In this case, too, a large number of abortive seeds were produced only by crossings in which the female parents were monosomics with regard to the *1D* chromosome and the pollen donors were nullisomics with regard to the *1D* chromosome. In

Table 13. Pollen fertility and selfed seed fertility in mono–trisomics of *(squarrosa)* Chinese Spring

Line					Pollen fertility	Selfed seed fertility
					%	%
Chinese Spring (euplasmic line)					97.6	97.4
(squarrosa) Chinese Spring					96.3	98.2
(squarrosa) monosomic– *1D* – trisomic— *1A*					31.7	83.7
"	"	*–2D*	"	*–2A*	91.8	95.5
"	"	*–3D*	"	*–3A*	96.4	85.7
"	"	*–4D*	"	*–4A*	97.6	87.3
"	"	*–5D*	"	*–5A*	95.4	72.1
"	"	*–6D*	"	*–6A*	96.8	87.9
"	"	*–7D*	"	*–7A*	97.9	82.1
"	"	*–1D*	"	*–1B*	34.6	71.4
"	"	*–2D*	"	*–2B*	88.3	96.2
"	"	*–3D*	"	*–3B*	96.3	75.0
"	"	*–4D*	"	*–4B*	95.2	87.0
"	"	*–5D*	"	*–5B*	97.2	79.7
"	"	*–6D*	"	*–6B*	96.1	91.7
"	"	*–7D*	"	*–7B*	96.5	88.4

Table 14. Seed sets in mono–trisomics of *(squarrosa)* Chinese Spring crossed with nulli–tetrasomics of Chinese Spring

Line				Seed sets crossed with corresponding nulli–tetra			Seed sets crossed with Chinese Spring (AABBDD)		
				No. of crossed florets	Normal seeds	Abortive seeds	No. of crossed florets	Normal seeds	Abortive seeds
(sq) Chinese Spring				506*	464	0	170	168	0
(sq) mono– *1D* – tri– *1A*				312	54	194	142	134	2
"	"	*–2D*	" *–2A*	138	118	11	56	51	2
"	"	*–3D*	" *–3A*	138	137	1	56	51	2
"	"	*–4D*	" *–4A*	82	69	0	78	68	2
"	"	*–5D*	" *–5A*	136	114	6	54	50	1
"	"	*–6D*	" *–6A*	188	175	0	80	77	0
"	"	*–7D*	" *–7A*	194	170	7	84	78	0
"	"	*–1D*	" *–1B*	244	56	155	78	74	1
"	"	*–2D*	" *–2B*	104	97	1	48	48	0
"	"	*–3D*	" *–3B*	96	87	1	50	45	0
"	"	*–4D*	" *–4B*	107	82	0	50	49	0
"	"	*–5D*	" *–5B*	112	98	2	52	49	0
"	"	*–6D*	" *–6B*	148	128	2	56	51	2
"	"	*–7D*	" *–7B*	106	88	2	76	69	0

* crossed with all fourteen lines of nullisomic–D – tetrasomic-A or B of Chinese Spring.

comparison with 54 normal seeds (22%), the abortive seeds numbered 194 (78%) in 312 crossed florets in the crossings of a (*squarrosa*) monosomic-*1D*-trisomic-*1A* line with the pollens of a nullisomic-*1D*-tetrasomic-*1A* line. Also in the crossings of a (*squarrosa*) monosomic-*1D*-trisomic-*1B* line with the pollens of a nullisomic-*1D*-tetrasomic-*1B* line, the abortion rate was high, 73%, in comparison with 27% for normal seeds. When the ABD genome pollens of Chinese Spring were used for the crossings, (*squarrosa*) mono-trisomic lines including the monosomic-*1D* lines, showed 90% or higher seed sets with only normal seeds. These data indicate that the large number of abortive seeds produced in the (*squarrosa*) monosomic-*1D*-trisomic-*1A* or -*1B* lines, in the crosses with the pollens of corresponding nulli-tetrasomic lines, were not due to the sterility of the female gametes but to the lethality of the zygotes to which the *1D* chromosome was not transmitted.

As for the selfed seed-sets, the values of two lines of (*squarrosa*) monosomic-*1D*-trisomic-*1A* and (*squarrosa*) monosomic-*1D*-trisomic-*1B* were 83.7% and 71.4%, respectively, and both consisted of normal seeds only (Table 13). This observation implies that all the zygotes fertilized by self-pollination had the *1D* chromosome in both (*squarrosa*) mono-trisomic lines. From the data of the crossing experiment using the pollens of nulli-tetrasomic lines, at least 70% of the female gametes of (*squarrosa*) monosomic-*1D*-trisomic-*1A* or -*1B* lines, did not transmit the *1D* chromosome (Table 14). Thus it is evident that the *1D* chromosome was transmitted selectively from the pollen to the zygotes through selfing. In other words, only male gametophytes with the *1D* chromosome in combination
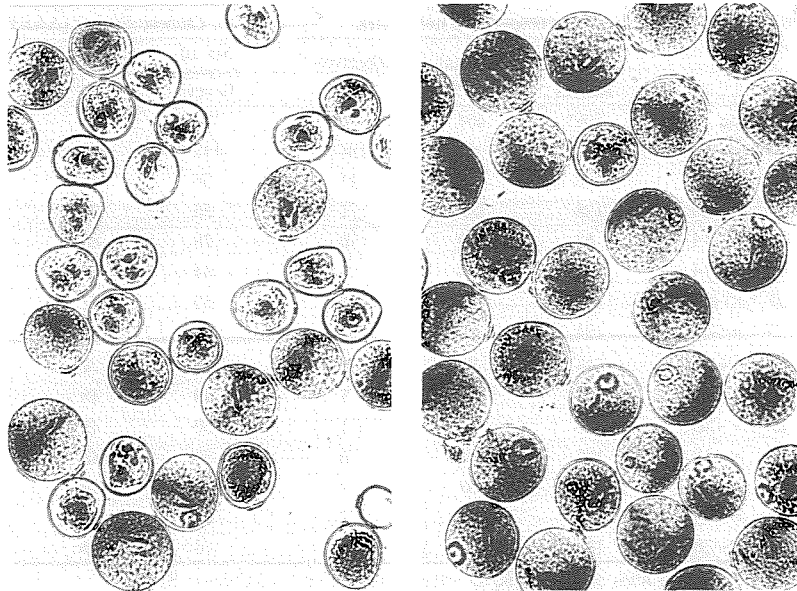

Fig. 7. Pollen grains of (*squarrosa*) monosomic-*1D* -trisomic-*1A* left and (*squarrosa*) monosomic-*2D* -trisomic-*2A* right lines.

with the *squarrosa* cytoplasm developed to sound pollens.   It can also be said that the sterile pollens that occurred at a rate of about 70% in the (*squarrosa*) monosomic-*1D*-trisomic-*1A* or -*1B* lines were caused by the absence of the *1D* chromosome (Table 13).

Fig. 7 compares the pollen grains of the (*squarrosa*) monosomic-*1D*-trisomic-*1A* line with those of the (*squarrosa*) monosomic-*2D*-trisomic-*2A* line.   The abnormality of the pollen grains in the (*squarrosa*) monosomic-*1D*-trisomic-*1A* line was caused by the male gametophytes to which the *1D* chromosome was not transmitted during the meiosis.

Fig. 8 shows the seed sets when the (*squarrosa*) monosomic-*1D*-trisomic-*1A* line was crossed with the pollens of the nullisomic-*1D*-tetrasomic-*1A* line.   These abortive seeds resulted from the lethality of the zygotes to which the *1D* chromosome was not transmitted through the female gametes.

Based on these findings, the *dx* chromosome of *Ae. squarrosa* responsible for the compatible relation between the *squarrosa* cytoplasm and the AB genome of Emmer wheats was identified as homologous to the *1D* chromosome of Dinkel wheat.   It can

Fig. 8.   Abortive seeds in (*squarrosa*) monosomic-*1D* -trisomic-*1A* line crossed with nullisomic-*1D* -tetrasomic-*1A* line.

thus be concluded that the *dx* chromosome in the original lines of (*squarrosa*) AABB+*dx* was the *1D* chromosome derived from *Ae. squarrosa*.

This experiment proves that the establishment of a compatible relation between the *squarrosa* cytoplasm and the ABD genome of Dinkel wheat always requires the presence of the *1D* chromosome.   Moreover, it was revealed that the *1C* chromosome of *Ae. caudata* (CC) carries the gene(s) equivalent to the gene(s) of the *1D* chromosome in the establishment of a compatible relation between the *squarrosa* cytoplasm and wheat genomes, based on the fact that the strain P168, a line of Dinkel wheat in which the *1D* chromosome is substituted by the *1C* chromosome, retained a normal fertility in both males and females when combined with the *squarrosa* cytoplasm (Section 1, Table 3).
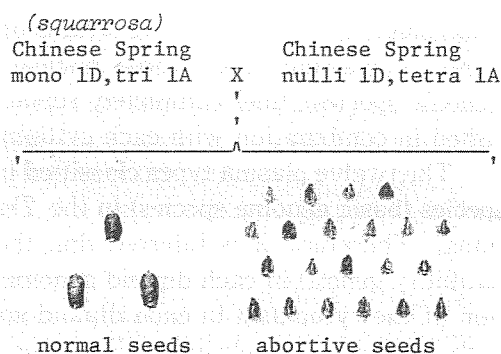
CHAPTER   II. Genetic analysis of the compatible relation
between tetraploid wheat genomes and
Aegilops squarrosa cytoplasm

Introduction

TSUNEWAKI[112] classified the cytoplasms of 33 species (75 strains) of *Triticum* and *Aegilops* into twelve plasma types, based on the cytoplasmic effects on the qualitative and quantitative characteristics observed in his study on various alloplasmic lines, using twelve strains of Dinkel wheat as the nuclear donors.   In these alloplasmic lines, whose nuclear donors were Dinkel wheat (AABBDD genome species), lines completely replaced by the AABBDD genome were established in combination with each cytoplasm.

The twelve plasma types classified by TSUNEWAKI correspond to ten diploid species (basic genome species) in the *Triticum-Aegilops* group, with some exceptions.   Therefore, it is inferred that the differentiation of the cytoplasm compatibility gene(s) in each diploid genome corresponded to the genetic differentiation of the cytoplasm in each diploid species, as shown in the *1D* chromosome with *squarrosa* cytoplasm[84].   However, in a compensatory relation, the cytoplasm compatibility gene(s) may be replaced with other gene(s) from different genomes, judging from the compatibility of wheat nuclei with the *squarrosa* cytoplasm.   Namely the gene(s) located on the *1C* chromosome in the C genome or the gene(s) from the AG genome harboured the compatibility with the *squarrosa* cytoplasm which is equivalent to the gene(s) located on the *1D* chromosome in the D genome[80].   Consequently, in order to clarify the genetic mechanisms of the compatibility between nuclear genome and cytoplasm, it is desirable to study the alloplasmic lines whose nuclear donors were derived from lower ploidy level.

The present experiments aimed at revealing the genetic differentiation of the cytoplasm and the genetic functions of the corresponding cytoplasm compatibility gene(s), on the basis of the genetic differentiation of the cytoplasm compatibility gene(s) among tetraploid wheats (AABB and AAGG genome species) with the *squarrosa* cytoplasm.

Materials and Methods

The alloplasmic lines of tetraploid wheats with the *squarrosa* cytoplasm used in the experiments were the lines with the genetic constitution (*squarrosa*) AABB+ *1D*, whose AABB genome donors were various Emmer wheats, i. e. *T. durum* var. *Reichenbachii, T. turgidum* var. *nigro-barbatum, T. polonicum* var. *vestitum, T. dicoccoides* var. *spontaneo-nigrum,* and *T. dicoccoides* var. *Aaron-sohni.*

The tetraploid wheat species used as the male parents in the cross experi-

ments were as follows : eight species (twelve strains) of Emmer wheat (AABB genome species), i. e. *T. dicoccoides* var. *spontaneo-nigrum, T. dicoccoides* var. *Aaronsohni, T. dicoccum* var. *farrum* (strain Hokudai), *T. durum* var. *Reichenbachii, T. turgidum* var. *nigro-barbatum, T. polonicum* var. *vestitum, T. palaeocolchicum* (=*georgicum*) var. *schwamilicum, T. persicum* (=*carthlicum*) var. *stramineum, T. persicum* var. *fuliginosum, T. persicum* var. *rubiginosum, T. pyramidale* var. *recognitum, T. pyramidale* strain Baladi, and two species (two strains) of Timopheevi wheat (AAGG genome species), i. e. *T. araraticum* var. *Thumaniani, T. timopheevi* var. *typicum*.   All the lines used were maintained by selfing in each generation at the Kihara Institute for Biological Research.

Crossing experiments, survey of chromosome number, and various morphological analyses were performed using materials cultivated in the autumn sowing in the greenhouse and field at Yokohama.

Results and Discussion

1. Zygotic lethality due to the combination of tetraploid wheat genomes and *squarrosa* cytoplasm

a) Different responses of AABB and AAGG genome species in crossing experiments to *(squarrosa)* AABB+ *1D* lines

The experiments in Chapter I revealed that the AB genome of the five strains (five species) of Emmer wheat is incompatible with the *squarrosa* cytoplasm, while the AG genome of the two strains (two species) of Timopheevi wheat is completely compatible with the *squarrosa* cytoplasm.   In other words, the combination of the *squarrosa* cytoplasm and the nuclei of the five species of Emmer wheat could not produce any plants with the genetic constitution (*squarrosa*) AABB, and the presence of the *1D* chromosome, the first homoeologous group chromosome of the D genome of *Ae. squarrosa*, was found to be indispensable for the survival of the alloplasmic lines.   Therefore, in the successive backcrossed lines in which these Emmer wheat species were used as the male parents in each generation, all the plants obtained had the genetic constitution (*squarrosa*) AABB+ *1D*, 2n=29, 14 II+1 I.   On the other hand, in the alloplasmic lines obtained from successive backcrossings in which two species of Timopheevi wheat were used as the male parents in each generation, all the plants showed the chromosome configuration 2n=28, 14 II indicating the genetic constitution (*squarrosa*) AAGG.   This phenomenon is ascribed to the fact that in the AG genome of the Timopheevi wheat species the cytoplasm compatibility gene(s) are equivalent to the gene(s) located' on the *1D* chromosome in the compatibility with the *squarrosa* cytoplasm.

