Hybrids and amphiploids of *Aegilops ovata* L. with *Secale cereale* L.: production, morphology and fertility

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**Abstract.** Hybrids (2n = 3x = 21) between *Aegilops ovata* and *Secale cereale* were produced via embryo rescue. Most hybrid morphological traits were intermediate between parents, the plants grew vigorously but were completely sterile. The average frequency of chromosome associations at metaphase I was 19.57-20.19 univalents and 0.40-0.86 rod bivalents. The fertility of the hybrids was restored by doubling their chromosome numbers by colchicine treatment and *in vitro* propagation. Selfed seeds were obtained from colchicine-doubled sectors and some callus regenerates. The seeds were mostly well formed and developed vigorous plants without embryo culture. Colchicine- and callus-derived amphiploids (2n = 6x = 42) resembled the F1 plants in overall morphology, but showed a lower tillering ability, broader leaves, thicker culms and larger spikes.

**Key words:** *Aegilops ovata*, amphiploids, embryo culture, fertility, hybrids, *Secale cereale*, tissue culture.

**Introduction**

*Aegilops ovata* L. is a species of particular interest for genetic and breeding research as an important gene donor for complex disease resistance, high grain protein content, salt tolerance and the sterilizing ability of its cytoplasm (GANEVA et al. 1992, LANDIEVA, GANEVA 1998, 1999). *Ae. ovata* has been extensively crossed with *Triticum aestivum* and F1 hybrids and amphiploids were produced to transfer the *Ae. ovata* chromosomes into bread wheat (LANDIEVA, GANEVA 1998). The hybrids between *Ae. ovata* and *Secale cereale* were obtained by LEIGHTY et al. (1926), KAGAWA and CHIZAKI (1934), KHALILOV and KASUMOV.
The aim of the experiment was to produce hybrids and amphiploids of *Ae. ovata* and *S. cereale*. The report deals with the crosses of *Ae. ovata* with *S. cereale*, production of hybrids and amphiploids, their morphology and fertility.

**Material and methods**

The plants of *Ae. ovata* (accession TO36) and *S. cereale* open-pollinated, spring cv. Strzekęcińskie and 5 winter inbred lines (Table 1) were used for intergeneric crosses. Seeds of *Ae. ovata* were kindly supplied by M. FELDMAN (Rehovot, Israel). Plants were grown and crossed in a greenhouse. The crosses with GA_{3} stigma treatment, embryo rescue and cytological examinations were performed according to WOJCIECHOWSKA and PUDELSKA (1995). Differentiated embryos were placed on medium B_{5} (GAMBORG et al. 1968) while the undifferentiated ones on B_{5} with 2,4-D (2 mg/l) for callus induction. Mitotic and meiotic chromosomes were stained by the Feulgen method. Tissue culture propagation of immature inflorescence, rachis and neck explants, and colchicine treatment of F_{1} tillers followed WOJCIECHOWSKA and PUDELSKA (1992). For *in vitro* propagation the explants were taken from F_{1} hybrid tillers: (a) non-treated by colchicine, (b) non-doubled by prior colchicine treatment, and (c) non-doubled by prior *in vitro* regeneration. Pollen stainability was determined in samples of 500 grains taken from mature anthers and stained in a solution of acid fuchsin in lactophenol (SASS 1964). The C symbol was used for marking the colchicine-derived amphiploids and R symbol for callus-derived regenerates.

**Results and discussion**

Results of crosses between *Ae. ovata* and *S. cereale* are shown in Table 1. Seed set and embryo yield were obtained from all 6 cross combinations and varied among combinations from 39.68 to 88.89% and from 15.79 to 44.44%, respectively. Most of isolated embryos (53.06%) were relatively well-developed. Relatively well-developed endosperm was present in most caryopses with well-developed embryos. However, only in one cross combination the plants were generated directly from cultured embryos. Most probably the reproductive barriers have been activated at various stages of hybrid seed development and even relatively well-developed embryos with signs of differentiation, surrounded by relatively well-developed, solid endosperm, have been unable to develop further. Callus was obtained from 47.82% undifferentiated embryos induced to form callus tissue but no regeneration was achieved. The results are in agreement with FUJIGAKI and
TOZU’s (1993) findings in Hordeum × Secale whose response to the immature caryopsis culture is quite different among different cross combinations and is independent of crossability.

