

Production of *Aegilops cylindrica* with *Ae. caudata* plasmon

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INTRODUCTION

The final aim of this investigation is to demonstrate the genetic effect of a co-existing alien genome on the plasmon. For this purpose, we are attempting to reconstruct *Aegilops caudata* (2n=14, genome constitution CC) by combining its genome and plasmon that were isolated from each other for more than half a century. This is an interim report of the work that led to production of an alloplasmic *Ae. cylindrica* (2n=28, CCDD) line, (*caudata*)-CCDD.

MATERIALS AND METHODS

An alloplasmic line of a common wheat, *Triticum aestivum* strain "Tve" (2n=42, AABBDD) having plasmon of *Ae. caudata*, was produced by 50-times repeated backcrosses of an *Ae. caudata* x "Tve" F1 hybrid with "Tve" as the recurrent pollen parent by Kihara (1959) and Tsunewaki et al. (1996). Chromosome doubling of the F1 hybrid, (*caudata*)-Tve x *Ae. caudata*, was successful by applying 0.5% aqueous colchicine solution to the hybrid for three days by Sears' method. Somatic chromosome number and meiotic chromosome pairing were observed by aceto-carmine squash and smear method applied to the root tips and anthers fixed with the Camoy's fixative, respectively.

RESULTS AND DISCUSSION

Transfer and maintenance of *Ae. caudata* plasmon in a common wheat "Tve"

The initial cross between *Ae. caudata* as female and "Tve" and the first 16 backcrosses (up to SB16 generation) of the hybrid by "Tve" as recurrent pollen parent were made in 1949 and from 1950 to 1965, respectively, by Kihara (1959). Later backcrosses from SB17 to SB50 generations were made by the senior author (Tsunewaki et al. 1996, unpublished). This alloplasmic line is shown by a formula, (*caudata*)-Tveⁿ, n referring to the number of repeated crosses made with the "Tve" pollen, including the initial hybridization. Until SB50, (*caudata*)-Tve retained complete male sterility with normal female fertility (67% average seed set by artificial pollination). The occurrence of germless grains and haploid and twin seedlings, in addition to complete male sterility, is the effect of the *caudata* plasmon consistently expressed in "Tve".

Production of a hybrid, (*caudata*)-ABCD, by crossing (*caudata*)-Tve as female to *Ae. caudata*

In total, 758 florets of (*caudata*)-Tve⁵¹ were pollinated with the *caudata* pollen, from which 96 seeds were obtained (Table 1).

The seed-setting rate was 13%. Of the 95 seeds sown, 15 were germinated, the germination rate being 16%. All of them grew

vigorously, although no seeds were produced by open pollination. Artificial pollination of 1,522 florets of those F1 hybrids with the *caudata* pollen, that was made in the 2002-03 season, did not set any seeds. Thus, production of (*caudata*)-ABCD was unsuccessful.

Production of (*caudata*)-AABBCCDD by colchicine treatment of (*caudata*)-ABCD

Three (*caudata*)-ABCD plants at the early tillering stage were treated with 0.5% aqueous colchicine solution by Sears' method. The results are shown in Table 2.

One of the three treated plants produced a highly fertile spike, from which eight seeds were obtained. Four seeds were sown and all germinated, and the resultant plants set seeds at an average of 54% by selfing. Their offspring showed variation on the 2n chromosome number, 56 (eu-octoploid), with a range of 53-57, proving their octoploid nature as well as meiotic instability. In accordance with the chromosome number fluctuation, their seed set under self-pollination varied between 3-67%.

Production of (*caudata*)-ABCCDD by crossing (*caudata*)-AABBCCDD with the *cylindrica* pollen

First, we attempted to cross (*caudata*)-AABBCCDD with the *caudata* pollen to produce (*caudata*)-ABCCD in the 2004-05 season. In total, 294 florets were pollinated without any success. We changed our approach to make a cross between (*caudata*)-AABBCCDD and *Ae. cylindrica* as pollen parent. The results are shown in Table 3.

This crossing was successful, producing 96 seeds from 1,002 florets pollinated, the seed-setting rate being 9.6% (Table 3). Of those seeds, 16 were sown and 12 of them germinated. Those hybrids had 42 chromosomes in root-tip cells, as expected from the supposed genome constitution, ABCCDD. They did not set any seeds in 566 florets by open pollination.

Backcrossing the (*caudata*)-ABCCDD as female to *Ae. cylindrica*

In total, 2,210 florets of (*caudata*)-ABCCDD plants were artificially pollinated with the *cylindrica* pollen, aiming to obtain an alloplasmic *Ae. cylindrica* line, (*caudata*)-CCDD. Results are given in Table 4.

