Cytoplasmic Male Sterility in Relation to Hybrid Wheat Breeding

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Summary. The method of substitution and restoration of nuclei is briefly described.

Three species, *Aegilops caudata*, *Ae. ovata* and *Triticum timopheevi*, were used as donors of male sterility cyto-plasms.

The characteristics of these three cytoplasms are summarized as follows:

*Caudata*-cytoplasm: This cytoplasm has in many respects deleterious effects on the manifestation of alien genomes. Substitution lines having hexaploid wheat genome constitution are mostly male sterile while the female organ is normal. Some lines set frequently germless seeds. Haploid and twin seedlings are common occurrence in other lines. Pistillody is common in the substitution lines with tetraploid wheat genomes.

*Ovata*-cytoplasm: No pistillody was found in the substitution lines, both with hexaploid and tetraploid wheats. Male sterility is always complete in the substitution lines of hexaploid wheats with the exception of *P. timopheevi-cytoplasm*. A variety of common wheat having a pair of sat-chromosomes of *Ae. caudata*. This variety restores male fertility completely. No effective restorers were found for the substitution lines of emmer wheat. Delayed heading is common in the 4x substitution lines. *Timopheevi*-cytoplasm: Substitution lines of 6x wheats are mostly male sterile, while those of 4x wheats are more or less male fertile. Only the genome of *T. spelta* *dulcis* cytoplasm restores completely pollen fertility.

Among the indispensable factors for the success of hybrid wheat, five were discussed. They were (1) heterosis, (2) selection of male sterile cytoplasms, (3) discovery of restoring genes, (4) production of hybrid seeds and (5) quality.

This paper deals with our investigations on cytoplasmic male sterility and the fertility restoring genes hitherto obtained in wheat and its allies. This line of work was started in Kyoto since 1935. The first report on this problem was written in Japanese (1949) and a full paper was published in Cytologia in 1951.

The finding of a male sterile cytoplasm in crosses with *Aegilops caudata* attracted the eyes of wheat geneticists and wheat breeders. Soon the cytoplasms of *Ae. ovata* and *Triticum timopheevi* became known to cause male sterility and later many new findings were added. All are briefly summarized in the follow-

Method

First of all I should like to describe the method of our investigations.

Substitutions of nucleus can be accomplished by successive backcrosses. Let us assume that two diploid species, *A* and *B*, are used in an experiment in which the nucleus of *BB* has to be transferred to the cytoplasm of *AA*. The first step will be to produce the *F*₁ hybrid *AB*, with *AA* as the female parent. The further step will consist of successive backcrosses of *BB* (ק) to *F*₁ and to the subsequent backcrossing products.

For the sake of simplicity we will assume that the chromosome pairing between the genomes *A* and *B* is complete. The first backcross, *BB* to *F*₁, namely *AB* × *BB* ֳָָָָ, will produce the genome complement *B*²*B*, in which *B*¹ represents the first combination genome, resulting from the meiotic divisions in *F*₁ (AB). Accordingly, the second backcross, *B*²*B* × *BB*, will result in *B*³*B* and so forth until the *n*th backcross, *B*n*B*. If *n* is large enough, the offspring of the *n*th backcross will have the genome *BB* in the *AA* plasma. This series of backcrosses may be called substitution backcrosses (SB). With the *n*th backcross the substitution of *AA* genomes with *BB* genomes would be completed.

On the other hand, if *AA* is backcrossed to the *F*₁ hybrid, *AB* × *AA* ֳָָָָ, we can expect to obtain after a sufficient number of backcrosses plants which will not be different, either in plasma or genome constitution, from *AA*. Here the restoration of the *AA* genomes to the *AA* plasma would have taken place. Hence the term: restoration backcrosses (RB).

Both above described procedures are given in the following schema:

<table>
<thead>
<tr>
<th>Number of backcrosses</th>
<th>Substitution backcrosses</th>
<th>Restoration backcrosses</th>
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<td>a<em>AB</em> × <em>BB</em> = a<em>B²B</em></td>
<td>a<em>AB</em> × <em>AA</em> = a<em>A²A</em></td>
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<td>a<em>B²B</em> × <em>BB</em> = a<em>B²B</em></td>
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<td>a<em>Bn⁻¹</em>B × <em>BB</em> = a<em>B²B</em></td>
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* denotes the plasma of species *AA*. The genome complements *A²A* and *B²B* will correspond to *AA* and *BB*, respectively, from which the experiments are supposed to have started, provided that *n* is large enough.