Somatic metaphases revealed the expected hybrid chromosome number 2n = 3x = 21. The plants showed vigorous vegetative growth and good tillering ability. Most morphological traits of the plants were intermediate between parental species. However, some characters, such as neck pubescence or spikelets with three to four flowers and brittle rachis resembled traits of the male or female parent, respectively. The hybrids were completely sterile, with dry and indehiscent anthers, unstained and empty pollen grains. Meiotic metaphase I (MI) plates in

<table>
<thead>
<tr>
<th>Cross combination</th>
<th>Florets pollinated No.</th>
<th>Seed set No. (%)</th>
<th>Embryos cultured</th>
<th>Embryos callused</th>
<th>Plants directly from embryo</th>
<th>Plants via callus culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ae. ovata × Strzeleckińskie</td>
<td>63</td>
<td>25 (39.68)</td>
<td>10 (15.87)</td>
<td>3 (4.76)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ae. ovata × 2480</td>
<td>60</td>
<td>40 (66.67)</td>
<td>19 (31.67)</td>
<td>3 (5.00)</td>
<td>4 (6.67)</td>
<td>0</td>
</tr>
<tr>
<td>Ae. ovata × 3510</td>
<td>24</td>
<td>11 (45.83)</td>
<td>7 (29.17)</td>
<td>2 (8.33)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ae. ovata × 2507</td>
<td>9</td>
<td>8 (88.89)</td>
<td>4 (44.44)</td>
<td>3 (33.33)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ae. ovata × 2488</td>
<td>19</td>
<td>9 (47.37)</td>
<td>3 (15.79)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ae. ovata × 2514</td>
<td>27</td>
<td>13 (48.15)</td>
<td>6 (22.22)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* (% of pollinated florets

The F1 hybrids of Ae. ovata × S. cereale reported by LEIGHTY et al. (1926), KAGAWA, CHIZAKI (1934), and SECHNYAK, SIMONENKO (1991) were completely sterile. Meiotic I analysis of the hybrids revealed 2-3 (KAGAWA, CHIZAKI 1934) or 1-5 (KHALILOV, KASUMOV 1989) rod bivalents of loose type and 1 trivalent per cell. SECHNYAK and SIMONENKO (1991) reported 1.49 bivalents, including 1.46 rods and 0.3 rings, and 0.12 trivalents per cell. CUÑADO (1992), by means of the C-banding technique, determined Aegilops-Aegilops and Aegilops-rye chromosome configurations: 1.83 rod bivalents and 0.09 trivalents of Aegilops-Aegilops, with a mean number 2.01 for Aegilops-Aegilops and 0.06 for Aegilops-rye chromosome associations per cell.

Because of low hybrid production frequency and complete hybrid sterility, tissue culture technique has been applied to propagate the Ae. ovata × S. cereale hy-
brids and to double their chromosome number. In vitro propagation revealed good (mean 82.54 %) callus induction of 2108 explants from immature inflorescences, rachides and necks of F1 hybrids. Regeneration frequency of 1.94% was very low, with a range 0.00-4.15%. The plants arose through organogenesis from green islands on a compact yellow-white callus of immature inflorescence, rachis and neck explants. Callus-derived regenerates exhibited the same (80.41%) as the original hybrids or doubled (19.51%) somatic chromosome numbers. Callus induction, regeneration ability and regenerate ploidy level was not affected by material (non-treated, colchicine-treated, in vitro regenerates) used for in vitro propagation.

A total of 8 callus-derived amphiploids (2n = 6x = 42) regenerated from R0 (6 plants) and R1 (2 plants) in vitro generation: 1 plant from immature inflorescence, 5 plants from rachis and 2 plants from neck explants. Two of the regenerates set no seeds while the other six set from 1 to 60 mostly well-developed selfed seeds (0.00-3.33 seeds/spike). The three most fertile callus-derived amphiploids had nearly completely dehisced anthers, pollen stainability varied from 44.0 to 68.20% and they set 1.18, 2.06 and 3.33 seeds per spike, respectively. Shape and size of stained pollen grains and kernels (Figure 1) were heterogenous and intermediate between parental species.