Table 1. Results of the crosses, (*caudata*)-Tve⁵¹ x *Ae. caudata* KU6-2 (2001-02)

| Female parent | No. florets pollinated | No. seeds set | % Seed-set | No. seeds sown | No. seeds germ. | % Germination |
|---------------|------------------------|---------------|------------|----------------|-----------------|---------------|
| 1 | 140 | 8 | 5.7 | 8 | 6 | 75.0 |
| 2 | 198 | 3 | 1.5 | 3 | 2 | 66.7 |
| 3 | 118 | 11 | 9.3 | 10 | 1 | 10.0 |
| 4 | 90 | 18 | 20.0 | 18 | 4 | 22.2 |
| 5 | 120 | 43 | 35.8 | 43 | 1 | 2.3 |
| 6 | 42 | 8 | 19.0 | 8 | 1 | 12.5 |
| 7 | 50 | 5 | 10.0 | 5 | 0 | 0.0 |
| Total | 758 | 96 | 12.7 | 95 | 15 | 15.8 |

Table 2. Result of the colchicine treatment of the F1 hybrid, (*caudata*)-Tve⁵¹ x *Ae. caudata* KU6-2, by Sears' method (2003-04)

| Plant No. | No. florets examined | No. seeds set | % Seed-set | No. seeds sown | No. seeds germinated | % Germination |
|-----------|----------------------|---------------|------------|----------------|----------------------|---------------|
| 1 | 638 | 1 | 0.16 | - | - | - |
| 2 | 612 | 10 * | 1.63 | 4 | 4 | - |
| 3 | 582 | 0 | 0.00 | - | - | - |
| Total | 1,832 | 11 | 0.60 | 4 | 4 | 100 |

* : Ten seeds were set in three ears as follows; 8/30, 1/30 and 1/28, where the numerator and denominator are numbers of seeds set and florets examined in each ear, respectively.

Table 3. Results of the cross, (*caudata*)-AABBCCDD x *Ae. cylindrica* KU7-1, -2 (2005-06)

| Female parent | No. florets pollinated | No. seeds set | % Seed-set | No. seeds sown | No. seeds germinated | % Germination |
|---------------|------------------------|---------------|------------|----------------|----------------------|---------------|
| 1 | 106 | 16 | 15.1 | 2 | 2 | - |
| 2 | 108 | 10 | 9.3 | 2 | 2 | - |
| 3 | 160 | 18 | 11.3 | 2 | 2 | - |
| 4 | 110 | 9 | 8.2 | 2 | 0 | - |
| 5 | 128 | 9 | 7.0 | 2 | 2 | - |
| 6 | 112 | 7 | 6.3 | 2 | 1 | - |
| 7 | 120 | 19 | 15.8 | 2 | 2 | - |
| 8 | 114 | 8 | 7.0 | 2 | 1 | - |
| 9 | 44 | 0 | 0.0 | - | - | - |
| Total | 1,002 | 96 | 9.6 | 16 | 12 | 75.0 |

Table 4. Results of the cross, (*caudata*)-ABCCDD x *Ae. cylindrica* KU7-1, -2 (2006-07)

| Female parent | No. florets pollinated | No. seeds set | % Seed-set | No. seeds sown | No. seeds germinated | % Germination |
|---------------|------------------------|---------------|------------|----------------|----------------------|---------------|
| 1 | 218 | 11 | 5.0 | 11 | 10 | 91 |
| 2 | 220 | 2 | 0.9 | 2 | 2 | 100 |
| 3 | 220 | 9 | 4.1 | 9 | 6 | 67 |
| 4 | 220 | 8 | 3.6 | 8 | 8 | 100 |
| □5 | 220 | 2 | 0.9 | 2 | 0 | 0 |
| 6 | 238 | 2 | 0.8 | 2 | 2 | 100 |
| 7 | 236 | 23 | 9.7 | 23 | 16 | 70 |
| 8 | 248 | 12 | 4.8 | 12 | 8 | 67 |
| 9 | 246 | 4 | 1.6 | 4 | 3 | 75 |
| Total | 2,066 | 73 | 3.5 | 73 | 55 | 75 |

Unfortunately, we did not find any $2n=28$ plants which should be eu-tetraploid (*caudata*)-CCDD that we sought. A possible reason for this was unexpectedly high univalent transmission rate, in other words, low univalent elimination rate in the (*caudata*)-ABCCDD. Estimated transmission rate of the univalent of the A or B genome was 92.8%, indicating almost no univalent elimination in this particular hybrid. This makes great contrast to only 54% transmission of a univalent in common wheat monosomics (Tsunewaki 1963), and is similar to about 90% transmission of a D-genome univalent in pentaploid wheat hybrids (Kihara and Wakakuwa 1935).

Production of the euploid (*caudata*)-CCDD and comparative organellar microsatellite analyses of *Ae. caudata*, (*caudata*)-Tve and (*caudata*)-CCDD

We will make the second backcross of the SB1 hybrids with the *cylindrica* pollen to obtain the euploid (*caudata*)-CCDD. In parallel, we will carry out a comparative microsatellite analysis of chloroplast and mitochondrial DNAs of *Ae. caudata*, (*caudata*)-Tve⁵⁵ and (*caudata*)-CCDD, using the primer sets developed by Ishii et al. (2001, 2006), to assay the constancy of the *caudata* organellar genomes under the presence of common wheat genomes for more than half a century.

ACKNOWLEDGEMENT

Ae. caudata accession KU6-2 and *Ae. cylindrica* accession KU7-1, -2 used in this study were provided by T. Kawahara, Plant Germplasm Institute, Kyoto University, under the auspices of the NBPR(Wheat) Project, Japan.

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