GENETIC DIVERSITY OF THE CYTOPLASM IN *TRITICUM* AND *AEGILOPS*.
V. CLASSIFICATION OF 23 CYTOPLASMS INTO
EIGHT PLASMA TYPES¹⁾

K. TSUNEWAKI, Y. MUKAI, T. RYU ENDO,
S. TSUJI AND M. MURATA

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

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From the results of previous workers (Kihara 1951, Fukasawa 1959, Wilson and Ross 1962, Muramatsu 1965, Tsunewaki and Endo 1973, and others), it became evident that there exists great genetic diversity of the cytoplasm among *Triticum* and *Aegilops* species. Furthermore, comparative studies of the genetic characteristics of the cytoplasms in related species provided valuable information for considering their phylogenetic relationships (Hori and Tsunewaki 1967; Suemoto 1968, 1973; Maan 1973a; Maan and Lucken 1970, 1971; Tsunewaki 1973; Endo and Tsunewaki 1975; Mukai and Tsunewaki 1975; Tsuji and Tsunewaki 1976). Such comparative genetic studies of the cytoplasms in these genera are now in progress in three countries: Drs. Maan and Lucken's group at North Dakota State University, Fargo, ND, U.S.A. (rf. Maan 1973a), Drs. Gotsov and Panayotov's group at the Wheat and Sunflower Institute, Tolbuhin, Bulgaria (rf. Panayotov and Gotsov 1975), and our group at Kyoto University, Kyoto, Japan.

Our present approach to this problem consists of the following three steps:

(1) Production of a series of nucleus-cytoplasm hybrids, using twelve common wheat tester nuclei, which cover most range of genetic diversity of common wheat, and the cytoplasms of all representative species of *Triticum* and *Aegilops* (at present, single strain for each species).

(2) Investigation of the reaction spectrum of individual cytoplasms to the twelve tester nuclei, for various morphological and physiological characters, using the above nucleus-cytoplasm hybrids.

(3) Analysis of the degree of the similarity of the reaction spectra among all the cytoplasms to estimate their genetic similarity, based on which phylogenetic relationship between the cytoplasms is presumed.

Though cytoplasm substitution was still incomplete in a large number of nucleus-cytoplasm combinations, we carried out for the first time a full scale experiment to grasp genetic characteristics of the cytoplasms of 23 *Triticum* and *Aegilops* species. From the results of this experiment, we found the presence of at least eight distinctly

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different plasma types among those species, and classification of individual species into each plasma type provided new information on their origin. The results will be reported in this article.

MATERIALS AND METHODS

The following species were used as the nucleus and cytoplasm donors to produce the nucleus-cytoplasm hybrids which were employed in this investigation. Genome constitutions of the donors are cited from Kihara and Tanaka (1970).

Nucleus donor—Twelve common wheats ($2n=42$, genome constitution AABBDD), namely, *Triticum aestivum* var. *erythrospermum* (Abbreviation Tve), *T. aestivum* strain P168 (P168), *T. aestivum* cv. Chinese Spring (CS), *T. aestivum* cv. Norin 26 (N26), *T. aestivum* strain Salmon (Slm), *T. aestivum* cv. Jones Fife (JF), *T. aestivum* cv. Selkirk (Sk), *T. aestivum* cv. S-615 (S615), *T. sphaerococcum* var. *rotundatum* (Sphr), *T. compactum* cv. No. 44 (Cmp), *T. spelta* var. *duhamelianum* (Splt), and *T. macha* var. *subletschchumicum* (Mch).

Cytoplasm donor—*T. boeoticum* ($2n=14$, AA), *T. dicoccoides* var. *spontaneo-nigrum* ($2n=28$, AABB), *T. dicoccum* cv. Vernal ($2n=28$, AABB), *T. dicoccoides* var. *nudiglumis* ($2n=28$, AAGG), *T. timopheevi* var. *typicum* ($2n=28$, AAGG), common wheat ($2n=42$, AABBDD), *Aegilops caudata* ($2n=14$, CC), *Ae. umbellulata* ($2n=14$, C^uC^u), *Ae. squarrosa* ($2n=14$, DD), *Ae. speltoidea* ($2n=14$, SS), *Ae. sharonensis* ($2n=14$, S¹S¹), *Ae. triuncialis* ($2n=28$, CCC^uC^u), synthetic *triuncialis* ($2n=28$, CCC^uC^u; produced from the cross *Ae. caudata* (♀) × *Ae. umbellulata* (♂) by Kondo (1941)), *Ae. cylindrica* ($2n=28$, CCDD), *Ae. biuncialis* ($2n=28$, C^uC^uM^bM^b), *Ae. columnari* ($2n=28$, C^uC^uM^cM^c), *Ae. ovata* ($2n=28$, C^uC^uM^oM^o), *Ae. kotschy* ($2n=28$, C^uC^uS^vS^v), *Ae. variabilis* ($2n=28$, C^uC^uS^vS^v), *Ae. juvenalis* ($2n=42$, C^uC^uDDMⁱMⁱ), *Ae. triaristata* ($2n=42$, C^uC^uMⁱMⁱMⁱ²Mⁱ²), *Ae. crassa* ($2n=42$, DDD²D²M^{cr}M^{cr}) and *Ae. vavilovii* ($2n=42$, DDM^{cr}M^{cr}S^pS^p). In the following sections, these cytoplasm donors will be indicated by their species names, except *T. dicoccoides* var. *nudiglumis* and synthetic *triuncialis*, which are shown as *nudiglumis* and syn-CC^u, respectively. Each nucleus-cytoplasm hybrid is indicated by the name of its cytoplasm donor in parentheses hyphenated with the name of its nucleus donor. And, when necessary, the number of backcrosses, including the initial cross, is indicated by a superscript to the name of nucleus donor.

