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# The Origin of the Cytoplasm of Tetraploid Wheats

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It is believed widely that the A genome of emmer wheats has been derived from einkorn wheat and the B genome of those from *Aegilops speltoides*, but we do not know which of the diploid species has contributed the cytoplasm to the emmer wheats. In 1966, KIHARA tentatively assumed that emmer wheat had the cytoplasm of *Ae. speltoides*.

In the present paper, the emmer and *timopheevi* wheats having einkorn cytoplasm are compared with those having *Ae. speltoides* cytoplasm, and also included is a discussion of which of the diploid species has contributed the cytoplasm to tetraploid wheats.

## MATERIALS AND METHODS

Nuclei and cytoplasms used in the present study are shown in TABLE 1. Substitution of a nucleus to an alien cytoplasm has been accomplished by successive backcrosses (KIHARA, 1951 and 1958). Original substitution lines were produced by the following procedures:

*T. boeoticum* cytoplasm lines
- *T. boeoticum* x *T. turgidum* (or *T. timopheevi*)
  - $F_1$ x *T. turgidum* (or *T. timopheevi*)
    - $BC_1$ x *T. turgidum* (or *T. timopheevi*)
      - :

*Ae. speltoides* cytoplasm lines
- *Ae. speltoides* x *T. turgidum* (or *T. timopheevi*)
  - $F_1$ x *T. turgidum* (or *T. timopheevi*)
    - $BC_1$ x *T. turgidum* (or *T. timopheevi*)
      - :

Initial crosses with *T. turgidum* started in 1964 and with *T. timopheevi* in 1965, so that $BC_3$ plants of *turgidum* having *boeoticum* and *speltoides* plasmas and the $BC_2$ plants of *timopheevi* having these plasmas are available in 1968. In

TABLE 1. Materials.

| Cytoplasm | Nucleus |
|---|---|
| *T. boeoticum* Boiss. ssp. *aegilopoides* (Link.) Schiemann | *T. turgidum* L. var. *nigrobarbatum* Körn. |
| *T. monococcum* L. var. *vulgare* | *T. timopheevi* zhuk. var. *typicum* |
| *Ae. speltoides* Jaub. et sp. | *T. dicoccum* Schubl. var. *rufum* Körn. cultivar. 'Vernal' |
| *Ae. longissima* Schweinf. et Musvhl. | *T. durum* Desf. var. *hordeiforme* (Host) Körn. |
| *Ae. sharonensis* Eig. var. *typica* $S^bS^bAA$* | *T. dicoccoides* Körn. var. *kotschyanum* Schulz. |
| | *T. dicoccoides* var. *spontaneonigrum* Flaksb. |
| | *T. pyramidale* Perc. var. *recognitum* |
| | *T. orientale* Perc. var. *insigne* Perc. |
| | *T. araraticum* Jakubz. var. *tumaniani* |
| | *T. vulgare* Vill. var. *erythrospermum* Körn. |
| | *T. spelta* var. *duhamerianum* |

* Dr. Sears induced from the crosses of *Ae. bicornis* (♀) x einkorn (♂)

addition to these main lines, many nuclear substitution lines have been obtained as shown in TABLE 4.

The effects of adding an alien cytoplasm to a nucleus were investigated with respect to changes in morphology and fertility. Fertility was estimated mainly on the basis of the percentage of seeds set and the percentage of good pollen. The settings of seeds on both backcross-pollinated and bagged ears were taken into consideration.

## RESULTS AND CONCLUSIONS

### 1. THE ORIGIN OF EMMER CYTOPLASM

*Cross-Ability between Einkorn and* Ae. speltoides

Many reciprocal crosses between einkorn and *Ae. speltoides* were carried out to obtain amphidiploids in 1964 (TABLE 2). The $F_1$ seeds could be obtained

TABLE 2. Number of seeds obtained from the reciprocal crosses between einkorn wheats and *Ae. speltoides* (1964).

| Combinations | No. of florets | No. of seeds | Percentage |
|---|---|---|---|
| *T. monococcum* x *Ae. speltoides* | 1367 | 0 | 0 |
| *T. boeoticum* x *Ae. speltoides* | 820 | 0 | 0 |
| Total | 2187 | 0 | 0 |
| *Ae. speltoides* x *T. monococcum* | 92 | 1 | 1·1 |
| *Ae. speltoides* x *T. boeoticum* | 316 | 10 | 3·1 |
| Total | 408 | 11 | 2·7 |

only in one direction, *speltoides* ( ♀ ) x einkorn ( ♂ ). In its reciprocal cross, einkorn ( ♀ ) x *speltoides* ( ♂ ), we could get no seed. From these results, it is assumed that emmer wheat has been derived from the $F_1$ hybrid of the former direction.