The difference in the compatibility with the *squarrosa* cytoplasm between the AB and AG genomes, directly came out as the difference in the nature of the F_1 seeds obtained from crossings using various alloplasmic lines of tetraploid wheat
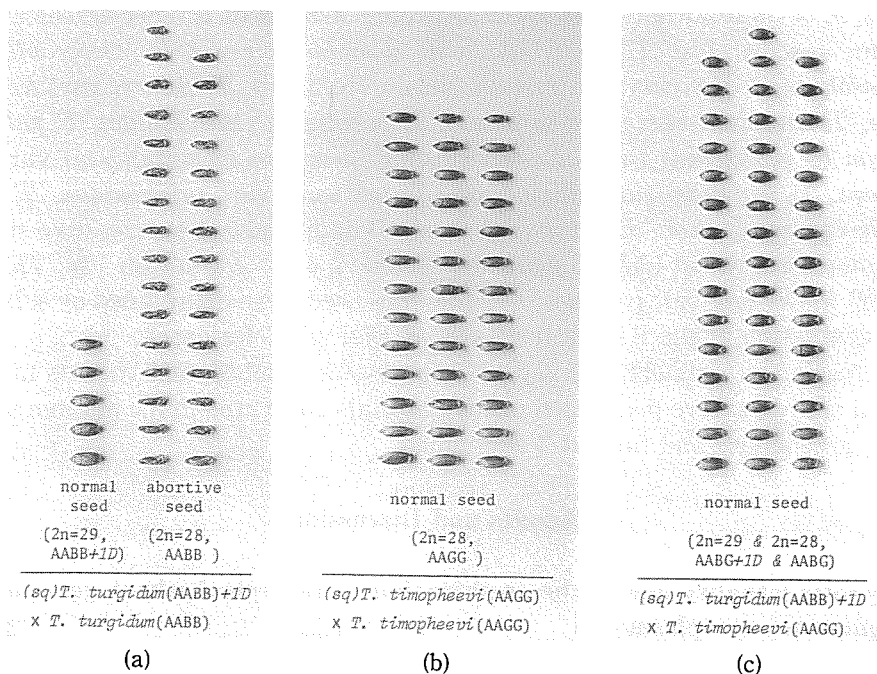
Fig. 9. Different responses of genomes AABB and AAGG to *squarrosa* cytoplasm appearing in the types of crossed seeds.

with the *squarrosa* cytoplasm as female parents and various species of tetraploid wheat as male parents.

Fig. 9 (a) shows the B_nF_1 seeds obtained from a crossing using an alloplasmic line of *T. turgidum* var. *nigro-barbatum,* the (*squarrosa*) AABB + *1D* line, as the female parent and a pure line of *T. turgidum* var. *nigro-barbatum* as the male parent. The five normal seeds were produced by the fertilization of the female gametes with the genetic constitution (*squarrosa*) AB + *1D,* and the plants obtained from the seeds showed the chromosome configuration 2n=29, 14 II+1 I, i. e. the genetic constitution of (*squarrosa*) AABB + *1D.* The thirty-one abortive seeds resulted from the zygotic lethality which was caused by the genetic constitution of (*squarrosa*) AABB (embryo) and (*squarrosa*) AAABBB (endosperm), produced by the fertilization of the female gametes of (*squarrosa*) AB.

Fig. 9 (b) shows the B_nF_1 seeds obtained from the alloplasmic line of *T. timopheevi* var. *typicum,* the (*squarrosa*) AAGG line. In this case, the genetic constitution of the female gametes was (*squarrosa*) AG, and the genetic constitution of the embryo and endosperm of the B_nF_1 seeds was (*squarrosa*) AAGG and (*squarrosa*) AAAGGG. Zygotic lethality did not appear despite the absence of the. *1D* chromosome, all of the seeds produced being normal.

Fig. 9 (c) shows the F_1 seeds obtained from a crossing using *T. timopheevi* var. *typicum* as the male parent as in (b) and (*squarrosa*) *T. turgidum* var. *nigrobarbatum* + *1D* line as female parent as in (a). This is an example of one spike with

fifty florets, forty-six normal uniform seeds being produced. Based on the results obtained in the experiments described in Chapter I, it is estimated that the female gametes of (*squarrosa*) AB+*1D* accounted for about 25% as a mean, the remaining 75% being (*squarrosa*) AB. Despite such a low transmission rate of the *1D* chromosome, no female sterility or zygotic lethality was observed in this crossing, the F_1 seeds obtained being normal without exception. This finding suggests that the seeds with the embryo and endosperm of (*squarrosa*) AABG and (*squarrosa*) AAABBG develop into completely normal seeds, as in the case of the seeds with the *1D* chromosome, presumably due to the cytoplasm compatibility gene(s) of the AG genome equivalent to the gene(s) located on the *1D* chromosome in the compatibility with the *squarrosa* cytoplasm.

b) Abnormality of F_1 seeds in the crosses to *(squarrosa)*AABB+*1D* lines with various tetraploid wheat species

By analysing the different responses to the zygotic lethality of the AABB and AAGG genome species in the crosses to (*squarrosa*) AABB+*1D* lines, the difference of compatibility with the *squarrosa* cytoplasm among various species or strains of tetraploid wheat could be examined without the production of their alloplasmic lines with the *squarrosa* cytoplasm.

Table 15. Segregation in seed morphology of F_1 in the crosses to *(squarrosa)* AABB+*1D* lines with various tetraploid wheat species

Male parent	No. of florets crossed	Seed morphology	
		viable (normal)	Abortive (lethal)
Emmer wheat (AABB genome)			
T. dicoccoides var. *spontaneo-nigrum*	432	27	258
" var. *Aaronsohni*	154	26	72
T. dicoccum var. *farrum*, strain Hokudai	248	41	66
T. durum var. *Reichenbachii*	294	24	208
T. turgidum var. *nigro-barbatum*	206	41	130
T. polonicum var. *vestitum*	270	99	118
T. palaeocolchicum (= *georgicum*) var. *schwamilicum*	172	102*	0
T. persicum (= *carthlicum*) var. *stramineum*	74	73*	0
" var. *fuliginosum*	88	49*	0
" var. *rubiginosum*	78	72*	0
T. pyramidale var. *recognitum*	92	66*	0
" var. - - - , strain Baladi	144	93*	0
Timopheevi wheat (AAGG genome)			
T. araraticum var. *Thumaniani* (KU-196-2)	254	179	0
T. timopheevi var. *typicum* (KU-107-1)	172	122	0

* Normal (plump) and shrivelled (germinable) seeds are included.

In other words, in cases where many abortive F_1 seeds are produced (as a result of zygotic lethality due to the absence of the *1D* chromosome) in the crossing of (*squarrosa*) AABB+*1D* lines (as female parents) with the pollens of various tetraploid wheat species, the male parent strains can be classified into type-I (incompatible with *squarrosa* cytoplasm) and in cases where the F_1 seeds obtained are normal irrespective of the *1D* chromosome transmission, the male parent strains can be classified into type-II (completely compatible with *squarrosa* cytoplasm).

Table 15 shows the segregation pattern in the F_1 seed morphology when the (*squarrosa*) AABB+ *1D* lines were crossed with the pollens of twelve strains (eight species) of Emmer wheat and two strains (two species) of Timopheei wheat. In every case in which five species (six strains) of the eight species of Emmer wheat, i. e. *T. dicoccoides, T. dicoccum, T. durum, T. turgidum,* and *T. polonicum* were used as male parents, many abortive seeds were produced. Therefore their response type to the *squarrosa* cytoplasm was classified as type-I. The two species (two strains) of Timopheevi wheat, i. e. *T. araraticum* and *T. timopheevi* were identified as type-II, which shows a complete compatibility with the *squarrosa* cytoplasm, because all their F_1 seeds were viable and normal. In the species shown above, the AABB genome species were identified as type-I and the AAGG genome species as type-II.

However, the three species (six strains) of Emmer wheat, i. e. *T. palaeocolchicum* (=*georgicum*), *T. persicum* (=*carthlicum*), and *T. pyramidale,* all of which hitherto classified as AABB genome species, showed responses different from that of type-I Emmer wheat, for all the F_1 seeds obtained from the crossings in which they were used as the male parents were viable. In addition, they showed responses clearly different from those of type-II Timopheevi wheat, for a clear morphological segregation was observed in the viable F_1 seeds produced as normal and shrivelled seeds. Therefore, with regard to the compatibility response to the *squarrosa* cytoplasm, Emmer wheat with the AABB genome can be classified into two types as follows : type-I showing segregation into normal seeds and abortive seeds (zygotic lethality) when crossed to the (*squarrosa*) AABB+*1D* lines whose AABB genomes were of type-I, and type-III showing segregation into normal and shrivelled seeds (viable) when crossed to the (*squarrosa*) AABB+*1D* lines of type-I Emmer wheat.

Fig. 10 shows the segregation into normal and shrivelled seeds in the F_1 seeds produced from the crossing using the (*squarrosa*) AABB+*1D* lines of type-I Emmer wheat as female parents and two species of type-III Emmer wheat, i. e. *T. palaeocolchicum* and *T. persicum,* as male parents. In the two examples shown, the (*squarrosa*) AABB+*1D* line of *T. turgidum* var. *nigro-barbatum* was used as female parents. Fig. 10 (a) is an example of one spike pollinated with *T. palaeocolchicum* var. *schwamilicum* ; in Fig. 10 (b), *T. persicum* var. *rubiginosum* was used as pollen parent. Forty florets were crossed per spike in each crossing.

In (a), five seeds were normal (left) and thirty-four shrivelled. In (b), five seeds were normal and thirty-three shrivelled. The shrivelled seeds differed clearly from the normal seeds as observed in the abortive seeds shown in Fig. 9. In comparison to the normal seeds, the shrivelled ones developed into an incomplete embryo and endosperm, their color being darker. As for the seed coat development, the seed coat tended to be thick as observed in the abortive ones.

Under the field conditions at Yokohama, where rainfall is abundant at the wheat ripening stage, the entire endosperm of the shrivelled seed was frequently attacked by fungi, for they are susceptible. Therefore, sometimes, it was difficult to distinguish them, especially when the development of the normal seeds was incomplete due to the environmental conditions.

However, in the seed sets obtained under favorable conditions in a greenhouse, as shown in Fig. 10, both types of seeds could be clearly distinguished, the ratio of normal seeds and shrivelled seeds corresponding to that of the normal seeds (with *1D* chromosome) and abortive seeds (without *1D* chromosome) produced in the crossing of (*squarrosa*) AABB+*1D* lines with pollens of type-I Emmer wheat. Therefore, it can be estimated that the normal seeds shown in Fig. 10 were derived from the zygotes to which the *1D* chromosome had been transmitted from female gametes, and the many remaining shreivelled seeds from the zygotes to which the *1D* chromosome had not been transmitted.

normal seed (viable)	shriveled seed (viable)	normal seed (viable)	shriveled seed (viable)

(*sq*)*T. turgidum nigro.*+*1D*
x *T. palaeocolchicum* (=*georgicum*) var. *schwamilicum*

(*sq*)*T. turgidum nigro.*+*1D*
x *T. persicum* (=*carthlicum*) var. *rubiginosum*

(a)   (b)

Fig. 10. Segregation in seeds of F_1 in the crosses to (*squarrosa*)AABB+*1D* line with *T. palaeocolchicum* (a) and *T. persicum* (b).

As described above, it is possible to classify the strains of tetraploid wheat used as male parents into three types in terms of the compatible response to the *squarrosa* cytoplasm, on the basis of the segregation in the F_1 seeds obtained from the crossing using (*squarrosa*) AABB+*1D* lines of type-I Emmer wheat as female parents, as follows.
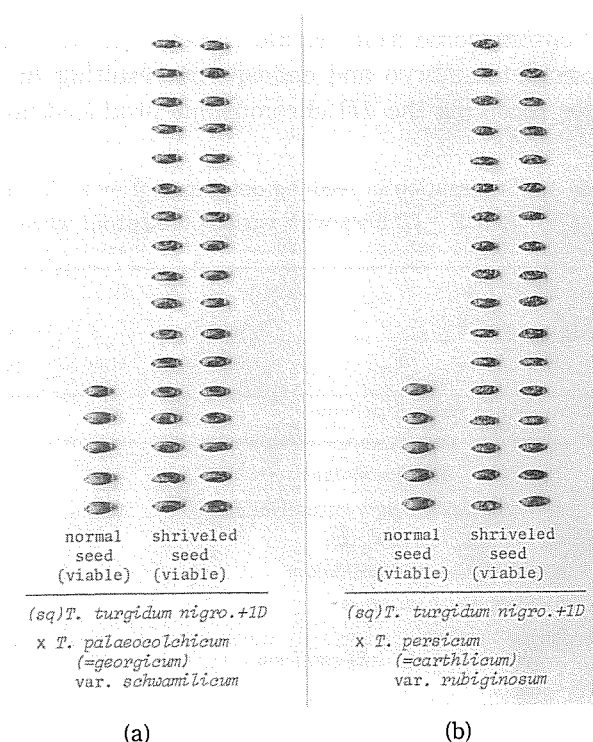
In the type-I, observed in typical species of Emmer wheat (AABB genome), where only the zygotes (2n=29, 3n=44) receiving the *1D* chromosome from female gametes developed into a complete embryo and endosperm to produce normal seeds, the zygotes (2n=28, 3n=42) which did not receive the *1D* chromosome displayed lethality, resulting in abortive seeds.

The type-II, observed in Timopheevi wheat (AAGG genome), is a response type in which all the zygotes developed into a complete embryo and endosperm, producing normal seeds irrespective of the *1D* chromosome transmission, and showing complete compatibility with the *squarrosa* cytoplasm.

Apart from these two types, the type-III found in some endemic species of Emmer wheat (AABB genome) can be regarded as a type partly compatible with the *squarrosa* cytoplasm, for the zygotes (2n=28, 3n=42) which did not receive the *1D* chromosome from female gametes did not show lethality but developed into incomplete embryo and endosperm, resulting in shrivelled seeds. Only the zygotes receiving the *1D* chromosome produced normal seeds, as in type-I.

Table 16. Segregation in seedling development of F_1 in the crosses to *(squarrosa)* AABB+ *1D* AABB+ *1D* lines with various tetraploid wheat species

Male parent	Seedlings*			
	Normal plants	Miniature plants**	Died after germi.	Not germi.
Emmer wheat (AABB genome)				
T. dicoccoides var. *spontaneo-nigrum*	21	0	0	6
〃 var. *Aaronsohni*	20	0	0	6
T. dicoccum var. *farrum*, strain Hokudai	40	0	0	1
T. durum var. *Reichenbachii*	20	0	0	4
T. turgidum var. *nigro-barbatum*	33	0	3	5
T. polonicum var. *vestitum*	99	0	0	0
T. palaeocolchicum (= *georgicum*) var. *schwamilicum*	20	14	25	43
T. persicum (= *carthlicum*) var. *stramineum*	6	35	18	14
〃 var. *fuliginosum*	9	17	12	11
〃 var. *rubiginosum*	5	26	35	6
T. pyramidale var. *recognitum*	9	47	6	4
〃 var. - - - , strain Baladi	14	45	21	13
Timopheevi wheat (AAGG genome)				
T. araraticum var. *Thumaniani* (KU-196-2)	159	0	6	14
T. timopheevi var. *typicum* (KU-107-1)	38	0	1	1

 * Seedlings from viable seeds.
** Miniature plants with extremely poor growth and severe chlorophyll
   variegation in winter.

2. Chlorophyll variegation and plant weakness caused by the interaction between tetraploid wheat genomes and *squarrosa* cytoplasm

a) Development of F_1 seedlings in the crosses to *(squarrosa)* AABB + *1D* lines

Table 16 shows the segregation pattern in the seedlings of the F_1 lines whose female parents were the (*squarrosa*) AABB + *1D* lines of type-I Emmer wheat and whose male parents were various strains of tetraploid wheat. Morphological segregation of the F_1 seeds is shown in Table 15. None of the abortive seeds (Table 15) germinated because the embryo and endosperm degenerated in spite of a well-developed seed coat. Thus, only the results for viable seeds are shown in Table 16.