In order to produce nucleus-cytoplasm hybrids in all possible combinations, individual cytoplasm donors were first crossed as female parent to one or several nucleus donors, and the F₁ hybrids were further backcrossed repeatedly with twelve nucleus donors as pollen parent. However, in the cases of the *caudata*, *umbellulata*, *squarrosa*, *ovata* and *timopheevi* cytoplasm, (*caudata*)-Tve¹⁰, (*umbellulata*)-CS⁷, (*squarrosa*)-CS¹, (*ovata*)-N26⁹, and (*timopheevi*)-Sk² were used as the source of those cytoplasm. Furthermore, the early part of our work to produce nucleus-cytoplasm hybrids with the *squarrosa*, *speltoidea*, *sharonensis*, *triuncialis*, *biuncialis*, *columnaris*, *kotschy*, *variabilis*, *juvenalis*, *triaristata*, *crassa* and *vavilovii* cytoplasm was accomplished with the generous help of Drs. S. Sakamoto and T. Hori, and Mr. T. Iwai.

Backcross generations of the nucleus-cytoplasm hybrids used in the present investigation are shown in Table 1.

Table 1. Backcross generations of the nucleus-cytoplasm hybrids used

Cytoplasm donor (haploid genome const.)	Total no. of crosses ¹⁾	Nucleus donor											
		Tve	P168	CS	N26	Slm	JF	Sk	S615	Sphr	Cmp	Sp1t	Mch
<i>boeoticum</i> (A)	8	2	—	4	1	4	0	—	—	—	3	7	0
<i>caudata</i> (C)	27	26	5	15	3	14	14	7	7	7	16	17	12
<i>umbellulata</i> (C ^u)	16	8	8	15	4	8	8	7	4	3	8	8	4
<i>squarrosa</i> (D)	7	3	4	6	3	4	5	3	1	1	3	5	2
<i>speltoides</i> (S)	5	4	3	4	3	4	4	4	3	3	3	4	3
<i>sharonensis</i> (S ¹)	7	1	1	3	1	3	6	3	1	1	1	3	0
<i>dicoccoides</i> (AB)	9	4	4	4	4	3	3	2	2	5	3	3	—
<i>dicoccum</i> (")	10	4	3	4	3	4	9	3	3	3	3	3	3
<i>nudiglumis</i> (AG)	5	3	4	3	3	1	3	2	3	2	3	4	4
<i>timopheevi</i> (")	13	9	2	7	5	10	5	12	4	7	9	10	10
<i>triuncialis</i> (CC ^u)	9	4	4	7	3	6	4	5	3	4	6	5	3
<i>synthetic</i> CC ^u (")	8	2	3	3	3	2	4	4	1	—	3	5	—
<i>cylindrica</i> (CD)	8	4	2	3	2	2	3	3	2	2	2	3	0
<i>biuncialis</i> (C ^u M ^b)	6	1	1	4	1	1	5	4	1	1	1	3	0
<i>columnaris</i> (C ^u M ^c)	7	4	1	3	3	3	6	3	1	1	1	3	6
<i>ovata</i> (C ^u M ^o)	21	11	3	11	20	11	11	7	3	7	11	3	0
<i>kotschy</i> (C ^u S ^v)	6	1	1	5	3	3	1	5	1	1	1	1	0
<i>variabilis</i> (")	7	1	1	5	6	3	1	1	1	1	1	1	0
<i>aestivum</i> (ABD)	—	s	s	s	s	s	s	s	s	s	s	s	s
<i>juvenalis</i> (C ^u DM ^j)	6	3	1	4	3	3	5	3	1	1	1	3	1
<i>triaristata</i> (C ^u M ¹ M ^{t2})	7	3	1	3	3	1	6	1	1	1	1	3	0
<i>crassa</i> (DD ² M ^{cr})	7	3	1	6	3	3	6	3	1	1	1	3	1
<i>vavilovii</i> (DM ^{cr} Sp)	4	—	—	3	2	0	—	0	—	—	—	—	—

1) Total number of backcrosses, including the initial cross, using all kinds of common wheat as the recurrent pollen parents

2) O: F₁ generation, 1-26: B₁-B₂₆ generation, S: selfed, —: Not tested

Total number of backcrosses, including the initial cross, made with common wheats as recurrent pollen parents was four at the lowest and 27 at the highest. Consequently, chromosomes of the cytoplasm donors were totally eliminated in most lines. However, in a small number of nucleus-cytoplasm hybrids with the *speltoides*, *sharonensis*, *triuncialis*, syn-CC^u, *cylindrica*, *biuncialis* and *juvenalis* cytoplasm, a single chromosome of the cytoplasm donors was still remaining, and because of this, 41-, 42-, and/or 43-chromosome plants came to segregate. Aneuploid plants, therefore, were cytologically checked out, and excluded from the observation.

Of 276 possible kinds of nucleus-cytoplasm hybrids, 15 could not be produced as yet, eleven were in the F₁ generation, 70 in the B₁ or B₂, 108 in the B₃ or B₄, and the remaining 72 in the B₅ or a later generation.

All lines were planted in field in a split-plot design with four replications and twelve

nuclei in the main plots, and 23 cytoplasms in the subplots. When sufficient number of seedlings were available, three plants constituted each subplot, but a few exceptional subplots contained only one or two plants.

Fifteen characters were observed for all plants, which are listed as follows:

1. Heading date (May 1st=1)
2. Number of tillers bearing ear/plant
3. Plant height
4. Length of 1st internode from top
5. " " 2nd " " "
6. " " 3rd " " "
7. " " 4th " " "
8. Length of flag leaf
9. Width of " "
10. Ear length
11. Number of spikelets/ear
12. Selfed seed fertility (Number of seeds set in 1st and 2nd florets/total number of 1st and 2nd florets)
13. Growth vigor observed in February (graded from 0 to 12; 0 for normal vigor to 12 for complete death)
14. Variegation in February (graded from 0 to 3; 0 for normal greening and 1 for weak, 2 for moderate, and 3 for strong variegation)
15. Pistillody (graded as either 0 for no occurrence, or 1 for occurrence of postillody)

For the characters from 1 to 12, data were taken from all plants in each subplot, and their average per plant was used to represent the subplot.