### *Chromosome Behaviours and Chromosome Numbers in Successive Backcross Generations*

The $F_1$ hybrids in both the substitution lines had a somatic chromosome number of 2n = 21 and complete pollen sterility. In subsequent generations, however, the numbers and behaviours of chromosomes in both the *boeoticum* and *speltoides* cytoplasm lines were quite different from each other (TABLE 3).

TABLE 3. Chromosome numbers in the backcross generations.

| Combination | $F_1$ Chromosome no. (2n) | $BC_1$ Chromosome no. (2n) | No. of plants | $BC_2$ Chromosome no. (2n) | No. of plants |
|---|---|---|---|---|---|
| *T. aegilopoides* x *T. turgidum* | | 27 | 1 | 27 | 1 |
| | 21 | 28 | 5 | 28 | 18 |
| | | 29 | 1 | 29 | 5 |
| *Ae. speltoides* x *T. turgidum* | | 21 | 1 | 27 | 1 |
| | 21 | 23 | 2 | 28 | 1 |
| | | 24 | 1 | | |

In the *boeoticum* cytoplasm line, we could get the $BC_1$ plants having 28 chromosomes and many $BC_2$ plants forming 14 bivalents in meiosis. On the other hand, the $BC_1$ plants in the *speltoides* cytoplasm lines had chromosome numbers of 2n = 21, 23 or 24. In the $B_2$ generation, two plants were obtained and one of them had 28 chromosomes but did not form 14 bivalents. In the $BC_3$ generation, only two out of thirteen plants showed the chromosome pairing of 14 bivalents.

### *Morphology*

In the *speltoides* cytoplasm line, not all plants showed morphological abnormalities. The $BC_1$ plants having other Sitopsis-group cytoplasm (*Ae. longissima, sharonensis,* or *bicornis*), also showed normal growth. On the other hand, the $BC_1$, $BC_2$ and $BC_3$ plants in the *boeoticum* cytoplasm line showed various morphological abnormalities such as weakness, bushy and stunted growth, delayed growth, and leaf variegation in seedlings (TABLE 4, Figs. 1 and 2). These abnormalities appeared in the $BC_1$ plants of *turgidum* having *T. monococcum* cytoplasm as well as in *turgidum* having *boeoticum* cytoplasm. The degree of these abnormalities varied from line to line and also from individual to individual even in the same line. *T. dicoccum* and *T. durum* having *boeoticum* plasma were much weaker than *T. turgidum* having the same plasma and most of them died during the winter time of 1968. Different degrees of weakness were also observed among the lines of *T. turgidum* having *boeoticum* plasma (TABLE 5). Lines 270 and 271 showed better growth than others. These lines were derived from the 29-chromosome plant forming 14 bivalents and one univalent (the $BC_1$ ancestor also had 29 chromosomes). It seems likely that a chromosome

**Table 4.** Effects of alein cytoplasm on the changes with respect to morphology and fertility.

| Cytoplasm | Nucleus | Cult no. (1968) | line variegation | Fertility (%) pollen | Fertility (%) selfed-seed | Fertility (%) backcrossed-seed |
|---|---|---|---|---|---|---|
| *T. boeoticum* | *T. boeoticum* × *T. turgidum*$^{4}$ | 263-271 | + | 0 | 0 | |
| | (*T. boeo.* × *turg.*$^{2}$ × *T. dicoccum*$^{2}$ | 283-285 | + | 0 | 0 | |
| | (*T. boeo* × *turg.*$^{2}$) × *T. durum*$^{2}$ | 281-282 | + | 0 | 0 | |
| | (*T. boeo.* × *turg.*$^{2}$) × *T. timopheevi*$^{2}$ | 286-292 | ± | | | |
| | (*T. boeo.* × *turg.*$^{2}$) × *T. monococcum*$^{1}$ | 152-153 ('67) | — | 0 | 0 | |
| | (*T. boeo.* × *turg.*$^{3}$) × *T. vulgare*$^{1}$ | 280 | + | 0 | 0 | |
| | *T. boeoticum* × *T. timopheevi*$^{3}$ | 306 | — | | | |
| *T. monococcum* | *T. monococcum* × *T. turgidum*$^{2}$ | 249-250 | + | 0–17 | | |
| | *T. monococcum* × *T. timopheevi*$^{2}$ | 307-309 | — | | | |
| *Ae. speltoides* | *Ae. speltoides* × *T. turgidum*$^{4}$ | 254-255 | — | 30–60 | 22 | |
| | (*Ae. spelt.* × *turg.*$^{3}$) × *T. vulgare*$^{1}$ | 262 | — | | | |
| | *Ae. speltoides* × *T. timopheevi*$^{3}$ | 297-304 | — | 0–7 | | |
| *Ae. longissima* | *Ae. longissima* × *T. turgidum*$^{2}$ | 244-245 | — | 2 | 0 | |
| *Ae. sharonensis* | *Ae. sharonensis* × *T. turgidum*$^{2}$ | 247-248 | — | 7 | 0 | |
| *Ae. bicornis* | ($S^bS^bAA$ × *dicoccum*) × *T. turgidum*$^{1}$ | 243 | ± | 7 | 0 | |