The completion of substitution or restoration will be assumed on grounds of morphology and fertility as well as conjugation of chromosomes in the backcross offspring. Both procedures, substitution and restoration, are clearly demonstrated in Fig. 1.

Should the genomes *A* and *B* be non-homologous, with only univalents and unreduced gametes in *F*₁, the backcross would give rise to *ABB* (**ABB** × **BB** ֳָָָָ = **A*B²B**; *A¹* and *B¹* are the first recombination genomes). The continuous backcrossing would result in placing the *BB* genomes in a plasma (Fig. 1). This process may be greatly simplified by doubling the chromosome number of *F₁* plants through colchicine treatment (Fig. 2). In such case the chance

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* Dedicated to Professor HANS STUBBE on the occasion of his 65th birthday.

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of crossing over between the paternal and maternal chromosomes during the $F_1$ meiosis would be practically eliminated.

It is assumed that crossing over occurs between the chromosomes of the non-homologous genomes $A$ and $B$. The theoretical decrease in the number of heterozygotes in the subsequent generations was calculated by KIMURA (1950). KIMURA showed that it is slow in several of the first backcross generations, depending on the haploid chromosome number and the amount of crossing over. But later the decrease of heterozygotes is quick like in calculations made on the basis of non-crossing over (see Fig. 3).

Our objectives of nucleus substitution are:

1) to know the effects of foreign cytoplasms on genome manifestations,
2) to study (if any) the mutagenic effects of foreign genomes on the cytoplasmic elements (and vice versa), and
3) to examine the possibility of cytoplasmic transmission from pollen to egg cells.

Restoration backcrosses are indispensable for our understanding of the relationship between genomes and cytoplasms. As the progress of nuclear substitution and restoration in the course of backcrosses proceeds in similar manner, the difference in genome manifestation of $SB$ and $RB$ strains may be attributed to cytoplasm. If $n$ is large enough, both lines will have quite identical genomes ($AA$).

**Results**

1. Experiments with *Ae. caudata* and *T. vulgare erythraserpum*. First experiment: In my first report (KIHARA 1951), a case of substitution was described, where the hexaploid genome complement ($VV$) of *Triticum vulgare erythraserpum* (abbreviated *T. v. e.*) was introduced to the cytoplasm of *Aegilops caudata*, a diploid species having the genome formula $CC$. By two successive backcrosses of the hybrid, *T. v. e. (‡) × Ae. caudata (‡)*, with *T. v. e.*, as the male parent we obtained an $SB_2$ plant having $21H + 21$. Its offspring were obtained from open pollination, as the third backcross was not available.

The offspring of this $SB_2$ plant were found to have $21H$ in later generations and the line was maintained by self-pollination in our collection as a pure strain ($P174$).

After karyological examination, this strain was proved to possess one *caudata* chromosome, called *C-Sat-2*, which carries a gene (or genes) for male fertility restoration in the *caudata* cytoplasm and a gene for black awns. The modified genome of $P174$ was designated $V^\beta$. *C-Sat-2* is homoeologous to chromosome XVII or 1D (KIHARA and MURAMATSU 1955, MURAMATSU 1959).

$P168$ was obtained from the cross *T. v. e. (‡) and P174 (‡)*. This strain has *vulgare* cytoplasm and the genomes of *P174*. Accordingly the plasmatic genome formulas for $T. v. e. , P174$ and $P168$ are:

old

\[
\begin{align*}
T. v. e. & = \beta V^V V \quad \text{(aestivum)} \quad V V \\
P168 & = \beta V^{\beta} V^b \quad \text{(aestivum)} \quad V^b V^b \\
\text{and} \quad P174 & = \alpha V^V V^b \quad \text{(caudata)} \quad V^b V^b
\end{align*}
\]

New

respectively, where $\alpha$ = plasma of *Ae. caudata* and $\beta$ = plasma of *T. v. e.*

As the number of male sterile cytoplasms available for our studies is increasing, we can no longer use Greek letters for distinguishing each of them. This was the reason why I am using now a new system for plasma-genome combinations. The new formulas are given above at the right side of the old ones. For a variety with an alien cytoplasm, for instance, Norin 26 with *ovata* plasma, the symbol should be (*ovata*) Norin 26.

Second experiment: Our backcrosses were stopped at the 2nd generation ($SB_2$) and we had no restoration lines. This means that our first investigations were not adequately planned. Therefore a new series of substitution as well as restoration backcrosses was started in 1949. Reciprocal hybrids between *T. v. e.* and *Ae. caudata* were used.

As far as the chromosome behavior in $F_1$ and the procedure of restoration or substitution are concerned, the reciprocal hybrids behaved quite simi-