When five species (six strains) of type-I Emmer wheat, i. e. *T. dicoccoides, T. diccocum, T. durum, T. turgidum, T. polonicum,* were used as the male parents, the abortive seeds, accounting for the greater part of the F_1 seeds produced, did not germinate, as stated above ; however, the F_1 seedlings germinating from the remaining normal seeds grew to normal plants. The F_1 normal plants showed the genetic constitution (*squarrosa*) AABB + *1D*, 2n = 29, 14 II + 1 I, as described in Chapter I.

When two species (two strains) of type-II Timopheevi wheat, i. e. *T. araraticum* and *T. timopheevi* were used as the male parents, the F_1 seeds were all normal, consisting of seeds of (*squarrosa*) AABG + *1D* and (*squarrosa*) AABG. No abnormality was observed in their germination rates and all the F_1 seedlings grew to normal plants.

In contrast to type-I and type-II, a clear segregation was observed in the F_1 seedlings, when three species (six strains) of type-III Emmer wheat, i. e. *T. palaeocolchicum, T. persicum,* and *T. pyramidale* were used as the male parents. In other words, of the F_1 seeds (normal and shrivelled seeds) obtained from crossings using six strains of type-III Emmer wheat as male parents and (*squarrosa*) AABB + *1D* lines of type-I Emmer wheat as female parents, the normal seeds produced normal plants without exception while the shrivelled ones produced miniature plants with an extremely slow growth. In addition, many of the shrivelled seeds, which accounted for the greater part of the F_1 seeds, did not germinate due to fungal attack. When they germinated, they showed a considerably slow growth, and died in many cases.

Table 17 shows the data obtained from the examination of the F_1 plants obtained from the crossings using (*squarrosa*) AABB + *1D* lines of type-I Emmer as female parents and various type-III AABB genome species as male parents. The chromosome number, plant height and fresh weight of the seedlings at 45 days after germination are shown in comparison with those obtained in crossings using type-II Timopheevi wheats as male parents.

When the two species (two strains) of type-II Timopheevi wheat, i. e. *T. araraticum* and *T. timopheevi* were the male parents, the F_1 plants showed normal

Table 17. Chromosome number, plant height and fresh weight of F_1 seedlings (normal and miniature plants) obtained from the crosses to *(squarrosa)*AABB + *1D* lines with different tetraploid wheat species

Male parent	No. of plants with different chromosome numbers				Plant height of seedlings* (cm)		Fresh weight of seedlings* (gr)	
	Normal plants		Miniature plants		Normal plants	Miniature plants	Normal plants	Miniature plants
	2n=28	2n=29	2n=28	2n=29	mean ±sd.	mean ±sd.	mean ±sd.	mean±sd.
T. palaeocolchicum (=georgicum) var. *schwamilicum*	0	7	6	0	23.0±6.7	4.8±···	4.48±1.40	0.09±···
T. persicum (=carthlicum) var. *stramineum*	0	5	35	0	19.2±2.5	6.6±1.8	3.63±1.63	0.17±0.07
T. persicum (=carthlicum) var. *fuliginosum*	0	9	16	0	21.3±2.6	5.6±1.5	4.13±1.38	0.17±0.09
T. pyramidale var. *recognitum*	0	4	19	0	19.2±2.0	6.8±1.3	4.21±1.73	0.14±0.05
T. pyramidale var. ···, strain Balabi	0	7	18	0	21.0±1.8	6.8±1.9	6.37±1.65	0.19±0.11
T. araraticum var. *Thumaniani*	20	7	···	···	17.7±2.1	···	2.67±0.73	···
T. timopheevi var. *typicum*	17	2	···	···	28.9±4.2	···	2.80±1.15	···

* Seedlings at 45 days after germination.

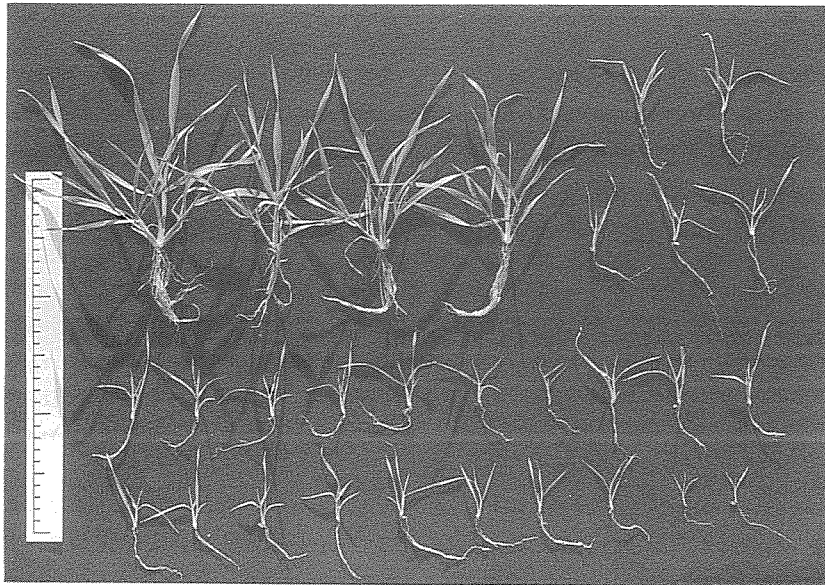**Fig. 11.** Segregation in seedling development of F_1 in the cross to *(squarrosa)* *T. durum* var. *Reich.* + *1D* line with *T. persicum* (= *carthlicum*) var. *stramineum* (as male), at 45 days after germination (Dec. 9, 1981).

*   Four normal seedlings exhibited the chromosome number of 2n = 29 (with *1D*) and other 25 miniature seedlings 2n = 28 (without *1D*).

* * Line No. 506 '82yg (cross No. 917 '81y).

growth with both chromosome numbers, 2n=28 and 2n=29. In addition, no noticeable differences in plant height and fresh weight were observed among the F_1 plants.

On the other hand, when the three species of type-III Emmer wheat, i. e. *T. palaeocolchicum, T. persicum,* and *T. pyramidale* were the male parents, normal and miniature plants were segregated in all the F_1 seedlings. The chromosome numbers of the F_1 normal plants were 2n=29 without exception, and those of the F_1 miniature plants examined were 2n=28 without exception. Therefore, it is assumed that all the F_1 normal plants received the *1D* chromosome from female gametes, and that the individuals which did not receiving the *1D* chromosome grew to miniature plants.

Fig. 11 shows an example of segregation in the F_1 seedlings at 45 days after germination in the cross of (*squarrosa*) AABB+*1D* lines of type-I Emmer wheat (as female parents) with type-III Emmer wheats (as male parents). In this case, *T. durum* var. *Reichenbachii* was the type-I AABB genome donor of the female line, *T. persicum* var. *stramineum* being the type-III Emmer wheat of the male parent. The four normal seedlings from normal seeds showed the chromosome number 2n=29, that is, they received the *1D* chromosome from female gametes. The remaining twenty-five miniature seedlings from shrivelled seeds did not harbour the *1D* chromosome, their chromosome number being 2n=28.

Fig. 12. F_1 normal plant (left, 2n=29) and F_1 miniature plant (right, 2n=28) obtained from the cross of (*squarrosa*) *T. durum* var. *Reichenbachii* + *1D* line × *T. palaeocolchicum* (= *georgicum*) var. *schwamilicum*, at 115 days after germination (Feb. 23, 1982).
* Normal plant : No. 503 '82yg -1, miniature plant : No. 504 '82yg -5.

Fig. 12 shows the normal and miniature F_1 plants at 115 days after germination obtained from the crossing using the female parent of the (*squarrosa*)

AABB+ *1D* line whose AABB genome donor was type-I species of *T. durum* var. *Reichenbachii* and type-III species of *T. palaeocolchicum* var. *schwamilicum* for the male parent.   These F_1 plants were obtained through the following process : sown at the end of October, 1981, reared outdoors at Yokohama from germination till the beginning of February, 1982, and grown in an unheated greenhouse for 20 days or so.   Because the F_1 normal plants with the *1D* chromosome (2n=29) continued to grow normally, and the F_1 miniature plants without it (2n=28) showed an extremely slow growth, the difference between them was more pronounced than in the seedling stage.   In addition, considerable chlorophyll variegation was observed in the F_1 miniature plants under the low temperature conditions in winter.   These phenomena were the same in all the F_1 combinations between the (*squarrosa*) AABB+ *1D* lines of type-I species (as female) and type-III species (as male).

b) Chlorophyll variegation in the F_1 miniature plants

An example of the F_1 miniature plants (2n=28) shown in the Plate was obtained from the crossing combination using the female parent of the (*squarrosa*) AABB+ *1D* line whose AABB genome donor belonged type-I species of *T. durum* var. *Reichenbachii* and type-III species of *T. palaeocolchicum* var. *schwamilicum* for the male parent.   The F_1 miniature plants exhibited a uniform morphology differing from the species of type-III Emmer wheat used as male parents.   All the leaves of the F_1 miniature plants, which had been exposed to the low temperature outdoors at Yokohama, exhibited severe chlorophyll variegation.   After cultivation in a greenhouse where the minimum night temperature was kept at about 15° C (under long day-length conditions), they developed new leaves without chlorophyll variegation, forming small spikes consisting of several spikelets, though they were poor plants.   For the control, some of the miniature plants were cultivated in a greenhouse where the minimum temperature was kept above 10°C (under short day-length conditions) after the seedling stage, and none of the individuals showed chlorophyll variegation.   These results suggest that the chlorophyll variegation observed in the miniature plants was due to low temperature conditions.

c) Segregation of plant type in B_1F_1 lines

The miniature plants of the F_1 hybrids between the type-I AABB genome species and type-III AABB genome species with the *squarrosa* cytoplasm, showed considerable weakness and marked chlorophyll variegation under the low temperature conditions in winter in the autumn sowing.   However, when cultivated in a greenhouse with the minimum temperature kept at above 15°C under long day-length conditions, the newly developed leaves and shoots did not show chlorophyll variegation, but grew to spike and bloom.   The mature plants displayed a considerably poorer growth than the normal plants with the *1D*
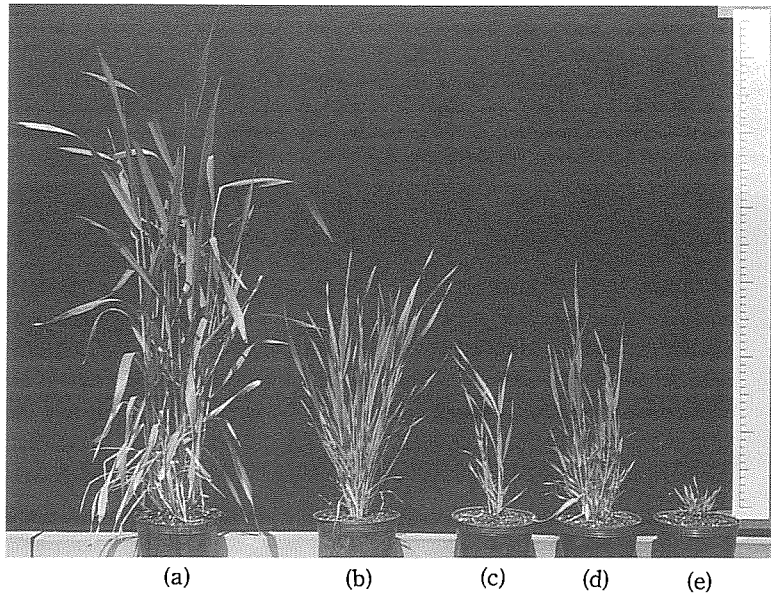
Fig. 13. Five types of B_1F_1 plants obtained from the crosses to F_1 plants (normal and miniature plants) of (*squarrosa*) *T. durum* var. *Reichenbachii + 1D* line × *T. persicum (= carthlicum)* var. *fuligi-nosum* with *T. durum* var. *Reich.* or *T. persicum* var. *fuligi..*
(a) Normal plant (2n=29), without chlorophyll variegation.
(b) Intermediate-I plant (2n=28), without chlorophyll variegation.
(c) Weak plant (2n=28), without chlorophyll variegation.
(d) Intermediate-II plant (2n=28), with chlorophyll variegation.
(e) B_1 miniature plant (2n=28), with chlorophyll variegation.

chromosome, and their spikes consisted of only several spikelets with the anthers did not show any dehiscence. However, backcrosses were successful between the F_1 miniature plants (as female parents) and type-I and type-III AABB genome species (as male parents).

Fig. 13 shows the typical plant types produced in the B_1F_1 lines from the F_1 normal (2n=29) and miniature (2n=28) plants backcrossed with type-I and type-III AABB genome species. The B_1F_1 line shown in Fig. 13 is a combination of *T. durum* var. *Reichenbachii* as the type-I species and *T. persicum* var. *fuliginosum* as the type-III species. In the lines, including another combination of *T. tur-gidum* var. *nigro-barbatum* as the type-I species and *T. palaeocolchicum* var. *schwamilicum* as the type-III species, the B_1F_1 plants were classified into five types on the basis of growth under the low temperature conditions in winter as follows :

(a) Normal type : the plant type segregated only in the B_1F_1 lines obtained from the cross with F_1 normal plants. There was no chlorophyll variegation and growth was normal. These characteristics appeared in the plants to which

Table 18. Segregations in seed morphology and seedling development of B_1F_1 in crosses to F_1 plants (normal and miniature plants) of *(squarrosa)T. durum* var. *Reichenbachii + 1D* line × *T. persicum (=carthlicum)* var. *fuliginosum* with *T. durum* var. *Reichenbachii* or *T. persicum* var. *fuliginosum*

Cross combination	No. of florets crossed	Seed morphology		Seedling development*					Died after germi.	Not germi.
		Viable	Abortive	Without variegation			With variegation**			
				Normal plants	Inter-mediate -I plants	Weak plants	Inter-mediate -II plants	Miniature plants		
F_1 miniature plant (2n=28) × T. *durum* var. *Reich.* (AABB, type-I)	136	47	48	0	0	0	0	42	5	0
F_1 miniature plant (2n=28) × T. *persicum* var. *fuligi.* (AABB, type-III)	156	89	0	0	22	19	26	22	0	0
F_1 normal plant (2n=29) × T. *dvrum* var. *Reich.* (AABB, type-I)	92	40	40	14	0	0	0	26	0	0
F_1 normal plant (2n=29) × T. *persicum* var. *fuligi.* (AABB, type-III)	62	52	0	8	11	10	9	13	0	1

* Seedlings from viable seeds.
** Severe chlorophyll variegation in winter.

Table 19. Segregations in seed morphology and seedling development of B_1F_1 in crosses to F_1 plants (normal and miniature plants) of *(squarrosa)T. turgidum* var. *nigro-barbatum+ 1D* line × *T. palaeocolchicum (=georgicum)* var. *schwamilicum* with *T. turgidum* var. *nigro-barbatum* or *T. palaeocolchicum* var. *schwamilicum*

Cross combination	No. of florets crossed	Seed morphology		Seedling development*					Died after germi.	Not germi.
		Viable	Abortive	Without variegation			With variegation**			
				Normal plants	Inter-mediate -I plants	Weak plants	Inter-mediate -II plants	Miniature plants		
F_1 miniature plant (2n=28) × T. *turgidum* var. *nigro.* (AABB, type-I)	50	15	13	0	0	0	0	15	0	0
F_1 miniature plant (2n=28) × T. *palaeocolchicum* var. *schwam.* (AABB,type-III)	50	14	0	0	5	4	3	2	0	0
F_1 normal plant (2n=29) × T. *turgidum* var. *nigro.* (AABB, type-I)	214	109	57	33	0	0	0	49	2	25
F_1 normal plant (2n=29) × T. *palaeocolchicum* var. *schwam.* (AABB,type-III)	90	57	0	10	8	4	10	7	1	17

* Seedlings from viable seeds.
** Severe chlorophyll variegation in winter.

the *1D* chromosome was transmitted (2n=29).