To estimate the effects of various sources of variation, ordinary method of analysis of variance, or χ^2 -test was applied. For evaluating the genetic similarities between the cytoplasms, cluster analysis was applied in the following ways: (1) principal component analysis of the data to obtain eigenvectors of individual characters for the first (Z_1) to the fourth (Z_4) principal components, (2) calculation of component scores for Z_1-Z_4 of individual cytoplasms, and (3) clustering of the cytoplasms using the distance between them, which was calculated from their component scores. This method is a combination of the cluster analysis and the principal component analysis, and appears to be suitable for the present case, where observed characters showed variable degrees of correlation with each other, and simple cluster analysis of Sokal and Sneath (1963) should result in overestimation of the differences caused by plasmagenes with pleiotropic effects.

RESULTS

1. *General description of the genetic characteristics of individual cytoplasms*

(1) The *boeoticum* cytoplasm: This cytoplasm strongly depressed vigor of wheat plants, resulting in low germination rate, feeble seedlings, and severe winter killing. Variegation of leaf color was prominent in winter. Due to these effects, the plants

rarely survived our winter; thus the series of nucleus-cytoplasm hybrids with this cytoplasm was hardly completed. In late spring, however, plants recovered vigor. Splt is the only one that stood well with this cytoplasm, and its backcrosses have been successfully continued. All the nucleus-cytoplasm hybrids so far produced became totally male sterile, though their female fertility was normal.

(2) The *caudata* cytoplasm: This cytoplasm did not affect plant growth. Pistillody and other types of anther malformation were frequently observed. Tve set germless grains, and Slm produced haploids and twins. Female fertility was normal. Most strains became completely male sterile.

(3) The *umbellulata* cytoplasm: This cytoplasm also depressed plant growth. In winter, plants showed severe variegation, with greatly reduced chlorophyll content. However, no winter killing took place. Tve set germless grains, and Slm produced haploids. Female fertility was not affected, but severe male sterility occurred.

(4) The *squarrosa* cytoplasm: No remarkable effects were detected. Both male and female fertilities were normal.

(5) The *speltoides* cytoplasm: Sphr became weak, bearing very compact ears. Other strains appeared normal. Occasionally, pistillody was found. Both male and female fertilities were normal in all lines, except Sphr that showed severe male sterility.

(6) The *sharonensis* cytoplasm: Most lines showed growth depression, and delayed heading. Partial female sterility was observed in all lines except CS and N26. Male sterility was also common.

(7) The *dicoccoides* cytoplasm: No particular effects were observed, and all lines looked totally normal, including fertility.

(8) The *dicoccum* cytoplasm: Similar to the *dicoccoides* cytoplasm.

(9) The *nudiglumis*: No remarkable effects were found, except frequent anther malformation and complete male sterility in most lines.

(10) The *timopheevi* cytoplasm: Similar to the *nudiglumis* cytoplasm.

(11) The *truncialis* cytoplasm: Plants showed growth depression, with some delay of heading. Variegation occurred in winter. Slm produced haploids and twins. Most lines showed severe male sterility.

(12) The syn-CC^a cytoplasm: No remarkable effects were detected on plant growth except frequent anther malformation and male sterility in most lines. Slm produced haploids and twins.

(13) The *cylindrica* cytoplasm: All lines showed normal growth and almost normal fertilities.

(14) The *biuncialis* cytoplasm: Growth was depressed, and plants became feeble, showing some delay of heading. Variegation occurred in winter. Most lines became male sterile, Tve set some germless grains, and Slm produced haploids.

(15) The *columnaris* cytoplasm: Similar to the *biuncialis* cytoplasm.

(16) The *ovata* cytoplasm: Plants grew vigorously, but heading was extremely delayed. Ear length and number of spikelets per ear increased. No clear effects were found on other characters, except complete male sterility in most lines.

(17) The *kotschy* cytoplasm: Slm and Splt showed reduced male fertility. Tve set some germless grains, and Slm produced haploids. No other effects were detected.

(18) The *variabilis* cytoplasm: Similar to the *kotschyi* cytoplasm.

(19) The *juvenalis* cytoplasm: No remarkable effects were detected on all characters, except slightly reduced male fertility in N26 and S1m.

(20) The *triaristata* cytoplasm: Plant growth was depressed, giving feeble plants with delayed heading. Variegation occurred in winter. Severe male sterility was induced in many lines. Haploids appeared in S1m.

(21) The *crassa* cytoplasm: No remarkable effects were detected.

(22) The *vavilovii* cytoplasm: Similar to the *crassa* cytoplasm.

2. Genetic relatedness among the 23 cytoplasms estimated from their effects on the character expression of CS

All the cytoplasms under investigation were successfully introduced into CS, and its cytoplasm substitution was, as a whole, most advanced among all tester nuclei; backcrosses were made at least three times in all the lines. For these reasons, genetic relatedness among the cytoplasms was first estimated using the nucleus-cytoplasm hybrids with this nucleus. Data of 15 characters, except haploid formation, were used: all of them showed significant effects of the cytoplasms at the 1% level.

