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GENETIC RELATEDNESS AMONG FIVE CYTOPLASMS IN TRITICUM AND AEGILOPS[1]

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Various cytoplasmic organelles, in addition to the nucleus, are known to carry genetic information. To fully understand the genetic mechanisms of evolution, it is therefore necessary to detect the genetic changes which have taken place during evolution not only in the nucleus but also in the cytoplasm. In the present investigation, a set of tester nuclei of common wheat was introduced by repeated backcrosses into four alien cytoplasms, and the cytoplasm-substitution lines thus produced were compared with their corresponding normal lines in order to detect the genetic differences which have occurred in the five cytoplasms of wheat and its relatives during their evolution.

MATERIALS AND METHODS

Materials used in the present investigation are shown in Table 1: eight cultivars or strains of common wheat and their cytoplasm-substitution lines with each of the four alien cytoplasms, which were introduced from *Aegilops caudata* (genome formula CC), *Ae. umbellulata* (C^uC^u), *Ae. ovata* ($C^uC^uM^oM^o$), and *Triticum timopheevi* (AAGG). The nucleus donors used were *Triticum aestivum* var. *erythrospermum* (abbreviated as Tve), strain Salmon (Slm), cv. Chinese Spring (Cns), cv. Selkirk (Sk), and cv. Jones Fife (JF); *T. compactum* cv. No. 44 (Cmp); *T. spelta* var. *duhamelianum* (Spl); and *T. vavilovii* var. *vaneum* (Vvl). They were repeatedly backcrossed as the recurrent pollen parent to the following donors of four alien cytoplasms: Tve with *Aegilops caudata* cytoplasm, Cns with *Ae. umbellulata* cytoplasm, *T. aestivum* cv. Norin 10 with *Ae. ovata* cytoplasm, and *T. aestivum* cv. Bison with *T. timopheevi* cytoplasm, which were originally produced by KIHARA (1951), MURAMATSU (1965), FUKASAWA (1959), and WILSON and ROSS (1962), respectively. The number of backcrosses made before use is also indicated in Table 1. Ears of four sets of the cytoplasm-substitution lines among the eight sets used are shown in Figure 1.

Table 1. Backcross generation of the cytoplasm-substitution lines used in the present investigation. Nucleus donors all hexaploid wheats (see text for meaning of abbreviations).

Nucleus	Cytoplasm donor			
donor	*caud.*	*umbell.*	*ovata*	*timoph.*
Tve	B22	B4	B7	B5
Slm	B11	B4	B7	B6
Cns	B11	B11	B7	B3
Sk	B3	B3	B3	B8
JF	B10	B4	B7	B1
Cmp	B13	B4	B7	B5
Spl	B13	B4	(missed)	B6
Vvl	B3	B3	B3	B3

The eight normal lines and their cytoplasm-substitution lines were planted according to a split-plot design with four replications. The eight nuclei

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constituted major plots, while the five cytoplasms (one original and four alien) formed minor plots. In each minor plot, five plants of the same line were planted

Figure 1. Ears of four sets of cytoplasm-substitution lines. (A) Cmp, (B) Cns, (C) Sk, and (D) Vv1. From left to right: normal line and four cytoplasm-substitution lines with *caudata*, *ovata*, *umbellulata*, and *timopheevi* cytoplasms.

in a row. Records were taken from the three central plants of each row. Twenty characters were subjected to observation (Table 2).

Analysis of variance was made for each character, and differences due to the primary genetic effects of the five cytoplasms and interactions between the five cytoplasms and eight nuclei were confirmed before making further analyses. To evaluate the genetic relatedness among the five cytoplasms, a clustering of genetic correlation coefficients was made based on the weighted variable-group method (SOKAL and SNEATH, 1963), details of which are given with the results.

RESULTS

Effects of Alien Cytoplasms on Wheat Characters

The over-all effects of each alien cytoplasm (average of eight lines having different nuclei) on the 20 characters studied are shown in Table 2. All alien cytoplasms exerted specific effects on

Table 2. General effects of five cytoplasms on 20 characters of hexaploid wheats; average of seven lines having different nuclei (excluding lines with Spl nucleus)

Character	Cytoplasm				
	caudata	*umbel-lulata*	*ovata*	*timo-pheevi*	Original
Germless grains (%)	4.4*	1.9*	0.0	0.0	0.0
Haploids (%)	4.1*	2.0*	0.1	0.0	0.0
Twins (%)	2.0*	1.2	0.0	0.0	0.0
Heading date (in May)	18.5	23.1*	29.1*	18.0	17.8
Plant height (cm)	133*	115*	127*	130*	135
Dry matter (gm)	126*	61*	116*	134	137
Tiller number	33.1	22.2*	29.2*	34.4	32.9
Pistillody (%)	11.6*	0.0	0.0	11.1*	0.0
Anther malformation (%)	69.1*	0.7	3.6	61.6*	0.0
Pollen fertility (%)	11.8*	34.8*	12.5*	0.0*	84.2
Selfed seed fertility (%)	12.1*	31.6*	12.4*	0.2*	87.2
Open-poll. seed fertility (%)	20.1*	36.6*	13.3*	13.6*	90.0
Top internode length (cm)	45.9*	40.7*	37.9*	47.5*	50.1
2nd internode length (cm)	31.8	27.9*	28.3*	30.9	31.3
3rd internode length (cm)	19.9	16.8*	18.9	19.1	19.3
Ear length (cm)	11.6*	10.2*	11.9*	11.8*	11.3
No. spikelets/ear	24.1	21.9*	26.8*	24.3	23.8
Flag leaf length (cm)	32.9*	28.0*	30.5	33.0*	31.5
Flag leaf width (cm)	8.62*	7.06*	8.26*	8.70*	8.37
Awn length (cm)	6.8	5.6*	4.8*	6.3	6.8

