Discarding of the postzygotic reproductive barrier between hexaploid wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.)

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Rye (*Secale cereale* L.) is a close relative of wheat (*Triticum aestivum* L.) that provides a vast genetic variation for commercially important traits such as stress tolerance, biomass, yield and photosynthetic potential not only for triticale, but also for wheat itself.

**Postzygotic isolating mechanisms** between the *Triticum* and *Secale* genera ensure that not every cross between wheat and rye results in a hybrid plant.

**Embryo lethality** is one of the first postzygotic barriers that strongly limits the transmission of desirable genes from rye to wheat.

**Embryo lethality**

- **tetraploid wheat x rye**
  - early stage endosperm abortion
  - embryo rescue
  - Hexaploid triticale via chromosome doubling

- **hexaploid wheat x rye**
  - ~ 96% normal embryo and endosperm development
  - wheat-rye hybrids or octoploid triticale
  - ~ 4% normal endosperm development
  - ungerminated seeds with abnormal embryos
Embryo lethality in crosses hexaploid wheat (Triticum aestivum L.) with certain inbred rye (Secale cereale L.) lines.
Genetic analysis of wheat-rye embryo lethality

- A 3-way segregation analysis was performed by crosses hexaploid wheat ‘Chinese Spring’ (CS) with different interline rye F₁ hybrids (L6xL2; L7xL2). For all cross combinations under investigation a 1 : 1 (segregation for normal 1,146 vs. nonviable embryos 1,193) in hybrid seeds was obtained according to expectation.

- Therefore, it was concluded that embryo lethality, in wheat/rye crosses, is determined in rye by one gene which was named $Eml$ (Embryo lethality).

- This gene has two alleles: compatible ($Eml$-$R1a$) and incompatible ($Eml$-$R1b$) with wheat genome.

Tikhenko et al., 2005, Russ J Genetics, 41:877–884
Chromosomal localisation of \textit{Eml-R1} rye gene

The set of 79 recombinant inbred lines (RIL) of an F5 population was produced from cross L7xL2 and served as testers in crosses with CS.

\textit{Eml-R1} maps to chromosome arm 6RL. The distance between \textit{Eml-R1} and the co-segregating markers \textit{Xgwm1103 (Xgwm732)} was found to be 8.8±3.5 cM, the distance to isozyme locus \textit{Est10 (EstR-5)} was 29.4±6.2 cM.

Mode of inheritance was studied by crosses CS wheat-rye additional lines (+6R, +6RL and +6RS) with rye L2 and was shown, that mutant allele \textit{Eml-R1b}, causing embryo lethality in crosses common wheat with rye, is dominant, and wild type allele \textit{Eml-R1a} is recessive.

According the Bateson-Dobzhansky-Müller model embryo lethality of wheat-rye F₁ hybrids could be the result of complement interaction the genes of parental genomes

The wheat gene complemented to rye *Eml-R1b* allele could be revealed in crosses the sets ‘Chinese Spring’ nullisomic-tetrasomic (CSNT) with line L2 or L535, carrying the rye *Eml-R1b* allele, if this gene (allele) located only in one of homeoeological genome – A, B or D.

We crossed the sets CSNT lines with rye line L2. Only the embryos in crosses with lines, nullisomic on 6A chromosome (*Nulli6A/Tetra6B* and *Nulli6A/Tetra6D*), have the normal embryogenesis.

This result allows to proposed, that 6A wheat chromosome carries gene complementing to *Eml-R1b* rye allele. So this wheat gene, involving in embryo lethality in crosses common wheat with rye was named *Eml-A1*.

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Localization of the *Eml-A1* incompatible allele on chromosome 6A of wheat.

Deletion mapping of *Eml-A1* gene of common wheat was performed using the set of DT and deletion CS lines for 6A chromosome of *Eml-A1* gene is located on the long arm of the 6A chromosome in telomere region from 0.90 to 1.00.
Identification of candidate genes which are involved in this reproductive barrier

In *silico* sequence homology analysis was performed between the wheat chromosome 6AL survey sequence and the set of candidate genes.

I – KNOX genes: Arabidopsis - *STM, CLV1, CLV2, CLV3*;
   maize – *KN1, TD1, FEA2*;
   rice – *OSH1, FON1, FON2, FCP1*

II – WOX genes: Arabidopsis - *WUS*;
   maize – *ZmWUS1, ZmWUS2*;
   rice – *OsWUS*

III - specific and independent of meristem regulators:
   Arabidopsis – *CUC1, CUC2, miR394*;
   rice – *SHO1, SHL1, SHL2, SHL3, SHL4/SHO2, HD-ZIPIII*;

IV - other gene or gene interactions which lead to embryo lethality:
   Arabidopsis – *AtFH, HPA1/HISN6A, HPA2/HISN6B, AtDEK1*;
   maize – *sml, dgr, dek1*;
   rice – *IPT, ADL1, ONI1*

**Summary:** 6AL wheat chromosome carries the sequences which are corresponded to *ADL1* and *miR394* genes (identity ≥ 75 % and alignment length ≥ 50 bp), but not in 0.9-1.0 region.
The main objectives of the current study:

I. How strong this barrier can be?

II. Developing a model for a wheat-rye lethality system involving differential expression of incompatible wheat *Eml-A1* and rye *Eml-R1b* alleles in an identical genetic background.
Methods

- *In vitro* embryo rescue
- Histological analysis and scanning electron microscopy
- Colchicine treatment
- Flow cytometric analysis of DNA content
- Genomic *in situ* hybridization (GISH)
- Fertility analyses of male and female gametophytes in amphidiploid (AD) plants
Plant material:

**Maternal forms:** hexaploid wheat cv ‘Chinese Spring’ (CS) with *kr1* and *kr2* genes

**Paternal forms:** marker rye inbred lines (L6, L7) which are the carriers of compatible with wheat genome *Eml-R1a* allele and inbred line L2 with mutant incompatible allele *Eml-R1b*.