In total, 54 tillers of the four hybrids were treated by colchicine and 28 of them survived. Root-tip chromosome counts in the survived plants showed 21 chromosomes by 20 (71.43%) plants, and chimeric numbers 21 and 42 chromosomes by 8 (28.57%) ones, but only three (10.71%) of these plants had sectors that produced seeds. The C0 chimeric plants set 1-2, in total 4 seeds. Anther dehiscence was very sporadic: in one or two flowers, in one or two spikes per plant, with pollen stainability 24.00% in one flower.

### Table 2. Mean configurations in MI euploid cells of *Ae. ovata × S. cereale* hybrids

<table>
<thead>
<tr>
<th>Hybrid No.</th>
<th>No. of cells</th>
<th>I</th>
<th>II</th>
<th>X ta/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Chromosome configuration</td>
<td>rod</td>
<td>ring</td>
</tr>
<tr>
<td>204 A</td>
<td>95</td>
<td>19.57</td>
<td>0.72</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(15-21)</td>
<td>(0-3)</td>
<td></td>
</tr>
<tr>
<td>204 B</td>
<td>101</td>
<td>20.07</td>
<td>0.47</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(17-21)</td>
<td>(0-2)</td>
<td></td>
</tr>
<tr>
<td>205</td>
<td>107</td>
<td>19.28</td>
<td>0.86</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(13-21)</td>
<td>(0-4)</td>
<td></td>
</tr>
<tr>
<td>210</td>
<td>89</td>
<td>20.19</td>
<td>0.40</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(17-21)</td>
<td>(0-2)</td>
<td></td>
</tr>
</tbody>
</table>
Callus– (R1) and colchicine– (C1) derived amphiploids showed vigorous vegetative growth and morphologically were mostly intermediate between the parental species. In comparison to the F1 hybrids, they exhibited lower tillering ability and somewhat larger spikes (Figures 2, 3), broader leaves and thicker culms. The spikes of F1 hybrids and amphiploids had a similar number of spikelets (Figures 2, 3) and flowers (2-4, mostly 3) per spike.

*Aegilops*-*Secale* amphiploids were obtained via regeneration mostly from immature inflorescence callus tissue in *Triticum tauschii × S. cereale* (FEDAK 1984), *Ae. squarrosa* and *Ae. ventricosa × S. cereale* (SECHNYAK et al. 1992), and *Ae. variabilis* and *Ae. kotschyi × S. cereale* (WOJCIECHOWSKA, PUDELSKA 1999). FEDAK (1984) reported on 75% *T. tauschii × S. cereale* callus regenerates with a doubled chromosome number. SECHNYAK et al. (1992) found over 50% of fertile callus regenerates in R0 depending on crossing combination of *Ae. squarrosa* or *Ae. ventricosa* with *S. cereale*.

Seed set was also much lower in C0 than in R0 generation of the *Ae. variabilis × S. cereale* plants (WOJCIECHOWSKA, PUDELSKA 1999). SECHNYAK and SIMONENKO (1993) reported in *Ae. ventricosa × S. cereale* amphiploids more successful meiotic stabilization in colchiamphiploid than in callus-regenerated ones, and they found also that the fertility of the amphiploids does not depend on chromosome pairing level but on PMC euploid level.

Amphiploids *Ae. ovata × S. cereale*, produced by colchicine treatment and *in vitro* propagation of F1 hybrids, will be examined in respect of their fertility in further generations and crossability with rye.
Figures 2, 3. Parental, hybrid and amphiploid spikes. 2. *Ae. ovata, Ae. ovata × S. cereale* hybrid (2x), *S. cereale*; 3. *Ae. ovata, Ae. ovata × S. cereale* amphiploid (2x), *S. cereale*

REFERENCES