From the principal component analysis, the following eigenvectors were obtained for the first four principal components, Z_1 — Z_4 , which inclusively covered just 90% of the total variance, as shown in Table 2. Contribution of individual characters to each principal component can be estimated from the eigenvectors given in the table: The

Table 2. Eigenvectors of individual characters obtained from principal component analysis (Eigenvalues and cumulative contribution of four principal components are also given)

	Principal component			
	Z_1	Z_2	Z_3	Z_4
Heading date	-.26	.01	.47	.03
Tiller number	.26	.20	-.46	-.07
Plant height	.32	-.10	.01	-.24
1st internode length	.21	-.41	-.08	.06
2nd " "	.24	-.30	.21	-.23
3rd " "	.29	.18	.15	-.12
4th " "	.29	.20	.15	-.37
Flag leaf length	.26	-.00	.23	.53
Flag leaf width	.28	-.06	.12	.48
Ear length	.31	.09	-.06	.13
No. spikelets/ear	.27	.05	.32	-.39
Selfed seed fertility	.17	-.50	-.12	-.02
Vigor	-.27	-.15	.42	-.07
Variegation	-.29	-.03	-.31	-.21
Pistillody	.06	.58	.09	.08
Eigenvalue	9.01	2.54	1.15	0.83
Cumulative contribution (%)	60	77	85	90

first component (Z_1) was determined by all characters at almost equal weight except two characters relating to reproduction, i.e., seed fertility and pistillody. The second component (Z_2) was mostly determined by these two reproductive characters and some characters expressed in late growth period, such as the lengths of first and second internode from the top. The third component (Z_3) was mainly determined by characters which were expressed in vegetative to early reproductive phase, i.e., vigor, variegation, tiller number and heading date. The fourth component (Z_4) was mostly determined by length and width of flag leaf and number of spikelets, which were expressed in late part of reproductive phase. The component scores were calculated for individual cytoplasms by weighing the effect of each character (transformed to standard score) by its eigenvector. Using these values, distances between all pairs of the 23 cytoplasms were calculated to make a distance matrix. Clustering of the cytoplasms was performed, using the average of distances (Sokal and Sneath 1963). The result is shown in the form of a dendrogram in Fig. 1.

Since reactions of individual cytoplasms were tested, in this case, only against a single nucleus, it is not sound to make fine grouping of the cytoplasms. Therefore, the 23 cytoplasms were grouped in the following seven groups separated by $d=1.5$, as shown in Fig. 1: (1) *kotschy*, *variabilis*, *speltoides*, *dicoccoides*, *dicoccum*, *aestivum*, *squarrosa*, *cylindrica*, *vavilovii*, *juvenalis*, and *crassa* cytoplasms, (2) *sharonensis* cytoplasm, (3) *ovata* cytoplasm, (4) *caudata* and syn-CC^u cytoplasms, (5) *nudiglumis* and

Fig. 1. A dendrogram showing the genetic relatedness among 23 cytoplasms; drawn from their effects on 15 characters of CS.

timopheevi cytoplasm, (6) *umbellulata*, *biuncialis*, *triaristata*, *triuncialis* and *columnaris* cytoplasm, and (7) *boeoticum* cytoplasm.

3. Genetic relatedness among the cytoplasm estimated from the fertility spectra

A reaction spectrum of each cytoplasm to the twelve tester nuclei, in regard to selfed seed fertility, will be called its fertility spectrum. Since fertility of a nucleus-cytoplasm hybrid is genetically determined by a specific interaction between plasmagenes of the cytoplasm donor and nuclear genes of the nucleus donors (Tsunewaki 1974), the fertility spectrum obtained against a set of tester nuclei is a reflection of some of the genetic characteristics of the cytoplasm under study.

The fertility spectra of the 21 cytoplasm, which were obtained in the present investigation, are shown in Fig. 2.

The *dicoccoides*, *dicoccum*, common wheat, *squarrosa*, and *crassa* cytoplasm showed

Fig. 2. Fertility spectra of 21 cytoplasm against the twelve tester nuclei.

1—12: Tester nuclei

1. Tve, 2. P168, 3. CS, 4. N26, 5. Slm, 6. JF, 7. Sk, 8. S615, 9. Sphr, 10. Cmp, 11. Splt, 12. Mch

A—U: Cytoplasm

A. *umbellulata*, B. *triuncialis*, C. *biuncialis*, D. *columnaris*, E. *triaristata* 6x (A—E: C^u type plasma), F. *squarrosa*, G. *cylindrica*, H. *juvenalis*, I. *crassa* 6x (F—I: D type plasma), J. *boeoticum* (A type plasma), K. *speltoides*, L. *dicoccoides* and *dicoccum*, M. *kotschyi*, O. *variabilis*, P. common wheat (K—P: S type plasma), Q. *caudata* (C type plasma), R. *nudiglumis*, S. *timopheevi* (R—S: G type plasma), T. *ovata* (M type plasma), U. *sharonensis* (S^t type plasma)

high seed fertilities against all the nuclei tested. Similarly, the *speltoides*, *kotschy*, *variabilis*, *cylindrica*, and *juvenalis* cytoplasm expressed normal fertilities with most nuclei, though they gave very low fertilities with a few exceptional nuclei; these are Sphr in the *speltoides* cytoplasm, Slm and Splt in both the *kotschy* and *variabilis* cytoplasm, Tve and JF in the *cylindrica* cytoplasm (P168 was not tested), and N26 and Slm in the *juvenalis* cytoplasm. However, none of the above ten cytoplasm induced complete sterility with any of the tester nuclei. In contrast, the remaining eleven cytoplasm caused complete sterility against different tester nuclei: The *boeoticum* cytoplasm showed complete sterility in reaction with all six nuclei tested. The *caudata* cytoplasm showed almost normal fertility against three nuclei of P168, S615 and Cmp, very low fertility against Sk, and complete sterility against the remaining eight nuclei. The *umbellulata* cytoplasm caused complete sterility in Tve, Slm, Sphr and Splt, and severe sterilities in N26, Sk, S615, Cmp and Mch, but gave almost normal fertilities in P168, CS and JF. Four other cytoplasm of *triuncialis*, *biuncialis*, *columnaris*, and *triaristata* showed fertility spectra similar to that of the *umbellulata* cytoplasm, though some minor differences were observed among them. The *sharonensis* cytoplasm gave low fertilities with most nuclei, except normal fertility with N26 and complete sterility with Slm. The *nudiglumis* and *timopheevi* cytoplasm showed identical spectra in which Splt and Mch gave normal fertilities, Sk and S615 very high or complete sterilities, and nine other nuclei complete sterility. Lastly, the *ovata* cytoplasm showed normal fertilities

Fig. 3. A dendrogram showing the genetic relatedness among 19 cytoplasm, that was obtained by a cluster analysis of the fertility spectra.

in P168 and CS, moderate sterility in Mch, and complete sterility in nine other nuclei. In total, we could recognize six major types of the fertility spectrum among the 21 cytoplasms we tested.