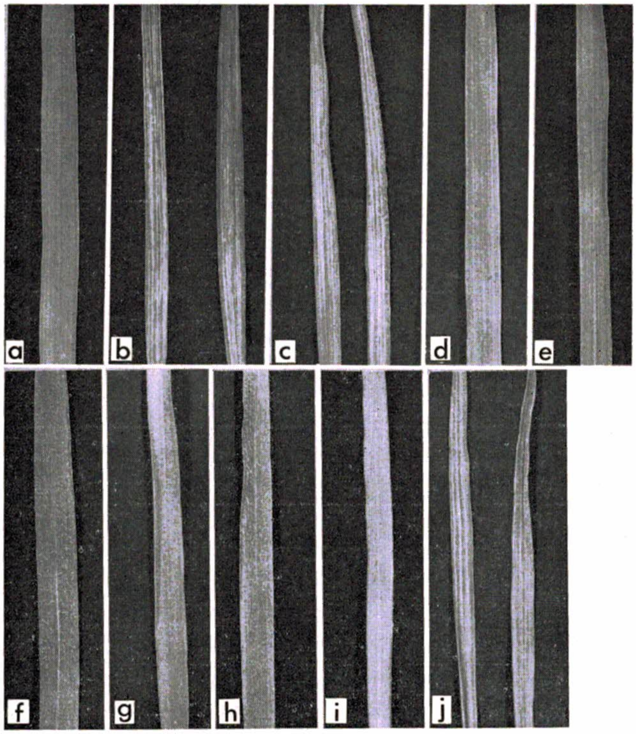


Fig. 1. Leaf variegation. a: control, *T. turgidum*. b: cult. no. 250, ♀ *T. monococcum* x ♂ *T. turgidum*$^{2}$. c: 270, ♀ *T. boeoticum* x ♂ *turgidum*$^{4}$. d: 244, ♀ *Ae. longissima* x ♂ *turgidum*$^{2}$. e: 255, ♀ *Ae. speltoides* x ♂ *turgidum*$^{4}$. f: control, *T. timopheevi*. g: 306, ♀ *boeoticum* x ♂ *timopheevi*$^{3}$. h: 303, ♀ *speltoides* x ♂ *timopheevi*$^{3}$. i: 292, ( ♀ *boeoticum* x ♂ *turgidum*$^{2}$) x ♂ *timopheevi*$^{2}$. j: 280, ( ♀ *boeoticum* x ♂ *turgidum*$^{3}$) x ♂ *T. vulgare*$^{1}$. b, c and j show leaf variegation but d, e, g, h and i do not show leaf variegation.

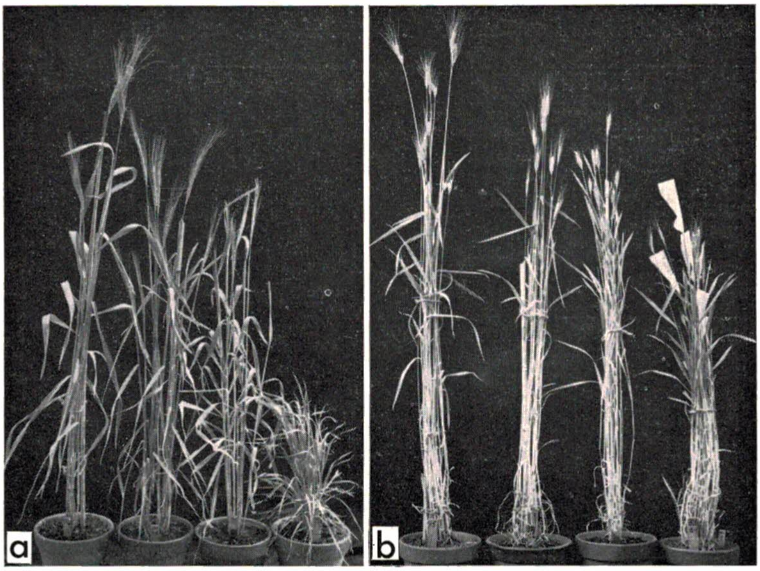


FIG. 2. Whole plant. a. Pictures arranged from left to right, as *T. turgidum*, ♀ *T. boeoticum* x ♂ *turgidum*[4], and two plants of ♀ *Ae. speltoides* x ♂ *turgidum*[4]. The last one shows extremely abnormal growth.