*Performance significantly different from that of original cytoplasm at the 5% level.

many characters which were significantly different from those of hexaploid wheat cytoplasm. It can be definitely said that all five cytoplasms are genetically different from each other.

Specific interactions between cytoplasms and nuclei were also detected for all the characters studied. An example is shown in Figure 2; some nuclei responded more sensitively to an alien cytoplasm than others as to the number of spikelets per ear.

Response of Individual Characters to Alien Cytoplasm

Various characters responded differently to the four alien cytoplasms; six main types were recognized, though some minor variations were also noted within each group. A representative case of each type is shown in Figure 3, where the ordinate indicates the average difference between cytoplasm-substitution lines and their corresponding normal lines, taking the maximum difference observed among four alien cytoplasms as the unit.

Type 1. *Caudata* cytoplasm exerted the most effect, followed by *umbellulata* cytoplasm with an effect about one-half that of *caudata*. The other two cytoplasms did not show any detectable effect. This type of response was recognized in three characters--frequencies of germless grains, haploids, and twin seedlings--all of which are determined at a very early stage of development.

Type 2. *Umbellulata* cytoplasm exerted the most effect, followed by *ovata* cytoplasm with about one-third as great an effect. The other two cytoplasms showed almost no effect. This type was found in the following six characters, determined mostly in the tillering to booting stages: length of third internode from the top, flag-leaf length and width, number of fertile tillers, dry-matter weight, and plant height. The effect of *ovata* cytoplasm, relative to that of *umbellulata* cytoplasm, gradually increased from the tillering to the booting stage, and their relation was eventually reversed in the next heading stage.

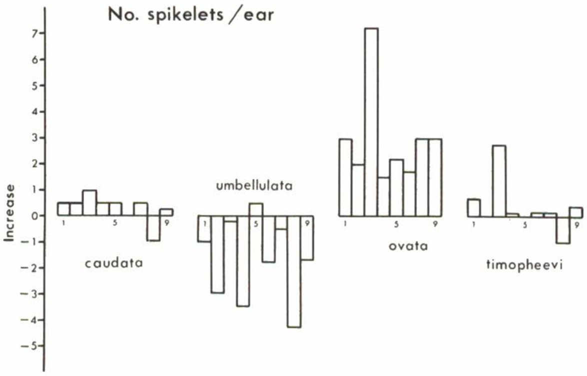

Figure 2. Deviation of spikelet number per ear of 31 cytoplasm-substitution lines from those of their corresponding normal lines. 1=Tve, 2=Slm, 3=Cns, 4=Sk, 5=JF, 6=Cmp, 7=Spl, 8=Vvl, and 9=average.

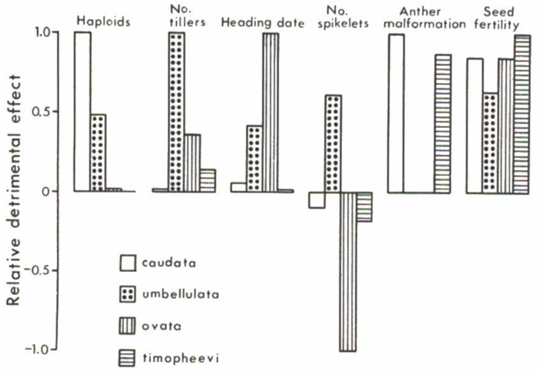

Figure 3. Six typical types of response of wheat characters to the four alien cytoplasms.

Type 3. *Ovata* cytoplasm exerted the greatest effect, followed by *umbellulata*, whose effect was about one-third that of *ovata*. The effects of the other two cytoplasms were much less. This type was noticed in three characters determined at the heading stage: first-internode length, heading date, and awn length.

Type 4. *Umbellulata* cytoplasm showed a pronounced retarding effect, while *ovata* cytoplasm had some stimulative effect. The other two cytoplasms exhibited no remarkable effect. This type was found in two characters--number of spikelets per ear, and ear length--both of which seem to be determined in the booting to heading stage.

Type 5. *Caudata* and *timopheevi* cytoplasms showed remarkable effects, while the other two cytoplasms exerted no effect. This type was seen in two characters of male organs: frequencies of pistilloid stamens and malformation of anthers.

Type 6. All four alien cytoplasms exerted drastic effects, though *umbellulata* much less than the others. This type was found for three characters, all relating to pollen fertility; namely, pollen fertility, and selfed and open-pollinated seed fertilities.