(b) Intermediate-I type : no chlorophyll variegation, slender plant body, slightly poor growth but vigorous shooting.

(c) Weak type : no chlorophyll variegation, slender plant body, poor growth, and poor shooting.

(d) Intermediate-II type : chlorophyll variegation, slender plant body, poorer growth than (b) (due to the effect of chlorophyll variegation) but shooting was vigorous.

(e) Miniature type : considerable chlorophyll variegation, dwarf plant, and poor growth.

Tables 18 and 19 show the segregation in the seed and seedlings of the B_1F_1 lines in the two crossings mentioned above.

When the F_1 miniature plants (2n=28) were used as the female parents and the type-I species as the male parents, half of the B_1F_1 seeds obtained were viable and the remaining half were abortive in both combinations.   The viable B_1F_1 seeds produced the miniature plants shown in Fig. 13 (e), with some exceptions (several plants died after germination).

On the other hand, when the F_1 miniature plants (2n=28) were crossed with the type-III species, the B_1F_1 seeds obtained were all viable.   In the B_1F_1 lines produced, four plant types, i. e. (b) intermediate-I, (c) weak, (d) intermediate-II, (e) miniature, were produced.   As shown in Tables 18 and 19, (b) 27, (c) 23, (d) 29 and (e) 24 individuals were segregated.   If the expected ratio is 1 : 1 : 1 : 1, the equation $\chi^2=0.883$ fits with the expectation at a probability of $0.80<p<0.90$.

In each B_1F_1 line in which the F_1 normal plants (2n=29, with *1D* chromosome) were used as female parents and type-I or type-III species as male parents, additional viable (normal) seeds and normal plants due to the transmission of the *1D* chromosome were produced.   However, if these effects are eliminated, the same segregation ratio could be obtained as in the B_1F_1 lines from the backcrosses with F_1 miniature plants as female parents.

In other words, if the effects associated with the transmission of the *1D* chromosome are excluded, the segregation of seed and plant types in these B_1F_1 lines (Tables 18 and 19) can be summarized as follows :

(1) When type-I species were used as male parents :

Viable seeds and abortive seeds were segregated.   In the two crosses to F_1 miniature plants with type-I species, 62 seeds were viable and 61 seeds abortive. If a 1 : 1 ratio is expected, the equation $\chi^2=0.0081$ fits with the expectation at a probability of $0.80<p<0.95$.   In the two crosses to F_1 normal plants with type-I species, 149 seeds were viable and 97 abortive ; however, if those produced due to the effects of the *1D* chromosome, i. e. a part of the normal plants in B_1F_1, are excluded, the count is 102 : 97.   If this is regarded as 1 : 1, the equation $\chi^2=0.1256$ fits with the expectation at a probability of $0.70<p<0.80$.

As for the segregation of plant type, in the four crosses to F_1 normal and

miniature plants with type-I species, a total of 47 normal plants were produced due to the transmission of the *1D* chromosome ; the remaining 132 plants were miniature plants showing extremely poor growth and chlorophyll variegation. (2) When type-III species were used as male parents :

Irrespective of *1D* chrosomosome transmission, zygotic lethality did not occur and all the seeds were viable.

If the normal plants due to *1D* chromosome transmission are excluded, in the four crosses to F_1 normal and miniature plants with type-III species, 46 plants were intermediate-I plants, 37 were weak plants, 48 were intermediate-II plants, and 44 were miniature plants. If this ratio is regarded as 1 : 1 : 1 : 1, the equation $\chi^2=1.571$ fits with the expectation at a probability of $0.50 < p < 0.70$.

The four plant types shown above are combinations of two characteristics, i. e. plant vigour and chlorophyll variegation under low temperature conditions.

If the classification was based on plant vigour, the total of the intermediate-I and intermediate-II plants, which showed some poorer growth, was 94, and that of the weak and miniature plants, whose growth was considerably poor, was 81. If this ratio is regarded as 1 : 1, the equation $\chi^2=0.9657$ fits with the expectation at a probability of $0.30 < p < 0.50$.

If the classification was based on chlorophyll variegation, the total of the intermediate-I and weak plants, which showed no variegation, was 83, and that of the intermediate-II and miniature plants, which showed variegation, was 92. If this ratio is regarded as 1 : 1, the equation $\chi^2=0.4629$ fits with the expectation at a probability of $0.30 < p < 0.50$.

3. Genetic differentiation of the cytoplasm compatibility in tetraploid wheat species

Three types of tetraploid wheat genomes were revealed in relation to the compatibility with the *squarrosa* cytoplasm. Type-I was completely incompatible with the *squarrosa* cytoplasm, as observed in five species of Emmer wheat, type-II was completely compatible with the *squarrosa* cytoplasm, as observed in two species of Timopheevi wheat, and type-III, newly found in three endemic species of Emmer wheat, showed partial compatibility with the *squarrosa* cytoplasm.

Gene analysis was conducted to study the difference in cytoplasm compatibility between type-I and type-III. The segregations were examined in the B_1F_1 seeds and plants obtained from backcrosses of F_1 normal (with *1D* chromosome) and miniature (without *1D* chromosome) plants produced by crosses of (*squarrosa*) type-I·AABB+*1D* lines × type-III AABB genome species, with type-I and type-III AABB genome species. The results suggest that two kinds of nuclear genes are responsible for the compatibility with the *squarrosa* cytoplasm in the type-III genome. One of the two alleles is related to the appearance of shrivelled seeds due to the incomplete development of the endosperm and the appearance of

chlorophyll variegation (incomplete development of chloroplast). The other, related to plant vigour in B_1F_1 plants, is considered to be the nuclear gene controlling the incomplete compatibility with the cytoplasm of *Ae. squarrosa*.

When these two kinds of alleles (the cytoplasmic compatibility genes) are designated as *Cp* (nuclear gene for chloroplast) and *Cv* (nuclear gene for plant vigour), the genotype, *Cp1* and *Cv1* is expected for the type I and *Cp2* and *Cv2* for the type-III, respectively. Segregations in the B_1F_1 lines can be interpreted as follows :

1) B_1F_1 lines backcrossed with type-I species

Cytoplasm-genome	Genotype	Phenotype
(*squarrosa*) AABB	*Cp1 Cp2, Cv1 Cv2*	Miniature plant :
(*squarrosa*) AABB	*Cp1 Cp2, Cv1 Cv1*	chlorophyll variegation, low plant vigour
(*squarrosa*) AABB	*Cp1 Cp1, Cv1 Cv2*	Abortive seed :
(*squarrosa*) AABB	*Cp1 Cp1, Cv1 Cv1*	zygotic lethality

2) B_1F_1 lines backcrossed with type-III species

Cytoplasm-genome	Genotype	Phenotype
(*squarrosa*) AABB	*Cp2 Cp2, Cv2 Cv2*	Intermediate-I plant : no chlorophyll variegation, slightly low plant vigour
(*squarrosa*) AABB	*Cp2 Cp2, Cv1 Cv2*	Weak plant : no chlorophyll variegation, low plant vigour
(*squarrosa*) AABB	*Cp1 Cp2, Cv2 Cv2*	Intermediate-II plant : chlorophyll variegation, slightly low plant vigour
(*squarrosa*) AABB	*Cp1 Cp2, Cv1 Cv2*	Miniature plant : chlorophyll variegation, low plant vigour

As for the *Cp* allele (the two genes for chloroplast development), *Cp1* is incompatible with the cytoplasmic factor of *Ae. squarrosa*, and the *Cp2* gene is partly compatible with the cytoplasmic factor of *Ae. squarrosa*. Therefore, the genotype of *Cp1 Cp1* with the *squarrosa* cytoplasm leads to abortive seeds or zygotic lethality (abortion of endosperm), and the genotype of *Cp1 Cp2* with the *squarrosa* cytoplasm to shrivelled seeds and chlorophyll variegation in plants as a result of incomplete development of the chloroplast.

As for the *Cv* allele (the two genes for plant vigour), *Cv1* shows a very low compatibility with the cytoplasmic factor of *Ae. squarrosa*, and the *Cv2* gene a partial compatibility with the cytoplasmic factor of *Ae. squarrosa*. Therefore, the genotype of *Cv2 Cv2* with the *squarrosa* cytoplasm leads to the subnormal plant vigour observed in the intermediate-I and intermediate-II plants, and the genotype of *Cv1 Cv2* with the *squarrosa* cytoplasm to the low plant vigour

observed in the weak and miniature plants.

In addition, it can be deduced that the type-II AG genome also harbours the two kinds of alleles (*Cp3* and *Cv3* genes) for the cytoplasmic factor(s) of Timopheevi wheat, and these two genes show a complete compatibility with the cytoplasmic factor(s) of *Ae. squarrosa*. Therfore, the F_1 zygotes of *Cp1 Cp3, Cv1 Cv3* with the *squarrosa* cytoplasm produce normal seeds and normal plants, without abnormality of the chloroplast and plant vigour.

CHAPTER III. Phylogenic relationships of polyploid wheat species based on responses to *Aegilops squarrosa* cytoplasm

Introduction

The phylogeny of polyploid wheat species has been intensively studied in terms of genetic relationships, geographical distributions and archaeological evidences[33,38,69,43,50,73,74,93,102,107]. However, the origin of Dinkel wheats (AABBDD genome species) has not yet been clarified completely, particularly regarding the tetraploid progenitor(s)[43] due to the genetic diversity of Emmer wheats (AABB genome species).

The present experiments were conducted to obtain further evidence on the genetic relationships among the AABB genomes of Emmer wheat and Dinkel wheat, with special reference to the compatibility with the cytoplasm derived from *Aegilops squarrosa*. In addition the origin of Dinkel wheat was, also, estimated.

Materials and Methods

A total of 68 strains of tetraploid wheat were supplied by Prof. M. TANAKA, Kyoto University and 21 by Prof. G. KIMBER, University of Missouri. Other strains of tetraploid or hexaploid wheat used in the present experiments have been maintained at the Kihara Institute for Biological Research.

The (*squarrosa*) AABB+*1D* lines used as female testers were : (*squarrosa*) *T. turgidum* var. *nigro-barbatum* +*1D* line and (*squarrosa*) *T. durum* var. *Reichenbachii* +*1D* (isochromosome) line, both bred in the experiments described in Chapter I, and (*squarrosa*) *T. dicoccoides* var. *spontaneo-nigrum* +*1D* line and (*squarrosa*) *T. dicoccoides* var. *Aaronsohni* +*1D* line, both later bred from (*squarrosa*) *T. durum* var. *Reichenbachii* +*1D* line. The (*squarrosa*) AABB+*1D-1A* lines also used as the testers were : (*squarrosa*) *T. dicoccoides* var. *spontaneo-nigrum* +*1D-1A* line and (*squarrosa*) *T. dicoccoides* var. *Aaronsohni* +*1D-1A* line, both newly derived from the original (*squarrosa*) AABB+*1D* line of *T. dicoccoides* var. *spontaneo-nigrum* described in Chapter I, and these were identi-

fied as the $+1D-1A$ monosomic substitution lines by a cross experiment using a disomic substitution line of *T. durum* cv. Langdon, the *T. durum* $1D(1B)$ line, bred by Prof. L. R. JOPPA[18], North Dakota State University.   The Emmer wheat strains which transmitted the AABB genome (type-I) to these alloplasmic lines are No. 94, No. 68, No. 34, No. 35, No. 34, No. 35, (presented in order of the tetraploid wheat code number shown in Table 21).

Cross experiments, morphological studies and a survey of the chromosome number were carried out using materials cultivated in a greenhouse and field in Yokohama.

Results and Discussion

1. Classification of tetraploid wheat strains based on response to *squarrosa* cytoplasm

a) Response types in relation to the genomic compatibility with *squarrosa* cytoplasm

It was disclosed in the experiments in Chapter II that the cytoplasm compatibility genes have been genetically differentiated in nuclear genomes of tetraploid wheat species.   This differentiation was observed in three types, based on the compatibility with the cytoplasm of *Ae. squarrosa*, i. e. incompatible (type-I), completely compatible (type-II), and partly compatible (type-III).

Table 20 shows the compatibility of the three types in the F_1 seed and seedling development in crosses using the (*squarrosa*) AABB$+1D$ lines as female parents with various tetraploid wheat strains as male parents.   The (*squarrosa*) AABB$+1D$ lines are alloplasmic lines with $1D$ chromosome in monosomic addition whose AABB genome donors belong to the type-I species.

Table 20. Types of tetraploid wheat nuclei compatible with *squarrosa* cytoplasm identified from seed and seedling development of F_1 in crosses to (*squarrosa*)AABB$+1D$ lines of type-I Emmer wheat species

Type of male parent	Seed			Seedling	
	Viable		Abortive (zygotic lethal)	Normal plant	Miniature plant (variegated)
	Normal	Shrivelled			
Type-I (AABB genome)	(*sq*)AABB$+1D$	——	(*sq*)AABB	(*sq*)AABB$+1D$ (2n= 29)	————
Type-II (AAGG genome)	(*sq*)AABG$+1D$ (*sq*)AABG	———	——	(*sq*)AABG$+1D$ (*sq*)AABG (2n=29 & 28)	——
Type-III (AABB genome)	(*sq*)AABB$+1D$	(*sq*)AABB	——	(*sq*)AABB$+1D$ (2n=29)	(*sq*) AABB (2n=28)

When type-I strains of tetraploid wheat were the male parents, only the F_1 seeds to which the $1D$ chromosome had been transmitted from female gametes were normal, those not receiving the chromosome being abortive due to zygotic lethality.   As a result, all the F_1 seedlings produced carried the $1D$ chromosome (chromosome number, 2n=29) and developed into normal plants with normal growth by the effects of cytoplasm compatibility gene(s) located on the $1D$ chromosome.

When type-II strains of tetraploid wheat were the male parents, the F_1 seeds did not show any segregation on the basis of $1D$ chromosome transmission and were all normal seeds.   In addition, all the seeds developed into normal plants, regardless of whether their chromosome number was 2n=29 (with $1D$ chromosome) or 2n=28 (without $1D$ chromosome).

When type-III strains of tetraploid wheat were the male parents, the F_1 seeds showed segregation into normal seeds with well-developed endosperm and shrivelled seeds with incompletely developed endosperm in accordance to the $1D$ chromosome transmission.   The F_1 seedlings also showed segregation into normal plants (with $1D$ chromosome) and miniature plants (without $1D$ chromosome) with considerable chlorophyll variegation under low temperatures and an extremely low plant vigour.

Data from cross experiments with (*squarrosa*) AABB+ $1D$-$1A$ lines, with $1D$ chromosome in monosomic substitution, are also given in this chapter.   In this case, the compatibility with the *squarrosa* cytoplasm, or segregations in F_1 seeds and plant types, were the same as in the former case (cross experiments with $1D$ monosomic addition lines), though the frequencies of abortive seeds, shriveled seeds, and miniature plants differed due to the difference in the $1D$ chromosome transmission rate ; in the former case, about 25%, in the latter, about 40%.