b. Pictures arranged from left to right, as *T. timopheevi*, ♀ *Ae. speltoides* x ♂ *timopheevi*[3], (♀ *T. boeoticum* x ♂ *turgidum*[2]) x ♂ *timopheevi*[2] and ♀ *T. boeoticum* x ♂ *timopheevi*[3].

TABLE 5. The germination and abnormal growth of *T. turgidum* having *T. boeoticum* cytoplasm ($BC_3$).

| Cult. nos. (plant) 1966 | Cult. nos. (plant) 1967 | Cult. nos. (line) 1968 | Percentage of seeds germinated | Percentage of seeds germinated Mean | Percentage of seedlings abnormally grown | Percentage of seedlings abnormally grown Mean |
|---|---|---|---|---|---|---|
| 115—3 | 133—1—2 | 263 | 90·0 | | 100·0 | |
| | —4 | 264 | 100·0 | | 95·3 | |
| | —3 | 266—1 | 92·3 | 95·5±2·5 | 91·7 | 94·9±1·4 |
| | —3 | —2 | 100·0 | | 93·3 | |
| 115—4 | 134—3—3 | 267—1 | 100·0 | | 90·0 | |
| | —3 | —2 | 100·0 | 98·3±1·0 | 90·0 | 91·5±1·4 |
| | —4 | 268 | 95·0 | | 94·4 | |
| 115—5 | 135—1—3 | 269 | 83·3 | 83·3 | 100·0 | 100·0 |
| 115—8 | 138—2—2 | 270—1 | 93·3 | | 64·3 | |
| | —2 | —2 | 73·3 | 80·6±6·4 | 73·7 | 72·7±4·5 |
| | —3 | 271 | 75·0 | | 80·0 | |

or chromosome segment from the *boeoticum* A genome remains in these lines, and the existence of the chromosome or chromosome segment in the *boeoticum* cytoplasm is the cause of the better growth of these lines.

TABLE 6 shows plant heights of the $BC_3$ plants in both the nuclear substitution lines. The plant heights of *turgidum* having *speltoides* plasma are greater than those of *turgidum* having *boeoticum* plasma and are close to that of control, *T. turgidum*.

TABLE 6. Plant height.

| *T. turgidum* having *T. boeoticum* plasma | | *T. turgidum* having *Ae. speltoides* plasma | | *T. turgidum* |
|---|---|---|---|---|
| Cult. no. | height (cm) | Cult no. | height (cm) | height (cm) |
| 263—1 | 86·3±13·4 | 255—1 | 152·6± 9·4 | 175·3±1·4 |
| 264—1 | 93·3±61·4 | 255—2 | 138·1±10·7 | |
| 266—1 | 45·3± 6·7 | | | |
| 267—1 | 70·0±40·7 | | | |
| 268—1 | 83·0±52·5 | | | |
| 269—1 | 85·2±11·1 | | | |
| 270—1 | 103·5±10·0 | | | |
| 271—1 | 104·6±13·2 | | | |

The photographs of ears of *T. turgidum* having both cytoplasms are given in Fig. 3 together with that of their parents.

FIG. 3. Ears. Pictures arranged from left to right, *T. boeoticum*, $BC_3$ of *T. turgidum* having *boeoticum* cytoplasma, *T. turgidum*, $BC_3$ of *T. turgidum* having *speltoides* cytoplasm and *Ae. speltoides*.