To estimate in a quantitative manner the genetic similarity of the fertility spectra among 19 cytoplasms, simple cluster analysis (Sokal and Sneath 1963) was carried out. The result is shown in Fig. 3, in which the *boeoticum* and *cylindrica* cytoplasms were excluded because their fertility spectra were incomplete.

If we use $d=1.5$ as the critical value for grouping the cytoplasms into different types, the *squarrosa*, *speltoides*, *dicoccoides*, *dicoccum*, common wheat, *juvenalis* and *crassa* cytoplasms constitute one group, the *kotschy* and *variabilis* cytoplasms a second group, the *umbellulata*, *sharonensis*, *triuncialis*, *biuncialis*, *columnaris*, and *triaristata* cytoplasms a third group, the *caudata* and *ovata* cytoplasms a fourth and fifth group, respectively, and the *nudiglumis* and *timopheevi* cytoplasms constitute a sixth group. The *boeoticum* cytoplasm should form a seventh group because it showed complete sterility to all nuclei tested.

DISCUSSION

1. Major plasma types found in *Triticum* and *Aegilops*

The results of two cluster analyses were totally agreeable with each other, except the following two minor points: (1) the *kotschy* and *variabilis* cytoplasms belonged to the same cluster as the *speltoides* and other cytoplasms in the dendrogram obtained from 15 characters of CS, while they formed different clusters in the dendrogram based on the fertility spectrum, and (2) the *sharonensis* cytoplasm formed an independent cluster from all others in the former test, while it was included in a cluster formed by the *umbellulata* and other cytoplasms in the latter test.

The first discrepancy is mainly due to the fact that the *kotschy* and *variabilis* cytoplasms reacted to the CS nucleus in the same manner as the *speltoides* and some other cytoplasms did, while they caused very severe sterility to SIm and SpHr. To solve this discrepancy, a supplementary cluster analysis was carried out using 45 nucleus-cytoplasm hybrids for 15 cytoplasms, including the *kotschy* and *variabilis* cytoplasms, and for three tester nuclei, namely, CS, N26 and SIm, all of which were the B_3 or later backcross generations. From this analysis, it was found that the *kotschy* and *variabilis* cytoplasms form a cluster with the *speltoides*, *dicoccoides*, *dicoccum*, common wheat, and *squarrosa* cytoplasms. Therefore, we would like to include all those cytoplasms in one plasma type, though it is evident that the *kotschy* and *variabilis* cytoplasms show minor genetic differences from the rest of the cytoplasms in the same plasma type.

The second discrepancy is mainly due to the fact that fertility spectra of the *sharonensis* and *umbellulata* cytoplasms were rather similar to each other, while these cytoplasms differed in their effects on plant characters, e.g., no variegation occurred in (*sharonensis*)-CS, but clear variegation was found in (*umbellulata*)-CS (Mukai and Tsunewaki 1976). They also differed in their ability to induce haploids in SIm; the

umbellulata cytoplasm is an effective inducer, while the *sharonensis* cytoplasm is a non-inducer (Tsunewaki *et al.* 1976). From these facts, we will classify them into different plasma types.

Based on the above considerations, the 23 cytoplasms involved in the present investigation can be grouped into the following seven groups:

- (1) *boeoticum* cytoplasm
- (2) *caudata* and syn-CC^u cytoplasms
- (3) *umbellulata*, *triuncialis*, *biuncialis*, *columnaris*, and *triaristata* (6x) cytoplasms
- (4) *squarrosa*, *speltoides*, *dicoccoides*, *dicoccum*, *cylindrica*, *kotschy*, *variabilis*, common wheat, *juvenalis*, *crassa* (6x) and *vavilovii* cytoplasm
- (5) *sharonensis* cytoplasm
- (6) *nudiglumis* and *timopheevi* cytoplasms
- (7) *ovata* cytoplasm

In the fourth group, two types of the cytoplasm donors are distinguished by their genome constitution; the one type includes *speltoides* (S), *dicoccoides* (AB), *dicoccum* (AB), *kotschy* (C^uS^v), *variabilis* (C^uS^v), common wheat (ABD), and *vavilovii* (DM^{cr}S^p), all of which have the S or its related genome (B, S^v and S^p), in their genome complements. The other type consists of *squarrosa* (D), *cylindrica* (CD), common wheat (ABD), *juvenalis* (C^uDM^j), *crassa* (DD²M^{cr}) and *vavilovii* (DM^{cr}S^p), all of which have the D genome in common. Kihara (1973) critically demonstrated that common wheat with the *squarrosa* cytoplasm did not differ from its normal line, while emmer wheat with this cytoplasm showed severe growth depression and sterility. He concluded from these results that the D genome present in common wheat nucleus harmoniously interacts with the *squarrosa* cytoplasm and conceals the otherwise deleterious effects of this cytoplasm on character expression of wheat plants. Tsunewaki and Hori (unpubl.) produced a nucleus-cytoplasm hybrid with the *dicoccum* nucleus and the common wheat cytoplasm, and found that this hybrid was totally like the normal *dicoccum*. From these findings, it is evident that emmer (*dicoccoides* and *dicoccum* are included) and common wheats have identical cytoplasm, while the *squarrosa* cytoplasm is extremely different from it. Though we did not test the effects of other cytoplasms on character expression of emmer wheat, the *cylindrica*, *juvenalis* and *crassa* cytoplasms are assumed to be similar to the *squarrosa* cytoplasm on the basis of the genome constitution of the cytoplasm donors. For the *juvenalis* and *crassa* cytoplasms this assumption was already proved to be valid from the works of Tsuji and Tsunewaki (1976). On the other hand, the cytoplasms of *speltoides*, *dicoccoides*, *dicoccum*, *kotschy*, *variabilis* and common wheat are assumed to be similar to one another. This assumption is well supported by the works of Suemoto (1968) and Mukai and Tsunewaki (1975). As to the *vavilovii* cytoplasm, it is not easy to decide whether it is similar to the *speltoides* or *squarrosa* cytoplasm, because both of their genomes are included in *vavilovii*'s nucleus. However, *vavilovii* is assumed to have originated from a cross between DDM^{cr}M^{cr} type plant (resembling 4x *crassa*) as female and SS type plant (a relative of *speltoides*) as male and, therefore, its cytoplasm is more likely to be the *squarrosa* type.