Using the three segregation patterns in F_1 seeds and plants as indications, the various strains of tetraploid wheat used as male parents in the cross experiments were classified into three types on the basis of the compatibility with the *squarrosa* cytoplasm, i. e. type-I, type-II, and type-III.

b) Classification of 104 strains of tetraploid wheat species on the basis of compatibility response to *squarrosa* cytoplasm appearing in the F_1 seeds and seedlings

Tables 21 and 22 show data obtained from the examination of the segregation in F_1 seeds and seedlings in crosses using 104 strains of tetraploid wheat, including wild and cultivated species of Emmer and Timopheevi wheat, as male parents and (*squarrosa*) AABB+ $1D$ and/or (*squarrosa*) AABB+ $1D$-$1A$ lines as female parents.   They were classified into three types based on the segregation patterns as mentioned before.

The 104 strains of tetraploid wheat were classified into three types on the basis of the *squarrosa* cytoplasm compatibility and the results obtained from the

Table 21. Segregations in F_1 seeds and seedlings in crosses to *(squarrosa)*AABB+*1D* lines with various tetraploid wheat species, and estimated types of the compatibility with *squarrosa* cytoplasm of their tetraploid wheat species

Code No.	Species (Genome formula) Variety (Genetic stock No.*)	Seeds Viable Normal	Seeds Viable Shrivelled	Seeds Abortive (zygotic lethal)	Seedlings Normal plants	Seedlings Miniature plants	Estimated type of the compatibility of male parent
	Wild Timopheevi wheat						
	Mesopotamian						
	T. araraticum (AAGG)						
1	var. *Nachitchevanicum* (KU-8478)	24	0	0	24	0	type-II
2	" (KU-8544)	29	0	0	28	0	"
3	" (KU-8601)	12	0	0	12	0	"
4	" (KU-8685)	16	0	0	16	0	"
5	" (KU-8700)	31	0	0	---	---	"
6	" (KU-8819)	10	0	0	---	---	"
7	" (KU-8732)	14	0	0	12	0	"
8	" (KU-8784)	33	0	0	---	---	"
9	" (KU-8821B)	136	0	0	25	0	"
10	" (KU-8822)	88	0	0	36	0	"
11	" (KU-8866)	28	0	0	27	0	"
12	" (KU-8884)	52	0	0	49	0	"
13	" (KU-8912)	16	0	0	16	0	"
14	" (KU-8919)	27	0	0	27	0	"
15	" (KU-8933)	15	0	0	15	0	"
16	" (KU-8940)	41	0	0	22	0	"
17	" (KU-8947)	8	0	0	8	0	"
18	var. *Thumaniani* (KU-8770)	27	102	0	13	29	type-III
19	" (KU-8944)	19	0	0	16	0	type-II
	Transcaucasian						
	T. araraticum (AAGG)						
20	var. *Thumaniani* (KU-196-1)	169	0	0	37	0	type-II
21	" (KU-196-2)	508	0	1	159	0	"
22	" (KU-1907A)	36	0	0	19	0	"
23	" (KU-1908A)	47	0	1	27	0	"
24	" (KU-1909A)	15	0	0	14	0	"
25	" (KU-1909C)	44	0	0	41	0	"
	Wild Emmer wheat						
	Mesopotamian						
	T. dicoccoides (AABB)						
26	var. *Kotschyanm* (KU-8539)	4	0	11	4	0	type-I
27	" (KU-8808)	29	245	1	12	48	type-III
28	" (KU-8937B)	1	0	5	---	---	type-I
29	var. *spontaneo-villosum* (KU-8736A)	---	---	---	---	---	---
30	var. *Aaronsohni* (KU-8821A)	28	146	1	18	17	type-III
31	" (KU-8821C)	40	181	0	25	10	"
32	var. *fulvo-villosum* (KU-8915A)	---	---	---	---	---	---
33	" (KU-8942)	3	0	26	---	---	type-I
	Syrio-Palestinian						
	T. dicoccoides (AABB)						
34	var. *spontaneo-nigrum* (KIBR)	57	0	412	29	0	type-I
35	var. *Aaronsohni* (KIBR)	64	0	228	24	0	"
36	var. *fulvo-villosum* (KIBR)	29	0	87	---	---	"
37	var. *Kotschyanm* (KIBR)	43	0	117	---	---	"

[Table 21. continued]

Male parent		Seeds			Seedlings		Estimated type of the compatibility of male parent
		Viable		Abortive (zygotic lethal)	Normal plants	Miniature plants	
Code No.	Species (Genome formula) Variety (Genetic stock No.*)	Normal	Shrivelled				
	Cultivated Timopheevi wheat						
	T. timopheevi (AAGG)						
38	var. *typicum* (KU-107-1)	262	0	0	66	0	type-II
39	" (KU-107-2)	34	0	0	32	0	"
40	" (KU-107-3)	66	0	0	31	0	"
41	" (KU-107-4)	10	0	1	9	0	"
42	" (KU-107-5)	22	0	0	13	0	"
43	" (KU-1818)	32	0	0	22	0	"
44	" (KU-1819)	70	0	1	32	0	"
45	var. *viticulosum* (KU-1820)	12	0	0	9	0	"
	" (KU-1821)	51	0	0	28	0	"
	Cultivated Emmer wheat						
	T. dicoccum (AABB)						
47	var. *farrum*, Hokudai (KIBR)	41	0	66	40	0	type-I
48	var. *arras*, Khapli (KU-112)	71	0	201	---	---	"
49	var. *farrum*, Emmer (KU-113)	30	0	73	---	---	"
50	var. *farrum* (KU-123)	5	0	12	---	---	"
51	" (TT25, G-800) (from Dr. G. Kimber as *T. amyleum*)	18	0	74	---	---	"
52	var. *atratum*, French 57 (KU-114)	22	0	100	---	---	"
53	" , Emmer (KU-115)	10	0	55	---	---	"
54	" (TT32, P71-52-1)	8	0	60	---	---	"
55	var. *rufum*, Russian 26 (KU-117)	19	0	49	---	---	"
56	" , Vernal (KU-124)	5	0	17	---	---	"
57	" (TT17, G-936)	19	0	70	---	---	"
58	var. *tricoccum* (TT14, G-497)	23	0	70	---	---	"
59	" (TT16, G-581)	12	0	52	---	---	"
60	var. *nigro-ajar* (TT15, G-597)	16	0	61	---	---	"
61	var. *macroantherium* (TT18, G-1007)	8	0	72	---	---	"
62	var.--- (TT31, G-799) (from Dr. G. Kimber as *T. abyssinicum*)	29	0	133	---	---	"
63	var. --- (---) (from China as "Russian perennial")	49	0	195	---	---	type-I
	T. palaeocolchicum (AABB) (=*georgicum*)						
64	var. *schwamilicum* (KIBR)	20	131	0	27	17	type-III
65	" (KU-190-2)	16	82	0	11	13	"
66	var. --- (KU-156)	13	47	0	6	3	"
67	var. --- (KU-191)	3	17	0	2	1	"
	T. durum (AABB)						
68	var. *Reichenbachii* (KIBR)	73	0	587	33	0	type-I
69	var. *coerulescens* (KU-126)	9	0	68	---	---	"
70	var. *hordeiforme* (KU-127)	11	0	39	---	---	"
71	" , Bansi (KU-130)	10	0	37	---	---	"
72	var. *melanopus* (KU-128-1)	33	0	75	21	0	"

(continued)

[Table 21. continued]

Code No.	Species (Genome formula) Variety (Genetic stock No.*)	Seeds Viable Normal	Seeds Viable Shrivelled	Abortive (zygotic lethal)	Seedlings Normal plants	Seedlings Miniature plants	Estimated type of the compatibility of male parent
	Male parent						
	(Cultivated Emmer wheat)						
	[*T. durum* (AABB)]						
73	var. *africanum* (KU-129-1)	23	0	70	---	---	type- I
74	var. *murciense*, pentad (KU-135)	21	0	44	---	---	"
75	var. *obscurum*, Stewart (KU-136)	8	0	53	---	---	"
76	var. *provinciale* (TT20, G-576)	16	0	88	---	---	"
77	var. *aestivum* (TT21, G-839)	6	0	42	---	---	"
78	var. *duro-compactum* (TT22, G-1063)	9	0	44	---	---	"
79	var. *libycum* (TT27, G-998) (from Dr. G. Kimber as *T. atratum*)	9	0	47	---	---	"
	T. persicum (AABB) (=*carthlicum*)						
80	var. *stramineum* (KIBR)	28	165	0	18	98	type-III
81	" (KU-1800)	12	85	0	8	19	"
82	" (KU-1801)	13	59	0	4	2	"
83	" (KU-1807)	30	0	135	9	0	type- I
84	" (KU-1808)	28	87	1	13	17	type-III
85	var. *fuliginosum* (KIBR)	21 (237)**	113	0	35	111	"
86	var. *rubiginosum* (KU-1632)	39	174	0	13	28	"
87	var. --- (TT42, RL5205)	33	126	1	9	27	"
88	var. --- (TT43, RL5320)	8	14	0	---	---	"
89	var. --- (TT45, RL5415)	5	42	0	5	30	"
	T. pyramidale (AABB)						
90	var. *recognitum* (KIBR)	33 (189)**	101	0	26	59	type-III
91	" (TT23, G-567)	15	63	0	9	2	"
92	var. *compiticum*, Dakker 52 (KIBR)	24	114	0	11	4	"
93	var. ---, Baladi 116 (KIBR)	32 (159)**	93	0	29	51	"
	T. turgidum (AABB)						
94	var. *nigro-baro-barbatum* (KIBR)	111	0	359	79	0	type- I
95	ssp. *compactum* (TT26, G-922)	16	0	24	---	---	"
96	" , SNS (KIBR)	14	0	34	---	---	"
	T. orientale (AABB) (=*turanicum*)						
97	var. *insigne* (KIBR)	33	0	133	13	0	type- I
98	var. *notabile* (TT24, G-568)	33	159	0	7	17	type-III
99	" (TT34, G-999) (from Dr. G. Kimber as *T. farrum*)	26	70	0	12	9	"
	T. aetiopicum (AABB)						type- I
100	var.--- (KIBR)	34	0	179	---	---	"
101	var. --- (KU-187)	55	0	169	6	0	"
	T.polonicum (AABB)						
102	var. *vestitum* (KIBR)	232	0	323	99	0	type- I
103	var. *nigro-barbatum* (TT29, G-991)	11	0	65	---	---	"
	T. isphahanicum (AABB)						
104	var. --- (KIBR)	15	0	107	---	---	type- I

* KU-: collection number in Plant Germ-plasm Institute of Kyoto University, TT-: collection number of Dr. G. Kimber, University of Missori, G-: collection number of University of California, Riverside, RL-: collection number of Dr. E. R. Kerber, Canada Agriculture Reserch Station, P-: ERS collection number, KIBR: indication for pure lines of Kihara Institute for Biological Research.

** Normal and shrivelled seeds were included, but could not be classified precisely into two categories.

Table 22. Segregations in F_1 seeds and seedlings in crosses to *(squarrosa)* AABB+ *1D* −*1A* lines with various tetraploid wheat species, and estimated types of the compatibility with *squarrosa* cytoplasm of their tetraploid wheat species

Code No.	Species (Genome formula) Variety (Genetic stock No.*)	Viable Normal	Viable Shrivelled	Abortive (zygotic lethal)	Normal plants	Miniature plants	Estimated type of the compatibility of male parent
	Wild Timopheevi wheat						
	Mesopotamian						
	T. araraticum (AAGG)						
1	var. *Nachitchevanicum* (KU-8478)	58	0	0	...	...	type-II
2	〃 (KU-8544)	53	0	0	...	...	〃
3	〃 (KU-8601)	72	0	0	22	0	〃
4	〃 (KU-8685)	69	0	0	...	...	〃
5	〃 (KU-8700)	46	0	0	21	0	〃
6	〃 (KU-8719)	72	0	0	26	0	〃
7	〃 (KU-8732)	86	0	0	19	0	〃
8	〃 (KU-8784)	36	0	0	20	0	〃
9	〃 (KU-8821B)	46	0	0	46	0	〃
10	〃 (KU-8822)	48	0	0	24	0	〃
	Transcaucasiam						
	T. araraticum (AAGG)						
21	var. *Thumaniani* (KU-196-2)	49	0	0	23	0	type-II
	Wild Emmer wheat						
	Mesopotamian						
	T. dicoccoides (AABB)						
26	var. *Kotschyanum* (KU-8539)	43	0	24	...	...	type-I
27	〃 (KU-8808)	(51)**		0	27	18	type-III
28	〃 (KU-8937B)	40	0	26	27	0	type-I
29	var. *spontaneo-villosum* (KU-8736A)	29	0	28	24	0	〃
30	var. *Aaronsohni* (KU-8821A)	(58)**		0	18	10	type-III
31	〃 (KU-8821C)	12 (37)**	13	0	24	14	〃
32	var. *fulvo-villosum* (KU-8915A)	13	0	6	12	0	type-I
33	〃 (KU-8942)	38	0	19	13	0	〃
	Syrio-Palestinian						
	T. dicoccoides (AABB)						
34	var. *spontaneo-nigrum* (KIBR)	165	0	163	...	......	type-I
35	var. *Aaronsohni* (KIBR)	95	0	80	...	...	〃
36	var. *fulvo-villosum* (KIBR)	36	0	11	...	...	〃
37	var. *Kotschyanum* (KIBR)	26	0	12	...	...	〃
	Cultivated Emmer wheat						
	T.dicoccum (AABB)						
47	var. *farrum*, Hokudai (KIBR)	26	0	21	...	...	type-I
48	var. *arras*, Khapli (KU-112)	20	0	18	...	...	〃
	T. persicum (AABB)						
80	var. *straminaeum* (KIBR)	14	11	0	14	11	type-III
	T. pyramidale (AABB)						
90	var. *recognitum* (KIBR)	(34)**		0	10	5	type-III
93	var. ---, Baladi 116 (KIBR)	32	30	0	25	13	〃

* KU-: collection number in Plant Germ-plasm Institute of Kyoto University, TT-: collection number of Dr. G. Kimder, University of Missouri, G-: collection number of University of California, Riverside, RL-: collection number of Dr. E. R. Kerber, Canada Agriculture Research Station, P-: ERS collection number, KIBR: indication for pure lines of Kihara Institute for Biological Research.
** Normal and shrivelled seeds were included, but could not be classified precisely into two categories.

segregation experiments on F_1 seeds and plant types were considered collectively. These differences are due to the differentiation of the cytoplasm compatibility genotype among the AABB and AAGG genomes of tetraploid wheat.

The wild Emmer wheat strains belonging to type-I consist of five strains among the eight Mesopotamian *T. dicoccoides* strains and all four Syrio-Palestinian *T. dicoccoides* strains. As for the cultivated Emmer wheat, all the seventeen *T. dicoccum* strains, all the twelve *T. durum* strains, one of the ten *T. persicum* (= *carthlicum*) strains, all the three *T. turgidum* strains, one of the three *T. orientale* (= *turanicum*) strains, both *T. aethiopicum* strains, and one *T. isphahanicum* strain were classified as type-I. In other words, more than half of the wild Emmer wheat strains and the majority of the cultivated Emmer wheat species with the AABB genome were classified as type-I strains.