## *Pollen and Seed Fertility*

Figures 4 and 5 show floral organs and pollen of various nuclear substitution plants. *T. turgidum* having *boeoticum* plasma has minute and dried anthers and

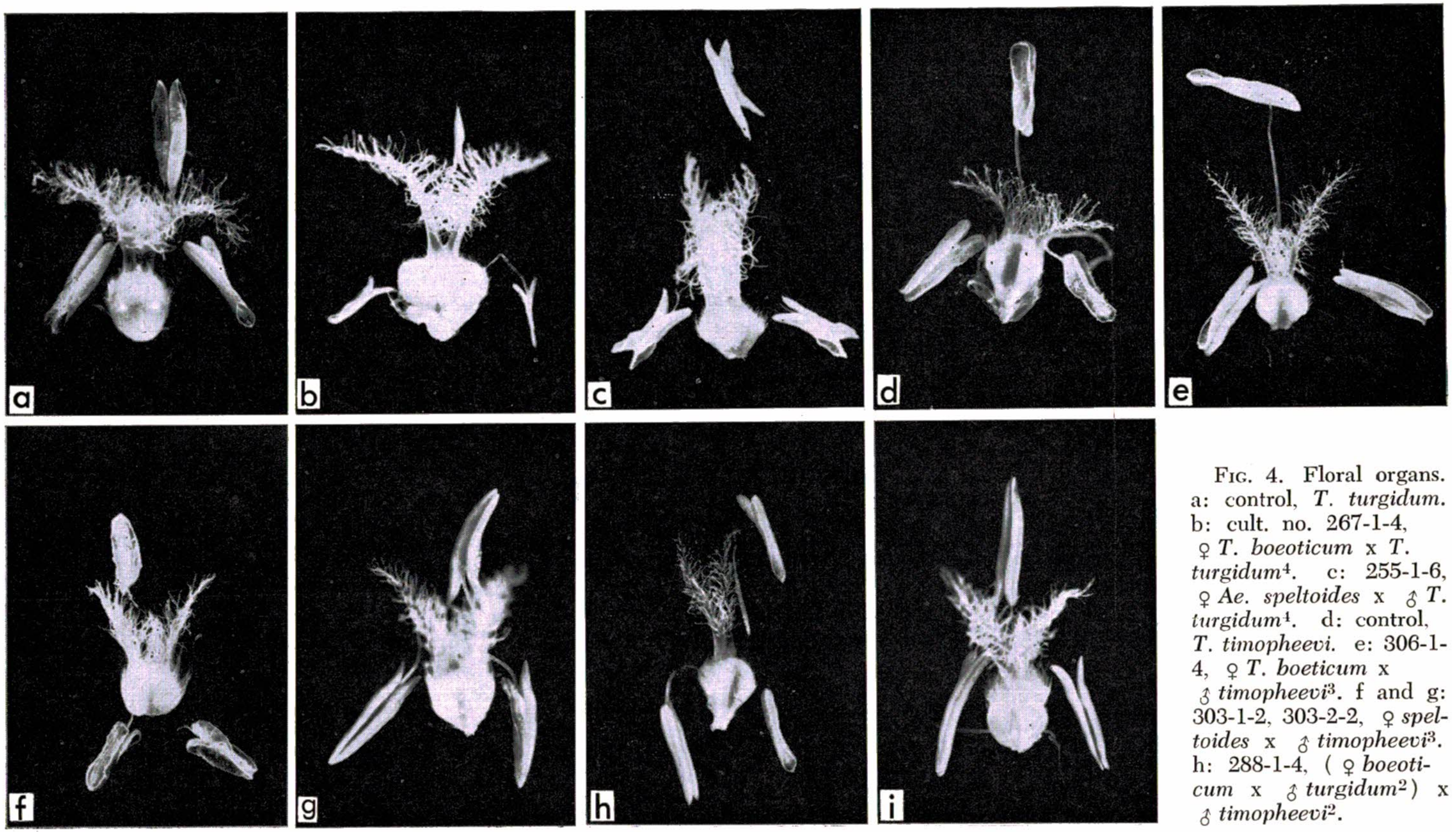

FIG. 4. Floral organs. a: control, *T. turgidum.* b: cult. no. 267-1-4, ♀ *T. boeoticum* x *T. turgidum*[4]. c: 255-1-6, ♀ *Ae. speltoides* x ♂ *T. turgidum*[1]. d: control, *T. timopheevi.* e: 306-1-4, ♀ *T. boeticum* x ♂ *timopheevi*[3]. f and g: 303-1-2, 303-2-2, ♀ *speltoides* x ♂ *timopheevi*[3]. h: 288-1-4, (♀ *boeoticum* x ♂ *turgidum*[2]) x ♂ *timopheevi*[2].