Fukasawa (1959) produced a nucleus-cytoplasm hybrid with the N26 nucleus and the *ventricosa* cytoplasm, finding that the hybrid was totally normal, including the

fertility. His result indicates that *ventricosa* has also the *squarrosa* type cytoplasm. Suemoto (1968) and Maan and Lucken (1970) demonstrated that *boeoticum* and *monococcum* (cultivated from of the former) have cytoplasm similar to each other. Maan and Lucken (1971) and Maan (1973b) showed that *speltoides*, *araraticum* (genome constitution AAGG, a wild form of *timopheevi*) and *zhukovskyi* (genome constitution AAAAGG) are cytoplasmically similar to *timopheevi*. The *speltoides* strain employed by them is different in source from ours.

Based on the results of these workers and on our own findings, we can recognize at least eight different plasma types in *Triticum* and *Aegilops*. We would like to name each plasma type by the genome symbol of its diploid carrier, i.e., A, C, C^u, D, S or S¹ type. However, no diploid species has been identified so far that carries the cytoplasm similar to the *ovata* cytoplasm. Mukai and Tsunewaki (1975) suggested that this cytoplasm was derived from the M genome donor. Therefore, it will be referred to M type. The *timopheevi* type cytoplasm was evidently derived from *speltoides* or its relative (Suemoto 1968, Maan and Lucken 1971). However, since the name S type was given to the cytoplasm of *speltoides*, emmer and common wheats, *kotschyi* and *varia-*

Table 3. Classification of the cytoplasm of 26 species into eight plasma types

Plasma type	Carrier species (haploid genomes)		
	Diploid	Tetraploid	Hexaploid
A	<i>boeoticum</i> (A) <i>monococcum</i> (A) ¹⁾		
C	<i>caudata</i> (C)		
C ^u	<i>umbellulata</i> (C ^u)	<i>triuncialis</i> (CC ^u) <i>biuncialis</i> (C ^u M ^b) <i>columnaris</i> (C ^u M ^c)	<i>triaristata</i> (C ^u M ¹ M ¹²)
D	<i>squarrosa</i> (D)	<i>cylindrica</i> (CD) <i>ventricosa</i> (DM ^v) ²⁾	<i>juvenalis</i> (C ^u DM ^j) <i>crassa</i> (DD ² M ^{cr}) <i>vavilovii</i> (DM ^{cr} S ^p)
G	<i>speltoides</i> (S) ³⁾	<i>nudiglumis</i> (AG) <i>araraticum</i> (AG) ³⁾ <i>timopheevi</i> (AG)	<i>zhukovskyi</i> (AAG) ³⁾
M		<i>ovata</i> (C ^u M ^o)	
S	<i>speltoides</i> (S)	<i>kotschyi</i> (C ^u S ^v) <i>variabilis</i> (C ^u S ^v) <i>dicoccoides</i> (AB) <i>dicccum</i> (AB)	<i>aestivum</i> (ABD)
S ¹	<i>sharonensis</i> (S ¹)		

1) After Suemoto (1968) and Maan and Lucken (1970)

2) After Fukasawa (1959)

3) After Maan and Lucken (1971) and Maan (1973b)

bilis, the *timopheevi* type cytoplasm will be designated G type.

Cytoplasm of 27 species discussed above are classified into the eight plasma types, as shown in Table 3, based on the results of the present investigation supplemented with those of previous workers mentioned above. Each plasma type can be characterized as follows: the A type by severe winter killing, variegation in winter, growth depression and severe sterility, being the most deleterious among the eight types; the C type by fertility restoration in P168, S615 and Cmp, pistillody, and haploid formation in Slm; the C^u type by remarkable growth depression, complete sterility in Tve, Slm, and Splt, variegation in winter and haploid formation in Slm, being the second most deleterious among all types; the D and S types by no remarkable effects on common wheat; the G type by remarkable fertility restoration in Splt and Mch, and anther malformation; the M type by extreme delay of heading, increased spikelet number and fertility restoration in P168 and CS; and the S¹ type by growth depression similar to the C^u type and complete sterility in Slm.

2. Origin of the cytoplasm of polyploid species

Genetic similarities of the cytoplasm found in genomically related diploid and polyploid species should be a reflection of the phylogenetic relationship of their cytoplasm. Since the cytoplasm is maternally transmitted in *Triticum* and *Aegilops*, we may trace back the origin of the polyploid's cytoplasm to its related diploids, comparing their plasma types. Possible descent of the cytoplasm of polyploid species was clarified by such comparisons, the results being shown in Fig. 4.