The type-II strains consisted of eighteen of the nineteen Mesopotamian *T. araraticum* strains, all the six Transcaucasian *T. araraticum* strains (wild Timopheevi wheat), and all the nine *T. timopheevi* strains (cultivated Timopheevi wheat, endemic in Transcaucasia). In other words, all but one exceptional Mesopotamian wild strain of the wild and cultivated Timopheevi wheat species with the AAGG genome were classified as type-II.

The type-III strains consisted of three of the eight Mesopotamian *T. dicoccoides* strains (wild Emmer wheat), all the four *T. palaeocolchicum* (= *georgicum*, endemic species of Transcaucasia) strains, nine of the ten *T. persicum* (= *carthlicum*, endemic species of Transcaucasia) strains, all the four *T. pyramidale* strains, two of the three *T. orientale* (= *turanicum*) strains (cultivated Emmer wheat) and one of the nineteen Mesopotamian *T. araraticum* strains (wild

Table 23. Classification of tetraploid wheat species based on difference of response of their nuclei to the cytoplasm of *Aegilops squarrosa*

	Type-I species	Type-II species	Type-III species
Wild	Syrio-Palestinian *T. dicoccoides*(AABB); 4 strains	Mesoptamian* *T. araraticum*(AAGG); 18 strains	Mesopotamian* *T. araraticum*(AAGG); 1 strain
	Mesopotamian* *T. dicoccoides*(AABB); 5 strains	Transcaucasian *T. araraticum*(AAGG); 6 strains	Mesopotamian* *T. dicoccoides* (AABB); 3 strains
Cultivated			
Husked	*T. dicoccum*(AABB); 17 strains	*T. timopheevi*(AAGG); 9 strains	*T. palaeocolchicum* (=*georgicum*)(AABB); 4 strains
Naked	*T. persicum* (=*carthlicum*)(AABB); 1 strain		*T. persicum* (=*carthlicum*)(AABB); 9 strains
	T. orientale (=*turanicum*)(AABB); 1 strain		*T. orientale* (=*turanicum*)(AABB); 2 strains
	T. durum(AABB); 12 strains		*T. pyramidale*(AABB); 4 strains
	T. turgidum(AABB); 3 strains		
	T. aethiopicum(AABB); 2 strains		
	T. polonicum(AABB); 2 strains		
	T. isphahanicum(AABB); 1 strain		

* Wild strains collected from northern highlands of Mesopotamia (Zagros Mountains) by Dr. M. Tanaka *et al.* during the Botanical Expedition of Kyoto University to the Northern Highlands of Mesopotamia (BEM) in 1970

Timopheevi wheat). In other words, part of the wild Emmer wheat, some endemic species (cultivated) of Emmer wheat, and one exceptional strain of the wild Timopheevi wheat were classified as type-III.

In Table 23, a total of 104 strains of tetraploid wheat species were classified according to the compatibility response to the *squarrosa* cytoplasm. No difference was observed among the strains belonging to the same species, with the exception of four strains of wild tetraploid wheat of Mesopotamia, one strain of *T. persicum* and one strain of *T. orientale*. These results suggest that the genetic differentiation in the cytoplasm compatibility among tetraploid wheat nuclear genomes corresponds to the phylogenic differentiation of tetraploid wheat species.

2. Identification of response types of AABB genome involved in Dinkel wheat strains on the basis of compatibility with *squarrosa* cytoplasm

In 104 tetraploid wheat strains, the genetic differentiation of the cytoplasm compatibility in their nuclear genomes (AABB and AAGG) was analysed using the *squarrosa* cytoplasm as a tester. The strains were classified into three types, i. e. type-I, type-II, and type-III.

In particular, it was demonstrated that in Emmer wheat, one or some of the cultivated species of which the AABB genome and cytoplasm were transmitted to Dinkel wheat (AABBDD) at the origin, type-I and type-III species had differentiated from the wild species, and that in the cultivated species those two kinds of differentiation had occurred at the species level, except in some cases.

To further clarify the phylogenic relationships between Emmer and Dinkel wheats, the AABB genome composing the AABBDD genome of various Dinkel wheat strains was identified as type-I or type-III. Dinkel wheat strains are all hexaploid species with the AABBDD genome, thus having a pair of *1D* chromosomes in their nuclei which ensures complete compatibility with the *squarrosa* cytoplasm. Consequently, in order to identify the cytoplasm compatibility of the AABB genome involved in various Dinkel wheat strains, it was necessary to examine the nuclear-cytoplasm compatibility under conditions in which the effects of the *1D* chromosomes had been eliminated.

a) Breeding of *(squarrosa)* AABBD pentaploid hybrids with various Dinkel wheat strains

Table 24 shows the crossings for the pentaploid hybrids (AABBD genome) with the *squarrosa* cytoplasm and with the cytoplasm of various Dinkel wheat strains.

The alloplasmic Dinkel wheat strains with the *squarrosa* cytoplasm used as the female parents were the lines bred by successive backcrossings as described in Chapter I. A total of four Dinkel wheat species (five strains) were used as the nuclear donor hexaploid species, i. e. one variety of *T. spelta* and one variety of

Table 24. Crossing experiments to produce *squarrosa* cytoplasm and euplasmic pentaploid (AABBD) hybrids

Female parent (AABBDD genome)		Male parent (AABB genome)	Florets crossed	Seed sets
		Type- I Emmer wheat		
(eu) T. spelta var. *duhamelianum**	×	*T. turgidum* var. *nigro-barbatum*	56	41
(sq) T. spelta var. *duhamelianum***	×	*" "*	135	91
		Type-III Emmer wheat		
(eu) T. spelta var. *duhamelianum**	×	*T. palaeocolchicum (=georgicum)*	65	37
		var. *schwamilicum*		
(sq) T. spelta var. *duhamelianum***	×	*" "*	79	58
		Type- I Emmer wheat		
(eu) T. macha var. *sub-letschumicum**	×	*T. turgidum* var. *nigro-barbatum*	68	45
(sq) T. macha var. *sub-letetschumicum***	×	*" "*	108	90
		Type-III Emmer wheat		
(eu) T. macha var. *sub-letschumicum**	×	*T. palaeocolchicum (=georgicum)*	110	15
		var. *schwamilicum*		
(sq) T. macha var. *sub-letschumicum***	×	*" "*	104	15
		Type- I Emmer wheat		
(eu) T. aestivum (= vulgare)	×	*T. turgidum* var. *nigro-barbatum*	86	63
var. *erythrospermum (T. v. e.)**				
(sq) T. aestivum (= vulgare)	×	*" "*	136	87
var. *erythrospermum (T. v. e.)***				
		Type-III Emmer wheat		
(eu) T. aestivum (= vulgare)	×	*T. persicum (=carthlicum)*	46	15
var. *erythrospermum (T. v. e.)**		var. *stramineum*		
(sq) T. aestivum (= vulgare)	×	*" "*	48	23
var. *erythrospermum (T. v. e.)***				
		Type- I Emmer wheat		
(eu) T. aestivum cv. Selkirk*	×	*T. durum* var. *Reichenbachii*	34	19
(sq) T. aestivum cv. Selkirk**	×	*" "*	32	23
		Type- I Emmer wheat		
(eu) T. aestivum cv. Selkirk*	×	*T. turgidum* var. *nigro-barbatum*	68	50
(sq) T. aestivum cv. Selkirk **	×	*" "*	64	37
		Type-III Emmer wheat		
(u) T. aestivum cv. Selkirk*	×	*T. persicum (=carthlicum)*	32	11
		var. *fuliginosum*		
(sq) T. aestivum cv. Selkirk**	×	*" "*	26	10
		Type- I Emmer wheat		
(eu) T. compactum var. *Humboldti**	×	*T. turgidum* var. *nigro-barbatum*	32	25
(sq) T. compactum var. *Humboldti***	×	*" "*	28	22

* Euplasmic *(eu)* lines of respective Dinkel wheats.
** Alloplasmic lines of respective Dinkel wheats with *squarrosa* cytoplasm.

Table 25. Experimental scheme for identification of AABB genome involved in Dinkel wheat in relation to compatibility with *spuarrosa* cytoplasm

Cross combination	B_1F_1 seed		B_1F_1 seedling		Type of AABB genome of Dinkel wheat
	Viable	Abortive	Normal	Variegated	
[(*squarrosa*)Dinkel wheat × type- I Emmer wheat] × type- I Emmer wheat	+ (with *1D*)	+ (without *1D*)	+ (with *1D*)	−	type- I
[(*squarrosa*)Dinkel wheat × type- I Emmer wheat] × type- I Emmer wheat	+ (with *1D* and/or type-III gene*)	+ (without *1D* and type-III gene*)	+ (with *1D*)	+ (without *1D* but with type-III gene*)	type-III
[(*squarrosa*)Dinkel wheat × type-III Emmer wheat] × type- I Emmer wheat	+ (with *1D* and/or type-III gene*)	+ (without *1D* and type-III gene*)	+ (with *1D*)	+ (without *1D* but with type-III gene*)	type- I
[(*squarrosa*)Dinkel wheat × type-III Emmer wheat] × type- I Emmer wheat	+ (with *1D* and/or type-III gene*)	−	+ (with *1D*)	+ (without *1D* but with type-III gene*)	type-III

* *Cp2* gene of type-III AABB genome species of Dinkel wheat or Emmer wheat.

T. macha (endemic species of Transcaucasia) as primitive husked Dinkel wheat, one variety and one cultivar of *T. aestivum* as the naked Dinkel wheat most common in modern wheat farming, and one variety of *T. compactum* as the naked Dinkel wheat of the old type.

Type-I Emmer wheat strains used as the male parents included *T. durum* var. *Reichenbachii* (code No. 68) and *T. turgidum* var. *nigro-barbatum* (No. 94), type-III Emmer wheat strains being *T. palaeocolchicum* var. *schwamilicum* (No. 64), *T. persicum* var. *stramineum* (No. 80), and *T. persicum* var. *fuliginosum* (No. 85).

In the crosses with *T. compactum,* euplasmic *T. compactum* and (*squarrosa*) *T. compactum* were used as the female parents and only type-I Emmer wheat as the male parent. In the crosses with the other three species (four strains) of Dinkel wheat, both type-I and type-III Emmer wheat species were used as the male parents to produce various euplasmic pentaploid hybrids and (*squarrosa*) pentaploid hybrids.

Table 25 shows the segregation expected in B_1F_1 seeds and plants in the crosses in which these (*squarrosa*) pentaploid hybrids (as female parents) were backcrossed with type-I Emmer wheat.

In the case of cross combinations such as {(*squarrosa*) Dinkel wheat × type -I Emmer wheat} × type-I Emmer wheat, the segregation in the B_1F_1 generation can be expected as follows :

(a) When the AABB genome of the Dinkel wheat belongs to type-I, only the zygotes to which the *1D* chromosome has been transmitted from female gametes produce viable B_1F_1 seeds, while those to which the *1D* chromosome has not been transmitted produce abortive B_1F_1 seeds. As a result, all the B_1

F_1 seedlings produced from these viable B_1F_1 seeds receive the *1D* chromosome ; thus all grow into normal plants.

(b) When the AABB genome of the Dinkel wheat belongs to type-III, not only the zygotes to which the *1D* chromosome has been transmitted from female gametes but also those to which the *Cp2* gene derived from the type-III AABB genome of Dinkel wheat has been transmitted produce viable B_1F_1 seeds. On the other hand, those receiving neither the *1D* chromosome nor the *Cp2* gene produce abortive B_1F_1 seeds and their percentage in the seed sets is about half that of (a). Two kinds of B_1F_1 seedlings are produced by these viable seeds, i. e. those to which the *1D* chromosome has been transmitted from female gametes and those to which only the *Cp2* gene has been transmitted. The former grow into normal plants and the latter into plants showing variegation at low temperatures.

In the case of cross combinations such as {(*squarrosa*) Dinkel wheat × type -III Emmer wheat} × type-I Emmer wheat, segregation in the B_1F_1 generation can be expected as follows :

(a) When the AABB genome of the Dinkel wheat belongs to type-I, the zygotes to which the *1D* chromosome and/or *Cp2* gene derived from the type-III Emmer wheat have been transmitted from female gametes produce viable B_1 F_1 seeds. Only those to which neither the *1D* chromosome nor the *Cp2* gene has been transmitted from female gametes produce abortive seeds and their percentage in the seed sets is about half that in (a) of the previous case, where only the transmission of the *1D* chromosome is related to.

(b) When the AABB genome of the Dinkel wheat belongs to type-III, all the B_1 ·F_1 seeds have received the *Cp2* gene, derived from type-III Emmer wheat, or from the type-III AABB genome of Dinkel wheat ; thus all the B_1F_1 seeds are viable, there are no abortive seeds, independently from the transmission of the *1D* chromosome.

As shown above, by examining the segregation modes expected to appear in B_1F_1 seeds and plants, the AABB genome of each Dinkel wheat can be identified as type-I or type-III based on the genetic differentiation of the cytoplasm compatibility gene(s).

b) B_1F_1 seeds in crosses to *(squarrosa)* pentaploid hybrids

Table 26 shows the segregation in the B_1F_1 seeds obtained from crosses in which each of the (*squarrosa*) pentaploid hybrids and euplasmic pentaploid hybrids was backcrossed with type-I Emmer wheat, to determine whether the seeds obtained were all viable or segregated into both viable and abortive types.

It may be considered that in some cross combinations some of these viable seeds developed by the action of the *Cp2* gene and not of the *1D* chromosome. However, it was impossible to detect morphological differences in the viable seeds obtained from crosses of pentaploid hybrids × tetraploid species, unlike in

Table 26. Seeds of B_1F_1 in crosses to *squarrosa* cytoplasm and euplasmic pentaploid (AABBD) hybrids with type- I Emmer wheat species

Dinkel wheat examined Cross combination	Percent of seed sets	Seeds	
		Viable	Abortive (%)
T. spelta var. *duhamelianum*			
[(*sq*)*T. spelta** × type- I Emmer wheat [1]] × type- I Emmer wheat [1]	38.6	219	111 (33.6%)
[(*eu*)*T. spelta*** × tyep- I Emmer wheat [1]] × type- I Emmer wheat [1]	56.9	141	0
[(*sq*)*T. spelta** × type-III Emmer wheat [2]] × type- I Emmer wheat [1]	35.8	236	51 (17.8%)
[(*eu*)*T. spelta*** × type-III Emmer wheat [2]] × type- I Emmer wheat [1]	41.4	264	0
T. macha var. *sub-letschumicum*			
[(*sq*)*T. macha** × type- I Emmer wheat [1]] × type- I Emmer wheat [1]	43.7	459	114 (19.9%)
[(*eu*)*T. macha*** × type- I Emmer wheat [1]] × type- I Emmer wheat [1]	52.0	579	0
[(*sq*)*T. macha** × type-III Emmer wheat [2]] × type- I Emmer wheat [1]'	45.7	649	0
[(*eu*)*T. macha*** × type-III Emmer wheat [2]] × type- I Emmer wheat [1]	50.3	474	0
T. aestivum (= vulgare) var. *erythrospermum (T. v. e.)* [(*sq*)*T. v. e.** × type- I Emmer wheat [1]] × type- I Emmer wheat [1]	24.0	152	82 (35.0%)

[continued]

the case of normal and shrivelled seed segregation in the F_1 seeds from crosses of (*squarrosa*) type-I · AABB+ *1D* lines × type-III · AABB genome species, because the viable seeds showed some fluctuations in the chromosome constitution as 2n= 28~35 along with various degrees of seed development aberrations. In addition, the seed sets in the backcrosses of (*squarrosa*) pentaploid hybrids were lower than those in the backcrosses of control euplasmic pentaploid hybrids, as a whole, presumably because in the pentaploid hybrids with the *squarrosa* cytoplasm, the fertility of the female gametes of n=14 or 15 is reduced, as reported by OHTSUKA and MATSUBARA[85].