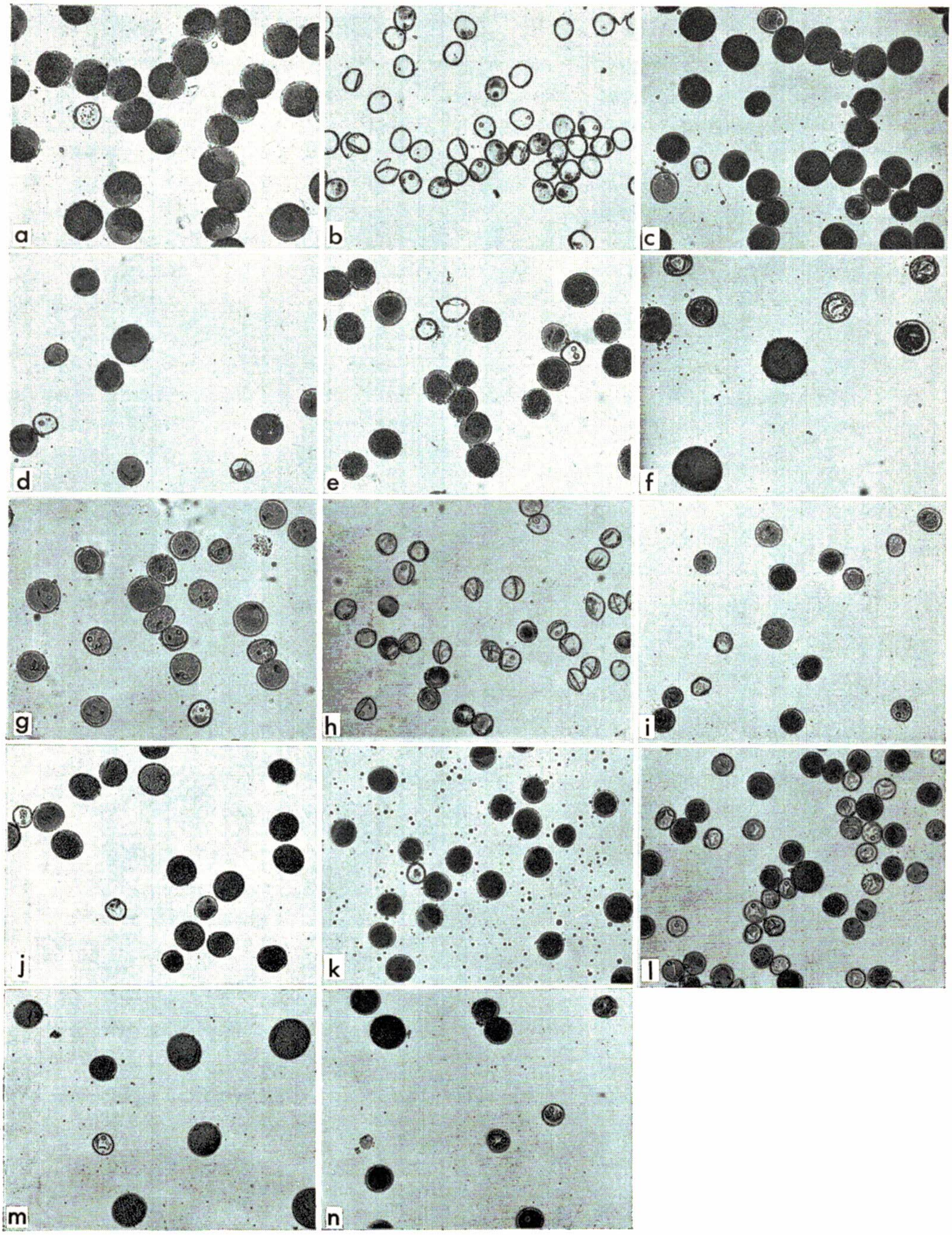



FIG. 5. Pollen grains. a: control, *T. turgidum*. b: cult. no. 267-1-4, ♀ *T. boeoticum* x ♂ *T. turgidum*[4]. c: 255-1-6, ♀ *Ae. speltoides* x ♂ *T. turgidum*[4]. d: 250-3-1, ♀ *T. monococcum vulgare* x ♂ *T. turgidum*[2]. e: 243-1-2, ♀ ( ♀ $S^bS^bAA$ x ♂ *T. dicoccum*[1]) x ♂ *T. turgidum*[1]. f: 244-2-1, ♀ *Ae. longissima* x ♂ *T. turgidum*[2]. g: 247-1-1, ♀ *Ae. sharonensis* x ♂ *T. turgidum*[2]. h: 280-1-1, ♀ ( ♀ *T. boeot.* x ♂ *T. turg.*[3]) x ♂ *T. vulgare*[1]. i: 262-1-1, ♀ ( ♀ *Ae speltoides* x ♂ *T. turgidum*[3]) x ♂ *T. vulgare*[1]. j: 272-1-1, ( ♀ *T. boeoticum* x *T. turgidum*[3]) x *T. dicoccoides kotschyanum*[1]. k: control, *T. timopheevi*. l: 306-4-3, ♀ *T. boeoticum* x ♂ *T. timopheevi*[3]. m: 303-1-2, ♀ *Ae. speltoides* x ♂ *T. timopheevi*[3]. n: 290-1-9, ♀ ( ♀ *T. boeot.* x ♂ *T. turg.*[2]) x ♂ *T. timopheevi*[2].