Combining the data on the descents of the constitutive genomes and cytoplasm of a polyploid species, we can easily decide which of the two parental species functioned

Fig. 4. Possible descent of the nucleus and cytoplasm in polyploid species of *Triticum* and *Aegilops*.

as the female parent in its origin. In Fig. 4, the female and male parents thus identified are indicated by solid and broken lines, respectively, to all tetraploid species. For hexaploid species, only their female parents are indicated by solid line, to avoid extra complication of the figure.

Three facts should be pointed out here. First, individual cytoplasms retained in most part their own characteristics during the evolution of tetra- and hexaploid species. This, of course, does not mean that the plasmagenes never mutated; occurrence of mutations in plasmagenes during speciation of the diploid species is evident because almost all diploid species have cytoplasms which are distinctly different from each other. Undoubtedly, similar mutations occur continuously after origin of the polyploid species, but apparently they did not alter so greatly the genetic characteristics of the cytoplasms which they received from the diploid, female parents. Secondly, a rather remarkable genetic differentiation of the cytoplasm appears to have taken place in the species of *speltoides*. Maan (1973b) carried out an extensive survey of the cytoplasmic differentiation in the *timopheevi* complex, including *nudiglumis*, *araraticum*, *timopheevi* and *zhukovskyi*, finding no sign of it. The present case of *speltoides* is the first indication of the intraspecific cytoplasmic differentiation found in *Triticum* and *Aegilops* that is well known in other crop species, such as *Zea mays* (Rogers and Edwardson 1952), *Sorghum bicolor* (Stephens and Holland 1954) and *Oryza sativa* (Shinjo 1975).

Thirdly, *biuncialis-columnaris* and *ovata* appear to have originated from the reciprocal crosses between *umbellulata* and a diploid M genome donor, such as *comosa*, because the former two species were found to have the C^u type plasma, while *ovata* has the M type plasma. This indicated that a difference of the cytoplasm has some role in speciation, though not on a very large scale. Among the diploid species under investigation, *umbellulata*, and *squarrosa* have a strong tendency toward self-pollination, while *boeoticum*, *caudata*, *comosa*, *speltoides*, and *sharonensis* can outcross facultatively or almost obligately (this information was kindly provided by Dr. M. Tanaka). It is

Table 4. Origin of 4x species in relation to the breeding behavior of the parental 2x species

Parental combination (♀ × ♂)			4x species (♀ × ♂)
Self-pollinating	×	Self-pollinating	(none)
Self-pollinating	×	Outcrossing	<i>triuncialis</i> (C ^u × C)
"	"	"	<i>biuncialis</i> (C ^u × M)
"	"	"	<i>columnaris</i> (")
"	"	"	<i>cylindrica</i> (D × C)
"	"	"	<i>ventricosa</i> (D × M)
Outcrossing	×	Self-pollinating	<i>ovata</i> (M × C ^u)
"	"	"	<i>variabilis</i> (S × C ^u)
"	"	"	<i>kotschyi</i> (")
Outcrossing	×	Outcrossing	<i>emmer</i> (S or B × A)
"	"	"	<i>timopheevi</i> (S or G × A)

Note) All outcrossing species are facultatively self-pollinating.

interesting to know the origin of tetraploid species in relation to the breeding behavior of their female and male parents. The results are summarized in Table 4. No established tetraploids originated from the crosses between two self-pollinating types; five tetraploids of four different genomic combinations were derived from the crosses between self-pollinating type (φ) and outcrossing type ($\otimes$); three tetraploids of two genomic combinations were derived from the crosses between outcrossing type (φ) and self-pollinating type ($\otimes$); and two tetraploids originated from the crosses between two outcrossing types. It is evident from these results that at least one of the two parents of all established tetraploids is the outcrossing type.

SUMMARY

We are attempting to produce nucleus-cytoplasm hybrids in all possible combinations between the nuclei of twelve strains of common wheat covering the wide range of its genetic variation, and the cytoplasm of 22 species of *Triticum* and *Aegilops* (including one synthetic amphiploid) representing almost all basic genome types known in the two genera. Though their production is still far from completion, comparative studies of the hybrids under development indicated that great genetic diversity of the cytoplasm exists among the species of the two genera, and their cytoplasm can be classified into several major plasma types. The results of the studies were reported here. First, brief description was given to individual cytoplasm as to their genetic effects on the character expression of common wheats. Second, genetic similarities between the cytoplasm were evaluated in two ways, (1) from their effects on 15 characters of CS and (2) from their effects on the selfed seed fertility of twelve common wheats; both evaluations were made in the form of genetic distance, based on which the cytoplasm were clustered. From these results, the following eight major plasma types were distinguished: (1) A type—the cytoplasm of *T. boeoticum* (haploid genome constitution A), causing growth depression, severe winter killing, variegation in winter and severe male sterility, (2) C type—the cytoplasm of *Ae. caudata* (C) causing pistillody, male sterility and haploid formation, (3) C^a type—the cytoplasm of *Ae. umbellulata* (C^a), *Ae. triuncialis* (CC^a), *Ae. biuncialis* (C^aM^b), *Ae. columnaris* (C^aM^c) and *Ae. triaristata* (C^aMⁱMⁱ²), causing growth depression, variegation in winter, male sterility and haploid formation, (4) D type—the cytoplasm of *Ae. squarrosa* (D), *Ae. cylindrica* (CD), *Ae. juvenalis* (C^aDM^j), *Ae. crassa* (DD²M^c), and *Ae. vavilovii* (DM^cS^p), causing no remarkable effects, (5) G type—the cytoplasm of *T. dicoccoides* var. *nudiglumis* (AG) and *T. timopheevi* (AG) causing anther malformation and male sterility, (6) M type—the cytoplasm of *Ae. ovata* (C^aM^o) causing extreme delay of heading and male sterility, (7) S type—the cytoplasm of *Ae. speltoides* (S), *Ae. kotschy* (C^aS^v), *Ae. variabilis* (C^aS^v), *T. dicoccoides* var. *spontaneonigrum* (AB), *T. dicoccum* (AB), and common wheat (ABD) with no remarkable effects, and (8) S¹ type—the cytoplasm of *Ae. sharonensis* (S¹) causing growth depression and male sterility. Five additional cytoplasm studied by previous workers were also classified according to these types. From the genetic similarities observed, descents of the cytoplasm in polyploid species were clarified, tracing their origin back to one of their parental species.