[**Table 26.** continued]

Dinkel wheat examined Cross combination	Percent of seed sets	Seeds	
		Viable	Abortive (%)
[*T. aestivum (= vulgare)*]			
[var. *erythrospermum (T. v. e.)*]			
[*(eu)T. v. e.*** × type- I Emmer wheat [1]] × type- I Emmer wheat [1]	35.7	137	0
[*(sq)T. v. e.** × type-III Emmer wheat [3]] × type- I Emmer wheat [1]	32.2	274	42 (13.3%)
[*(eu)T. v. e.*** × type-III Emmer wheat [3]] × type- I Emmer wheat [1]	21.5	101	1
cv. Selkirk			
[*(sq)*Selkirk* × type- I Emmer wheat [4]] × type- I Emmer wheat [4]	36.8	494	286 (36.7%)
[*(eu)*Selkirk** × type- I Emmer wheat [4]] × type- I Emmer wheat [4]	49.3	354	1
[*(sq)* Selkirk* × type-III Emmer wheat [5]] × type- I Emmer wheat [4]	32.0	304	54 (15.1%)
[*(eu)*Selkirk** × type-III Emmer wheat [5]] × type- I Emmer wheat [4]	36.3	434	0
T. compactum var. *Humboldti*			
[*(sq)T. compact.** × type- I Emmer wheat [1]] × type- I Emmer wheat [1]	35.8	236	69 (22.6%)
[*(eu)T. compact.*** × type- I Emmer wheat [1]] × type- I Emmer wheat [1]	61.7	293	3

1) *T. turgidum* var. *nigro-barbatum*   2) *T. palaeocolchicum (= georgicum)* var. *schwamilicum*
3) *T. persicum (= carthlicum)* var. *stramineum*
4) *T. durum* var. *Reichenbachii* and *T. turgidum* var. *nigro-barbatum*
5) *T. persicum (= carthlicum)* var. *fuliginosum*
 * Alloplasmic lines of respective Dinkel wheats with *squarrosa* cytoplasm.
** Euplasmic lines of respective Dinkel wheats.

In the cross combinations using *T. spelta* var. *duhamelianum* as Dinkel wheat, abortive seeds were segregated at a rate of about 34% in the B_1F_1 seeds produced from the cross of {(*squarrosa*) Dinkel wheat × type-I Emmer wheat} × type-I Emmer wheat, and at a rate of about 18% in the case of {(*squarrosa*) Dinkel wheat × type-III Emmer wheat} × type-I Emmer wheat. Therefore, based on the mode of segregation in the B_1F_1 seeds, stated in section a), the AABB genome

of *T. spelta* var. *duhamelianum* was identified as type-I.

In the cross combinations using *T. macha* var. *sub-letschumicum* as Dinkel wheat, abortive seeds were segregated at a rate of about 20% in the B_1F_1 seeds produced from the cross of {(*squarrosa*) Dinkel wheat × type-I Emmer wheat} × type-I Emmer wheat, whereas no abortive seeds segregated in the case of {(*squarrosa*) Dinkel wheat × type-III Emmer wheat} × type-I Emmer wheat. As stated above, both the reduction of the abortive seed percentage in the B_1F_1 seeds in the former cross combination and the lack of abortive seeds in the B_1F_1 seeds in the latter combination are due to the effects of the *Cp2* gene from the AABB genome of Dinkel wheat in the cross combinations. Therefore, the AABB genome of the Dinkel wheat supplied as a material, that is, of *T. macha* var. *sub-letschumicum,* was identified as type-III.

In the cross combinations using two *T. aestivum* strains, i. e. var. *erythro-spermum* (*T. v. e.*) and cv. Selkirk as Dinkel wheat, abortive seeds were segregated in the B_1F_1 seeds in both crosses of {(*squarrosa*) Dinkel wheat × type-I Emmer wheat} × type-I Emmer wheat and {(*squarrosa*) Dinkel wheat × type-III Emmer wheat} × type-I Emmer wheat, as mentioned before for *T. spelta.* The percentages of abortive seeds were 35% and 37% in the former combination and 13% and 15% in the latter, i. e. almost the same values as those with *T. spelta.* Therefore, the AABB genome of the two strains of *T. aestivum* supplied as materials, i. e. var. *erythrospermum* and cv. Selkirk, was identified as type-I.

When *T. compactum* var. *Humboldti* was used as a Dinkel wheat, B_1F_1 seed segregation was examined only in the cross of {(*squarrosa*) Dinkel wheat × type -I Emmer wheat} × type-I Emmer wheat. The percentage of abortive seeds in the B_1F_1 seeds was about 23%, lower than in the type-I Dinkel wheat, i. e. *T. spelta* and two *T. aestivum* strains (34%, 35%, and 37% or so, respectively) and was closer to that of type-III Dinkel wheat, *T. macha,* (approximately 20%). There-fore, the AABB genome of *T. compactum* var. *Humboldti* was estimated to belong to type-III.

c) B_1F_1 plant types in crosses to *(squarrosa)* pentaploid hybrids

Table 27 shows the plant type segregation in the B_1F_1 lines in cross combina-tions in which the segregation of the plants with chlorophyll variegation was affected by the type of the AABB genome in the Dinkel wheat (type-I or type-III) ; that is, some of the viable B_1F_1 seeds obtained from the crosses of {(*squarrosa*) Dinkel wheat × type-I Emmer wheat} × type-I Emmer wheat and some of the B_1F_1 seeds from the euplasmic cross combination were used.

In the B_1F_1 lines of {(*squarrosa*) Dinkel wheat × type-I Emmer wheat} × type -I Emmer wheat in which *T. macha* and *T. compactum* were used as Dinkel wheat in the cross combination, plants showing chlorophyll variegation under low temperatures were segregated (13% and 9.3% respectively) in contrast to the control euplasmic B_1F_1 lines. It is considered that these plants with chlorophyll

Table 27. Plant types of B_1F_1 in crosses to *squarrosa* cytoplasm and euplasmic pentaploid (AABBD) hybrids with type- I Emmer wheat species

Dinkel wheat examined Cross combination	Plant type			
	Non varieg. plants	Varieg. plants* (%)	Died after germi.	Not germi.
T. spelta var. *duhamelianm*				
[*(sq)T. spelta*** × type- I Emmer wheat [1]] × type- I Emmer wheat [1]	131	0	4	0
[*(eu)T. spelta* *** × type- I Emmer wheat [1]] × type- I Emmer wheat [1]	132	0	6	3
T. macha var. *sub-letschumicum*				
[*(sq) T. macha* ** × type- I Emmer wheat [1]] × type- I Emmer wheat [1]	133	31 (13.0%)	40	35
[*(eu)T. macha* *** × type- I Emmer wheat [1]] × type- I Emmer wheat [1]	226	0	11	0
T. aestivum cv. Selkirk				
[*(sq)*Selkirk** × type- I Emmer wheat [2]] × type- I Emmer wheat [2]	294	0	28	7
[*(eu)*Selkirk*** × type- I Emmer wheat[2]] × type- I Emmer wheat [2]	329	0	19	6
T. compactum var. *Humboldti*				
[*(sq)T. compact.* ** × type- I Emmer wheat [1]] × type- I Emmer wheat [1]	192	22 (9.3%)	20	2
[*(eu)T. compact.* *** × type- I Emmer wheat[1]] × type- I Emmer wheat [1]	172	0	4	9

1) *T. turgibum* var. *nigro-barbatum*

2) *T. durum* var. *Reichenbachii* and *T. turgidum* var. *nigro-barbatum*

* Seedlings with chlorophyll variegation at low temperatures.

** Alloplasmic lines of respective Dinkel wheats with *squarrosa* cytoplasm.

*** Euplasmic lines of respective Dinkel wheats.

variegation have not received the *1D* chromosome derived from Dinkel wheat in each cross combination, and have received the *Cp2* gene derived from the type–III AABB genome of Dinkel wheat. Based on these results, it was observed that the AABB genome of the Dinkel wheat used in the cross combination, i. e. *T. macha* var. *sub-letschumicum* and *T. compactum* var. *Humboldti,* belongs to type–III.

In the B_1F_1 lines of { *(squarrosa)* Dinkel wheat × type-I Emmer wheat} × type –I Emmer wheat in which *T. spelta* and *T. aestivum* (cv. Selkirk) were used as the

Dinkel wheat in the cross combination, no plants with chlorophyll variegation were segregated even at low temperatures as in the control euplasmic B_1F_1 lines. Therefore, based on the expectation of segregation in the B_1F_1 generation as stated in section a) (Table 25), the AABB genome of the Dinkel wheat strains in the cross combination, i. e. *T. spelta* var. *duhamelianum* and *T. aestivum* cv. Selkirk, was identified as type-I.

d) Experiment with *(squarrosa)* mono-trisomic lines of *T. aestivum* cv. Chinese Spring

In the experiments described in Chapter I, 3, gametophytic male sterility due to the absence of *1D* chromosome under *squarrosa* cytoplasm occurred in the monosomic-*1D*-trisomic-*1A* and monosomic-*1D*-trisomic-*1B* lines of (*squarrosa*) *T. aestivum* cv. Chinese Spring, due to the degeneration of pollen grains (male gametophyte). Zygotic lethality due to the lack of *1D* chromosome also occurred in these mono-trisomic lines when they were crossed with nullisomic-*1D*-tetrasomic-*1A* and nullisomic-*1D*-tetrasomic-*1B* lines of *T. aestivum* cv. Chinese Spring, due to the degeneration of the endosperm.

These results indicate that the AABB genome of *T. aestivum* cv. Chinese Spring is completely incompatible with the *squarrosa* cytoplasm. In other words, the AABB genome of this Dinkel wheat strain (AABBDD) was identified as type -I, based on the compatibility with the *squarrosa* cytoplasm.

e) Classification of Dinkel wheat strains on the basis of compatibility of their AABB genome with *squarrosa* cytoplasm

Based on the results obtained from former experiments with the *squarrosa* cytoplasm, various species of Dinkel wheat (AABBDD) have been classified into two groups with regard to the genetic differentiation of the cytoplasm compatibility of their AABB genome, as follows.

Type-I Dinkel wheat : *T. spelta* (spelt wheat or true Dinkel ; var. *duhamelianum*), a species of primitive husked Dinkel wheat, and *T. aestivum* (bread wheat or common wheat ; var. *erythrospermum,* cv. Selkirk, and cv. Chinese Spring), a species of naked Dinkel wheat. The AABB genome of their strains showed a type-I response, that is, they were all completely incompatible with the *squarrosa* cytoplasm. Therefore, these Dinkel wheat strains have been included in the type -I Dinkel wheat.

Type-III Dinkel wheat : *T. macha* (macha wheat ; var. *sub-letschumicum*), another species of primitive husked Dinkel wheat, and *T. compactum* (club wheat ; var. *Humboldti*), a relict species of ancient naked Dinkel wheat. The AABB genome of their strains showed a type-III response, that is, they were all partly compatible with the *squarrosa* cytoplasm. Therefore, these Dinkel wheat strains have been included in the type-III Dinkel wheat.

In other words, it has been demonstrated that the cytoplasm compatibility in

the AABB genome is diphyletically differentiated in Dinkel wheat strains, as in the Emmer wheat strains, which are the tetraploid progenitor(s) (AABB genome and cytoplasm donor) of Dinkel wheat.

3. Evolutionary pathway of polyploid wheat species based on the genetic differentiation of cytoplasm compatibility

a) Genetic differentiation in tetraploid wheat species

Genetic differentiation of cytoplasm compatibility gene(s) in tetraploid wheat was analysed in the present experiments using the cytoplasm of *Ae. squarrosa* as a tester. Classification of 104 strains of tetraploid wheat into three response types (type-I, type-II, and type-III), on the basis of the compatibility with *squarrosa* cytoplasm, is summarized in Table 23 {Chapter III, 1, b)}.

As shown in Table 23, no difference was observed among the strains belonging to the same species, with the exception of four strains of Mesopotamian wild tetraploid wheat, one strain of *T. persicum* (= *carthlicum*), and one strain of *T. orientale* (= *turanicum*). This observation suggests that the genetic differentiation of the cytoplasm compatibility gene(s) in tetraploid wheat species corresponds to their phylogenic differentiation[82].

In the above-mentioned Mesopotamian wild tetraploid wheat, *T. persicum* and *T. orientale,* in which exceptional strains were observed, there have often been arguments since their discovery, as to their phylogenic status.

The Mesopotamian wild tetraploid wheat, which has been discovered comparatively recently[2,88,119], was regarded at first all as *T. dicoccoides* (AABB), a wild Emmer wheat species. However, many wild tetraploid wheat strains were collected in that area by TANAKA *et al.* in 1970 (Botanical Expedition of Kyoto University to the Northern Highlands of Mesopotamia) and subsequent cytogenetical studies revealed that the greater portion consisted of *T. araraticum* (AAGG), a wild Timopheevi wheat species, while the smaller portion of *T. dicoccoides*[104]. Later, it was shown that the two Mesopotamian wild tetraploid wheat species (wild Emmer and Timopheevi wheats) are morphologicaly similar, and that their variation is continuous[105]. In addition, it was disclosed that there are some Mesopotamian strains with cytogenetically intermediate characters between the two representative wild species, Syrio-Palestinian *dicoccoides* and Transcaucasian *araraticum*[106,107].

Among the three Mesopotamian *T. dicoccoides* strains, which showed responses of type-III, unlike the other *dicoccoides* strains (Syrio-Palestinian *dicoccoides* and the majority of Mesopotamian *dicoccoides,* type-I), the two strains (KU-8821A, code No. 30 and KU-8821C, code No. 31) corresponded to those identified by TANAKA *et al.*[106,107] as intermediate between typical AABB genome and typical AAGG genome species. ' Based mainly on the presence of these two intermediate strains (KU-8821A and KU-8821C), TANAKA *et al.*[106,107] suggested

that two series of wild tetraploid wheat (AABB and AAGG genome species) had originated monophyletically in Mesopotamia as amphidiploids between the SS genome and AA genome species and differentiated later into wild Emmer and wild Timopheevi wheats. In the present experiment, not only the three Meso-potamian *T. dicoccoides* strains (the other five among the eight Mesopotamian strains examined were of type-I) but also one Mesopotamian *T. araraticum* strain (the other eighteen among the nineteen Mesopotamian strains examined were of type-II) showed a type-III response, which further suggests the genetic continuity between both wild species (AABB and AAGG genomes) in Mesopotamia.