empty pollen but has normal female organs, while *turgidum* having *speltoides* plasma has dehiscent anthers, good pollen and normal female organs.

Figure 6a shows the changes in pollen and seed fertility in the course of successive backcrosses in both substitution lines. In the *boeoticum* cytoplasm line, the pollen and selfed seed fertilities are constantly zero throughout successive backcross generations, but the backcrossed seed fertility is remarkably high. These results indicate that *turgidum* having *boeoticum* plasma has complete male sterility and normally functioned female organs. This male sterility seems to be caused by the alien cytoplasm.

On the other hand, in the *speltoides* cytoplasm line, the restoration of pollen fertility is accompanied with the increase in backcrossed seed fertility throughout successive backcrossed generations as shown in Fig. 6b. Furthermore, two

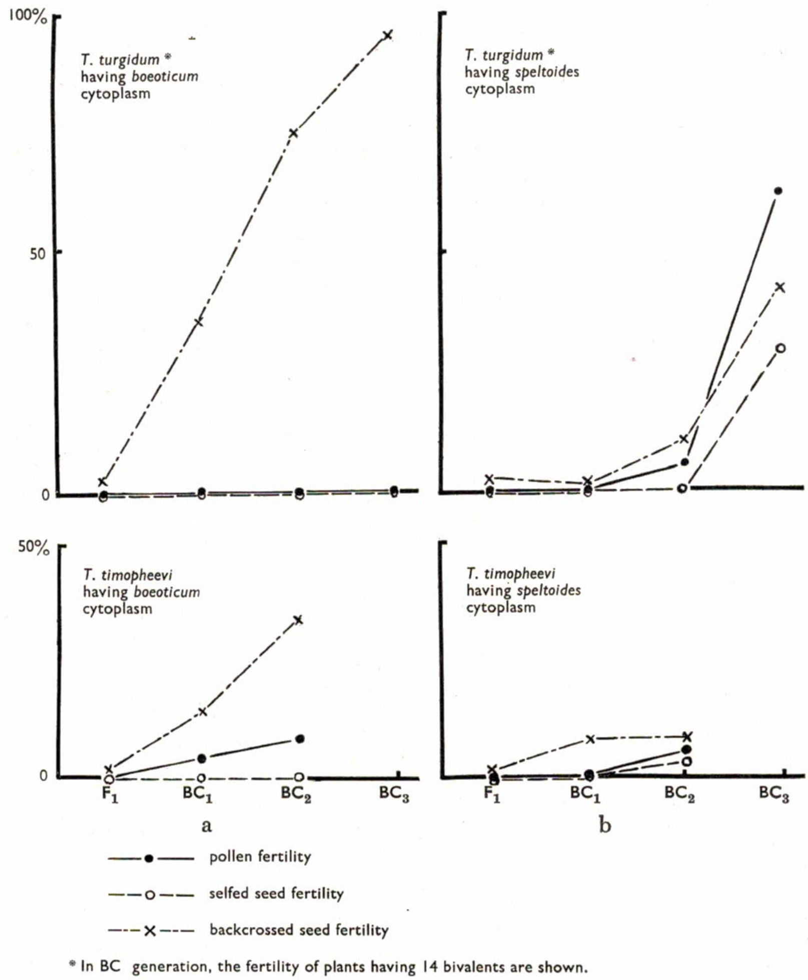


FIG. 6. Pollen and seed fertility in the successive backcross generations.

$BC_3$ plants having 14 bivalents had dehiscent anthers and produced some seeds in the bagged ears (Fig. 4). The sterility observed in the earlier back-crossed generations of this line seems to be due to the chromosome behaviour in meiosis, but not to the alien cytoplasm.