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LITERATURE CITED

- Endo, T. R. and K. Tsunewaki, 1975 Genetic diversity of the cytoplasm in *Triticum* and *Aegilops*.
I. On the origin of the cytoplasm of *Aegilops triuncialis* L. *Seiken Ziho* **25-26**: 55-66.
- Fukasawa, H., 1959 Nucleus substitution and restoration by means of successive backcrosses in wheat and its related genus *Aegilops*. *Japan. J. Bot.* **17**: 55-91.
- Hori, T. and K. Tsunewaki, 1967 Study on substitution lines of several emmer wheats having the cytoplasm of *Triticum boeoticum*. *Seiken Ziho* **19**: 55-59.
- Kihara, H., 1951 Substitution of nucleus and its effects on genome manifestations. *Cytologia* **16**: 177-193.
- Kihara, H., 1973 Characteristics of *Aegilops squarrosa* cytoplasm. *Proc. IV Int. Wheat Genet. Symp.*: 351-353.
- Kihara, H. and M. Tanaka, 1970 Addendum to the classification of the genus *Aegilops* by means of genome-analysis. *Wheat Inf. Serv.* **30**: 1-2.
- Kondo, N., 1941 Chromosome doubling in *Secale*, *Haynaldia* and *Aegilops* by colchicine treatment. *Japan. J. Genetics* **17**: 46-54.
- Maan, S. S., 1973a Cytoplasmic variability in *Triticinae*. *Proc. IV Int. Wheat Genet. Symp.*: 367-373.
- Maan, S. S., 1973b Cytoplasmic and cytogenetic relationships among tetraploid species. *Euphytica* **22**: 287-300.
- Maan, S. S. and K. A. Lucken, 1970 Interaction of *Triticum boeoticum* cytoplasm and genomes of *T. aestivum* and *T. durum*: Restoration of male fertility and plant vigor. *Euphytica* **19**: 498-508.
- Maan, S. S. and K. A. Lucken, 1971 Nucleo-cytoplasmic interactions involving *Aegilops* cytoplasm and *Triticum* genomes. *J. Heredity* **62**: 149-152.
- Mukai, Y. and K. Tsunewaki, 1975 Genetic diversity of the cytoplasm in *Triticum* and *Aegilops*.
II. Comparison of the cytoplasm between four 4x *Aegilops* Polyeides species and their 2x relatives. *Seiken Ziho* **25-26**: 67-78.
- Mukai, Y. and K. Tsunewaki, 1976 Genetic diversity of the cytoplasm in *Triticum* and *Aegilops*.
IV. Distribution of the cytoplasm inducing variegation in common wheat. *Theoret. Appl. Genet.* **48** (in press).
- Muramatsu, M., 1965 Substitution of wheat nucleus into *Aegilops umbellulata* cytoplasm by the backcross method. *Japan. J. Genetics* **40**: 406.
- Panayotov, I. and K. Gotsov, 1975 Results of nucleus substitution in *Aegilops* and *Triticum* species by means of successive backcrosses with common wheat. *Wheat Inf. Serv.* **40**: 20-22.

- Rogers, J. S. and J. R. Edwardson, 1952 The utilization of cytoplasmic male-sterile inbreds in the production of corn hybrids. *Agron. J.* **44**: 8-13.
- Shinjo, C., 1975 Genetical studies of cytoplasmic male sterility and fertility restoration in rice, *Oryza sativa* L. *Bull. College Agr. Univ. Ryukyus* **22**: 1-57.
- Sokal, R. R. and P. H. A. Sneath, 1963 Principles of Numerical Taxonomy. (Freeman and Co.) pp. 359.
- Stephens, J. O. and R. F. Holland, 1954 Cytogenetic male-sterility for hybrid sorghum seed production. *Agron. J.* **46**: 20-23.
- Suemoto, H., 1968 The origin of the cytoplasm of tetraploid wheats. *Proc. III Int. Wheat Genet. Symp.*: 141-152.
- Suemoto, H., 1973 The origin of the cytoplasm of tetraploid wheats. *Proc. IV Int. Wheat Genet. Symp.*: 109-113.
- Tsuji, S. and K. Tsunewaki, 1976 Genetic diversity of the cytoplasm in *Triticum* and *Aegilops*. III. On the origin of the cytoplasms of two hexaploid species. *Japan. J. Genetics* **51**: 149-159.
- Tsunewaki, K., 1973 Genetic differentiation of the cytoplasms in wheat and *Aegilops* (Abstr.). *Genetics* **74**: s280-s281.
- Tsunewaki, K., 1974 Monosomic analysis of two restorers to *Ae. caudata* and *Ae. umbellulata* cytoplasms. *Japan. J. Genetics* **49**: 425-433.
- Tsunewaki, K. and T. Endo, 1973 Genetic relatedness among five cytoplasms in *Triticum* and *Aegilops*. *Proc. IV Int. Wheat Genetics Symp.*: 391-397.
- Tsunewaki, K., Y. Mukai, T. R. Endo, S. Tsuji and M. Murata, 1976 Genetic diversity of the cytoplasm in *Triticum* and *Aegilops*. VI. Distribution of the haploid-inducing cytoplasms. *Japan. J. Genetics* **51**: 193-200.
- Wilson, J. A. and W. M. Ross, 1962 Male sterility interaction of the *Triticum aestivum* nucleus and *Triticum timopheevi* cytoplasm. *Wheat Inf. Serv.* **14**: 29-30.