   T. persicum (= *carthlicum*), discovered in Transcaucasia as an endemic cultivar of wheat, was regared by VAVILOV[116] as an independent species based on its peculiar disease resistance, and classified by LILIENFELD and KIHARA[56] as Emmer wheat (AABB). However, the morphology of this species is different from that of the other Emmer wheat species and the species is very similar to *T. aestivum*, a species of Dinkel wheat (AABBDD). Therefore, VAVILOV[117] did not consider it as a true Emmer wheat but as a secondary species of tetraploid wheat derived from a pentaploid hybrid between the species of Dinkel and Emmer wheats, and it received many genes from the Dinkel wheat.

   T. orientale (= *turanicum*), which is endemic in oasis farming in the Khorasan district, Iran, has been regarded as a strain produced by introgressive hybridiza-tion between cultivated Emmer wheat and *T. dicoccoides* since its discovery by PERCIVAL[87], based on the fact that the species is morphologically isolated from the other species of cultivated Emmer wheat and has many characteristics (for example ; large-long seed) peculiar to *T. dicoccoides,* a wild Emmer wheat.

   In each of these two species, which appear to be of hybrid origin, two response types, i. e. type-I and type-III, coexist in the same species. Their taxonomic position should be determined on the basis of phylogenic relationships among all polyploid wheats, including Dinkel wheat.

b) Polyphyletic origin of cultivated Emmer wheat and diphyletic origin of Dinkel wheat

   The results obtained in the present experiments revealed that two response types (type-I and type-III) were genetically differentiated with regard to the *squarrosa* cytoplasm compatibility of the AABB genome of Dinkel wheat as well as the AABB genome of Emmer wheat. Dinkel wheat inherited the cytoplasm from Emmer wheat when it was formed as an amphidiploid species between Emmer wheat and *Ae. squarrosa*[36]. Therefore, type-I Dinkel wheat has inherited the cytoplasm and AABB genome from type-I Emmer wheat and type-III Dinkel wheat from type-III Emmer wheat, respectively. This observation may indicate the diphyletic maternal origin in the phylogeny of Dinkel wheats[83].

   Based on results obtained from the examination of the response to the *squarrosa* cytoplasm, a hypothesis is proposed as to the evolutionary pathway of
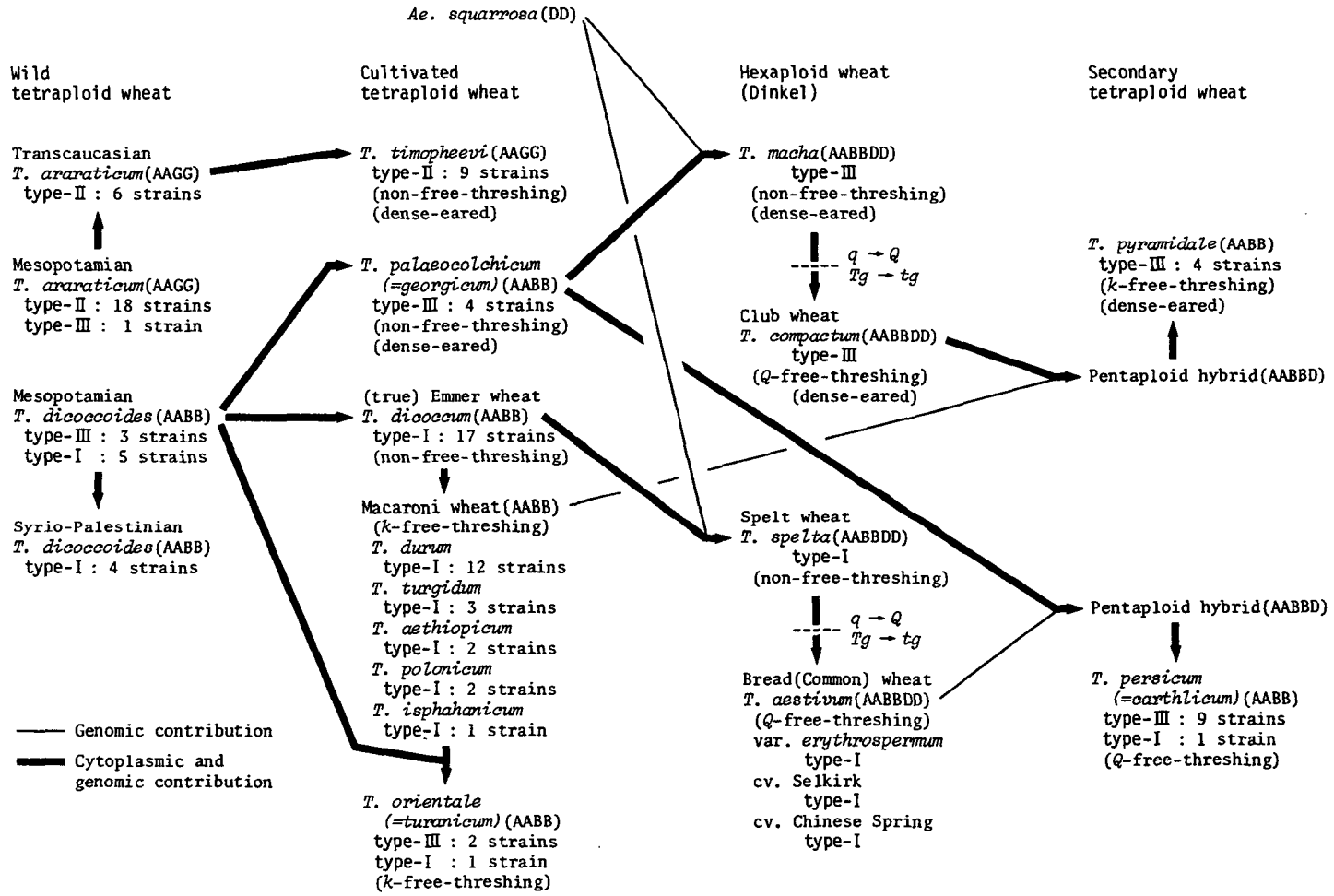
Fig. 14. Evolutionary pathways of polyploid wheats based on the response of their first and second genomes to *Ae. squarrosa* cytoplasm.

polyploid wheats in Fig. 14, with special references to the geographical distribution[13], archaeological evidence[11,89,93], and the genetic mechanism of the free-threshing character[19,69,120]. The maternal (thick arrows) and paternal (thin arrows) phylogeny is shown in Fig. 14.

In the present hypothesis, the establishment of cultivated Emmer wheats is explained from the point of view of polyphyletic origin.

Archaeological evidence suggests that the cultivation of Emmer wheat began in the mountainous districts in northern Mesopotamia. Also, the present results show that the type-I and type-III wild species of *T. dicoccoides* coexist in accordance with the two genetic types of cultivated Emmer wheat, in the northern Mesopotamia highlands. Therefore, it can be deduced that the two primitive cultivated husked Emmer wheat species, *T. dicoccum* (type-I) and *T. palaeocolchicum* (= *georgicum*, type-III) independently originated from Mesopotamian type -I *T. dicoccoides* and Mesopotamian type-III *T. dicoccoides* strains, respectively.

T. durum, or macaroni wheat, a widely cultivated *k*-free-threshing Emmer wheat species and its endemic species, i. e. *T. turgidum,* *T. aethiopicum,* *T. polonicum,* and *T. isphahanicum,* were all identified as type-I species. Based on these results, it is plausible that these free-threshing Emmer wheat species evolved from *T. dicoccum,* type-I husked Emmer wheat species.

The origin of *T. orientale* (= *turanicum*), in which type-I and type-III strains coexisted, can be explained from the point of view of hybridization between *T. durum,* a cultivated Emmer wheat, and *T. dicoccoides,* a wild species. It can be conjectured that the hybridization occurred reciprocally between *T. durum* (type -I species) and type-III *T. dicoccoides* of Mesopotamia, and that *T. orientale,* their descendant, received *k*-free-threshing genes from *T. durum* and many remarkable characters from *T. dicoccoides,* and that the strains originated from the crosses with type-III *T. dicoccoides* as female parent also inherited the cytoplasm and cytoplasm compatibility gene(s) derived from the type-III Emmer wheat.

In the conventional classification, *T. pyramidale* has often been regarded as a local variety of *T. durum*[13,70], or has been designated as "tetraploid club wheat" in contrast to Dinkel club wheat (*T. compactum*) on the basis of its extremely compact spike type[11]. However, in the present experiments, the four *T. pyramidale* strains examined were all identified as type-III, thus being clearly distinguished from *T. durum* (all the twelve strains examined were identified as type -I), and suggesting genetic relationships with *T. compactum,* a type-III Dinkel wheat species. As for *T. persicum,* a peculiar endemic Emmer wheat in Transcaucasia, which exhibits intermediate morphology and characters between Dinkel and Emmer wheats as pointed out by VAVILOV[117] and KUCKUCK[54], and which is the only Emmer wheat species having the *Q* gene-free-threshing characteristic of bread wheat[69], all but one of the strains were identified as type-III. This species was frequently considered to be the tetraploid progenitor of bread wheat (*T. aestivum*)[69,102,103]. However, since the present experiments revealed that there is

a genetic differentiation between the AABB genome of *T. persicum* and *T. aestivum,* type-III and type-I, respectively, it is difficult to consider *T. persicum* as tetraploid progenitor of *T. aestivum.* In the present hypothesis, both species (*T. pyramidale* and *T. persicum*) are regarded as secondary species of tetraploid wheat derived from pentaploid hybrids between Emmer and Dinkel wheats.

The origin of *T. macha* and *T. spelta,* primitive husked Dinkel wheat, is explained on the basis of independent diphyletic amphidiploid origins. It is possible that *T. macha,* a husked type-III Dinkel wheat endemic in Transcaucasia, was derived from a cross of *T. palaeocolchicum* (a husked type-III Emmer wheat endemic in Transcaucasia) × *Ae. squarrosa,* and that *T. spelta* (type-I), another husked Dinkel wheat, was derived from a cross of *T. dicoccum* (type-I, another husked Emmer wheat) × *Ae. squarrosa.*

As for the two naked (*Q*-free-threshing) Dinkel wheat species, i. e. *T. compactum* (type-III) and *T. aestivum* (type-I), it can be deduced that they have evolved independently from *T. macha* (type-III) and *T. spelta* (type-I), respectively, through parallel mutation as $q \rightarrow Q$[76] and $Tg \rightarrow tg$[19].

There are two hypotheses on the area of origin of Dinkel wheat. One is VAVILOV's[117], in which Afghanistan is regarded as "the center of origin" of Dinkel wheats, especially of bread wheat. The other proposed by KIHARA[38], suggests Azerbaijan (a part of Transcaucasia) and its neighboring districts as "the possible birthplace" of Dinkel wheat.

Both *T. macha* and *T. palaeocolchicum,* identified on the basis of the present results as a primary Dinkel wheat and its tetraploid progenitor, respectively, are endemic species whose cultivation occurs only in a limited area, i. e. western Georgia in Transcaucasia, the western end of *Ae. squarrosa* distribution[15]. *T. spelta,* identified as another primary Dinkel wheat, was also discovered as a relict species in Transcaucasia[53,55]. *T. dicoccum,* identified as the tetraploid progenitor of *T. spelta,* was abundantly cultivated in Transcaucasia until the beginning of this century[117]. Moreover, NISHIKAWA et al.[79], in their study on α-amylase isozymes whose genes are located on the chromosomes in the D genome, suggest that the D genome of var. *strangulata* distributed only in and around Transcaucasia, among those of *Ae. squarrosa,* is closest to the D genome of Dinkel wheat.

If these findings are sustained coordinately, it can be concluded that Dinkel wheat originated in and around Transcaucasia, as suggested by KIHARA[38].

SUMMARY

The present experiments showed that there was a genetic differentiation in the compatible relationship between nuclear gene(s) of tetraploid wheat species and the cytoplasm of *Ae. squarrosa* (DD). This differentiation was classifed into three types (type-I, type-II and type-III).

The nucleus of the majority of Emmer wheats (AABB) was incompatible with the *squarrosa* cytoplasm and designated as type-I. Zygotic lethality caused by the cytoplasm-genome constitution of (*squarrosa*) AABB and gametophytic sterility of (*squarrosa*) AB in the male were detected.

Genetic analysis showed that the *1D* chromosome, the chromosome of the first homoeologous group of the D genome, harbours indispensable gene(s) for the compatible relationship between the nuclear genome of type-I Emmer wheat and the *squarrosa* cytoplasm, in relation to the viability of the zygotes and fertility of the male gametophytes (pollen grains).

On the other hand, the nucleus of Timopheevi wheat (AAGG) was completely compatible with the *squarrosa* cytoplasm, and classified as type-II. The AAGG genome of Timopheevi wheat had gene(s) whose function was similar to that of the *1D* chromosome in the compatibility with the *squarrosa* cytoplasm.

The nucleus of four endemic species and some wild strains of Emmer wheat (AABB) showed partial compatibility with the *squarrosa* cytoplasm. These were classified as type-III. Shrivelled seeds with incomplete development of the endosperm and miniature plants with chlorophyll variegation were produced, when the type-III AABB genome was combined with the *squarrosa* cytoplasm. Gene analysis of the compatibility of the type-III genome with the *squarrosa* cytoplasm disclosed that the compatibility was controlled by two independent nuclear genes, i. e. a gene for the development of the chloroplast and the other for plant vigour.

All the alloplasmic lines of Dinkel wheat (AABBDD) with the *squarrosa* cytoplasm developed normal plants showing complete male and female fertility and normal growth. This is because compatibility and complete affinity were ensured between the AABBDD genome and the *squarrosa* cytoplasm, not only by the function of the *1D* chromosome but also by the presence of the whole D genome.

The present results suggested that a compatible relationship between nuclear genes and cytoplasmic factors should be considered in attempts to utilize alien cytoplasm in plant breeding.

The relationship between the genetic differentiation in cytoplasm compatibility gene(s) among polyploid wheat species and the phylogenic differentiation of these polyploid species was discussed.

No difference in the response type to the *squarrosa* cytoplasm was observed among the tetraploid wheat strains belonging to the same species, with some exceptions. The genetic differentiation in the compatibility among tetraploid wheat species was considered to correspond to the phylogenic differentiation of the tetraploid wheat species.

Two types (type-I and type-III) of genetic differentiation with regard to the compatibility response to the *squarrosa* cytoplasm also occurred in the AABB genome which is comprised in the AABBDD genome of Dinkel wheat, correspond-

ingly with the Emmer wheat.

The polyphyletic origin of cultivated Emmer wheat and diphyletic origin of Dinkel wheat were inferred, on the basis of the genetic relationships of the AABB genome among Emmer and Dinkel wheats. And, the area of origin of Dinkel wheat was assumed as Transcaucasia.

ACKNOWLEDGEMENTS

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(a)

(b)

Explanation of Plate

Chlorophyll variegation in F_1 miniature plants in winter (scale indicated in millimeters).

(a) F_1 miniature plant (No. 504 '82yg -5) obtained from the cross of (*squarrosa*) *T. durum* var. *Reichenbachii* $+1D$ line $\times$ *T. palaeocolchicum* ($=georgicum$) var. *schwamilicum* (Feb. 23, 1982).

(b) A part of the leaf of normal plant (left, 2n=29, No. 503 '82yg -1) and the leaves of miniature plant (right, 2n=28, No. 504 '82yg -5), of the F_1 plants of (*squarrosa*) *T. durum* var. *Reichenbachii* $+1D$ line $\times$ *T. palaeocolchicum* var. *schwamilicum* (Mar. 8, 1982).