Pollen and seed fertility in various nuclear substitution lines are seen in TABLE 4. The $BC_1$ plants of *turgidum* having *monococcum* cytoplasm also showed complete male sterility, but only one out of eleven $BC_1$ plants produced unexpectedly good pollen. The reason why this plant has such a good pollen fertility is not known, but the fertility may drop, as backcross progresses, as KIHARA had reported on the fertility of *T. vulgare* having *Ae. caudata* cytoplasm (KIHARA, 1958). All the $BC_1$ plants having *Ae. longissima, sharonensis* or *bicornis* cytoplasm show considerable high pollen and backcrossed seed fertility. This fertility agrees with the results from *turgidum* having *speltoides* plasma. In TABLE 4 and Fig. 5, the fertility of the $F_1$ hybrid between *turgidum* having *boeoticum* plasma (♀) and *T. vulgare* (♂) and the $F_1$ of *turgidum* having *speltoides* plasma (♀) x *T. vulgare* (♂) are shown. The former shows complete pollen sterility while the latter shows a considerable pollen fertility (22%) but lower than those of usual pentaploid hybrids (91-54%). Moreover, the former $F_1$ shows severe grades of weakness, variegation and delayed growth.

### *Restoring Gene or Genes.*

In order to obtain restoring genes for the male sterility caused by the *boeoticum* cytoplasm, the crosses between the $BC_2$ plants of *turgidum* having *boeoticum* plasma and six varieties of tetraploid wheats were carried out in 1967. Results are shown in TABLE 7. Two combinations between the $BC_2$ and *T. dicoccoides* var. *kotschyanum* and *T. araraticum* showed some pollen fertility restoration and these $F_1$'s produced some seeds on bagged ears.

TABLE 7. The fertility of the $F_1$ hybrids between *T. turgidum* having *T. boeoticum* cytoplasm and 6 varieties of tetraploid wheats.

| Combination | | Fertility pollen (%) | Fertility selfed-seed (%) |
|---|---|---|---|
| (*T. boeoticum* x *T. turgidum*$_3$) | x *T. dicoccoides kotschyanum* | 27—67 | |
| | x *T. dicoccoides spontaneonibrum* | 0 | 0 |
| | x *T. orientale* | 0 | 0 |
| | x *T. pyramidale* | 0 | 0 |
| | x *T. araraticum* no. 1 | 0 | 0 |
| | x *T. araraticum* no. 2 | —4·0 | |

### *Conclusion*

From these results, it is concluded that the cytoplasm of emmer wheats has been derived from *Ae. speltoides* or, if not, from its relatives, and the cytoplasm of common wheats has been also derived from the same donor through emmer wheats. The flowering habit of *Ae. speltoides* seems to be additional support for this conclusion. *Ae. speltoides* holds its glume open for a long time after flowering, but the glume of einkorn is closed after the flowering. Thus in the past when einkorn wheat was cultivated and *speltoides* had also been growing with it as a weed, *speltoides* might have been pollinated by einkorn pollen.

### 2. THE ORIGIN OF THE CYTOPLASM OF *T. timopheevi*

We have four substitution lines with respect to *T. timopheevi* nucleus (TABLE 4). The original two lines started from the crosses between *T. boeoticum* or *Ae. speltoides* x *T. timopheevi*. Another two lines were produced by substitution backcrosses with *timopheevi* nucleus to the $BC_1$ or $BC_2$ plants of *T. turgidum* having each of the *boeoticum* and *speltoides* cytoplasm.

#### *Morphology and Fertility*

In the $BC_1$ generation, we found good pollen in *T. timopheevi* having *boeoticum* plasma but did not find any good pollen in *timopheevi* having *speltoides* plasma. However, in the $BC_2$ generation, one plant of *timopheevi* having *speltoides* plasma showed a pollen fertility of % and had dehiscent anthers, and one plant of those having *boeoticum* plasma also had dehiscent anthers (Fig. 4). All plants of both the substitution lines did not show abnormal growth in general, but the growth of some plants having *boeoticum* plasma was somewhat delayed. The $BC_2$ plants from $BC_1$ *turgidum* having *boeoticum* cytoplasm x *timopheevi* showed a pollen fertility of %, possessed dehiscent anthers and grew better than their ancestral lines (Figs. 2 and 4). Furthermore, the $BC_1$ plants of *T. timopheevi* having *T. monococcum* cytoplasm showed extremely abnormal growth, small and narrow leaves, bushy and stunted growth and did not head yet at early July.

#### *Conclusion*

From these results we cannot discuss which of *T. boeticum* or *Ae. speltoides* contributed the cytoplasm to *timopheevi*, but the cytoplasm of *T. timopheevi* seems to be more related to the cytoplasm of *Ae. speltoides* than to the cytoplasm of einkorn and seems to be slightly different from emmer cytoplasm.

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