Developmental Characterization of Four Alien Cytoplasms

The relative genetic effects of the four alien cytoplasms on 20 characters are diagrammatically shown in Figure 4, where these

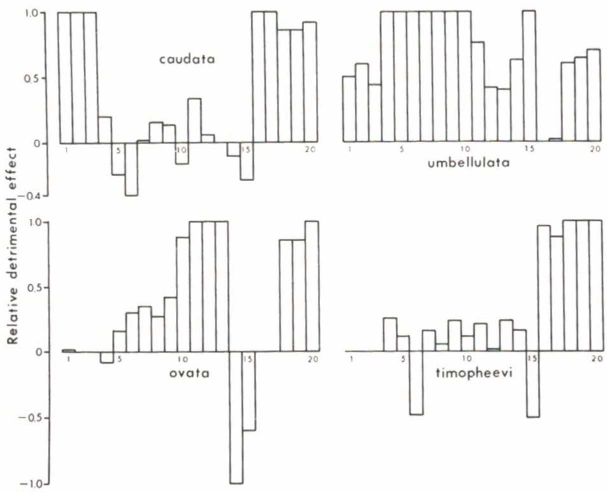

Figure 4. Developmental characteristics of four alien cytoplasms; the ordinate indicates the relative effect of each cytoplasm, compared to that of hexaploid-wheat cytoplasm. 1 - 20: Frequencies of germless grains, twins and haploids, flag-leaf width, third-internode length, flag-leaf length, tiller number, dry matter, plant height, second-internode length, first-internode length, heading date, awn length, spikelet number/ear, ear length, frequencies of pistillody and malformation of anthers, pollen fertility, and selfed and open-pollinated seed fertilities

characters were arranged on the abscissa roughly in order of the developmental stages when they are determined. *Caudata* cytoplasm was characterized as showing strong influences on reproductive traits, thus affecting eight characters observed in male organs, male gametophytes and seeds or seedlings, with very little effect on vegetative characters. On the other hand, *umbellulata* cytoplasm influenced almost all vegetative characters most severely, with very little effect on male organs and male gametophytes. *Ovata* cytoplasm affected drastically a late part of the vegetative period in either direction, inhibitory or stimulative, depending upon the characters investigated. *Timopheevi* cytoplasm was characterized by its strong detrimental effects limited to male organs and male gametophytes.

Genetic Relatedness among the Five Cytoplasms

In estimating the genetic relatedness among the five cytoplasms, a precaution was taken to avoid a bias caused by the pleiotropic effects of the same cytoplasmic genes. The genetic correlation between all pairs of 20 characters was calculated, and among a group of characters showing a higher correlation than 0.80 with each other, only one representative was selected. As a consequence, data on only 16 characters were used for the estimation of genetic relatedness among the five cytoplasms.

Next, the raw data of those characters were converted to a standardized score, x, after a formula, $x = (X - \bar{X})/s_c$, where X, $\bar{X}$ and s_c indicate the observed value of each line, the mean of five lines with the same nucleus, and the standard deviation obtained with them, respectively. Based on this score, the correlation coefficient (r) was calculated according to the following formula:

$$r = \frac{\sum x_1 \cdot x_2 - \frac{1}{n}\sum x_1 \cdot \sum x_2}{\sqrt{[\sum x_1^2 - \frac{1}{n}(\sum x_1)^2][\sum x_2^2 - \frac{1}{n}(\sum x_2)^2]}}$$

where x_1 and x_2 represent standardized scores of each character of a pair of lines having two different cytoplasms. The coefficient was calculated for every pair of five cytoplasms. *Caudata* and *timopheevi* cytoplasms showed the closest relation, with r = 0.076, then *umbellulata* and *ovata* cytoplasms with r = -0.169, the four cytoplasms tending to form two clusters. Based on such clustering, the correlation coefficients between the two clusters and hexaploid-wheat cytoplasm were recomputed; here, the closest relation was found between hexaploid-wheat cytoplasm and the *caudata-timopheevi* cluster at a level of r = -0.301; thus they were clustered secondarily. This secondary cluster consisting of *caudata*, *timopheevi*, and hexaploid-wheat cytoplasms was related to the other cluster comprised of *umbellulata* and *ovata* cytoplasms at a level of r = -1.000. Based on these results, a dendrogram was constructed among the five cytoplasms as shown in Figure 5. It must be cautioned, however, that such genetic relatedness of the different cytoplasms does not necessarily mean their phylogenetic relationship.

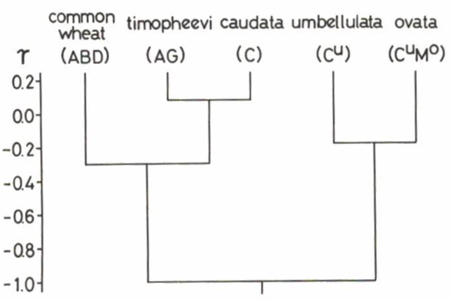

Figure 5. A dendrogram showing the genetic relatedness among five cytoplasms of *Triticum* and *Aegilops*

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