**Wheat-rye** hybrid embryos at age 14 and 16 days after pollination (DAP).

*Kr1/2:* high crossability genes
Results: Embryogenesis in callus culture from immature abnormal embryos

Crosses between CS and rye inbred lines with *Eml-R1a* (L6,L7) and *Eml-R1b* alleles were performed.

Embryo rescue wheat-rye of normal and abnormal embryos at age 14 and 16 DAP.

Morphogenesis in callus culture

- Organogenesis
- Somatic embryogenesis
Table 1. Regenerative capacity of immature wheat-rye embryos

<table>
<thead>
<tr>
<th>Cross</th>
<th>Protocol</th>
<th>Age of embryos (DAP)</th>
<th>Percent embryos with embryogenic callus</th>
<th>Regenerative capacity per embryo: mean number of adventive buds</th>
<th>plantlets</th>
<th>regenerative events</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>adventive buds</td>
<td>plantlets</td>
<td>regenerative events</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mean</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>min - max</td>
</tr>
<tr>
<td>CS x L6 wt</td>
<td>1</td>
<td>14 (n=60)</td>
<td>93.3</td>
<td>3.1</td>
<td>5.2</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16 (n=60)</td>
<td>95.0</td>
<td>5.6</td>
<td>6.2*¹)</td>
<td>11.8</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>14 (n=70)</td>
<td>85.7</td>
<td>8.5</td>
<td>6.1*¹)</td>
<td>14.6**¹)²)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16 (n=60)</td>
<td>95.0</td>
<td>5.4</td>
<td>4.9</td>
<td>10.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>14 (n=70)</td>
<td>100.0</td>
<td>4.6</td>
<td>9.9**²)</td>
<td>14.5**²)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16 (n=60)</td>
<td>83.3</td>
<td>4.7</td>
<td>8.9</td>
<td>13.7</td>
</tr>
<tr>
<td>CS x L2 mutant</td>
<td>1</td>
<td>14 (n=70)</td>
<td>96.1</td>
<td>3.8</td>
<td>8.8</td>
<td>12.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16 (n=79)</td>
<td>91.1</td>
<td>2.2</td>
<td>5.6</td>
<td>7.8</td>
</tr>
<tr>
<td>CS x CS (control)</td>
<td>2</td>
<td>14 (n=77)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>16 (n=79)</td>
<td></td>
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</tr>
</tbody>
</table>

* - significant differences in p<0.05; ** - significant differences in p<0.01;
¹) - variance analysis for two different genotypes (CSxL6, CSxL2) in 2 different ages for Protocol 1;
²) - variance analysis for abnormal embryos (CSxL2) which were incubated in two different protocols.
Chromosome doubling in embryo culture

1. Cultivation on CIM1 medium (2 µ/l 2,4-D) for 21 days before colchicine application.

2. Cold-treatment at 4°C for 36 h.

3. Colchicine treatment on the CIM1 medium with 0.5%, 0.4% and 0.2% colchicine for 48 h.

4. CIM1 medium without colchicine 14 days, than incubation according to protocol 2.

<table>
<thead>
<tr>
<th>Cold-treatment</th>
<th>0.5%</th>
<th>0.4%</th>
<th>0.2%</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ CS xL2</td>
<td>50.7*</td>
<td>44.6</td>
<td>32.1</td>
</tr>
<tr>
<td>-</td>
<td>(75)</td>
<td>(74)</td>
<td>(81)</td>
</tr>
<tr>
<td>+ Cs x L6</td>
<td>53.5</td>
<td>-</td>
<td>34.2</td>
</tr>
<tr>
<td>-</td>
<td>(99)**</td>
<td>(23)</td>
<td>(147)</td>
</tr>
</tbody>
</table>

* - percent diploid plantlets, ** - total number of plantlets

In total, more than 2540 amphihaploid and 229 amphidiploid plants from incompatible cross CSxL2 were produced and analyzed. The ploidy of 20 randomly selected ADL2 plants and 10 haploid plants was confirmed through analysis of meiotic chromosomes.
Chromosome pairing at metaphase I of meiosis in F1 interspecific wheat-rye hybrids (amphihaploid) and amphidiploids using the GISH method

(a, b) Amphidiploids (ADL2) from a cross between CS wheat (AABBDD) and the rye line L2 (RR) after chromosome doubling display 7 yellow rye bivalents with homologous pairing.

(c) An F1 amphihaploid derived from a CSxL2 cross exhibits 7 yellow rye univalents. GISH painting: ABD genomes, red; R genome, yellow
The presence of exogenous cytokinin in the environment is not a prerequisite for the beginning of morphogenesis in culture of abnormal embryos.

Morphogenic zones spring up on CIM1 medium containing 2 mg/l auxin 2,4-D in the dark and end with formation of normal plants without or with colchicine application in callus culture.

Totally 229 amphidiploid plants from cross CSxL2 were received.

8 amphidiploid plants are fertile in different rate, what is 3.5% from all tested amphidiploids plant.

These fertile amphidiploid plants can reproduce itself under the strong isolation.
Fertility of amphidiploid plants with “lethal” genotype (CSxL2)

The quality of pollen of amphidiploid plants

<table>
<thead>
<tr>
<th>Group of AD plants</th>
<th>Percent pollen grains</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>fertile</td>
<td>sterile</td>
</tr>
<tr>
<td></td>
<td></td>
<td>empty or single-celled</td>
</tr>
<tr>
<td>Fertile (n = 8)</td>
<td>35,8-79,0</td>
<td>6,1-15,9</td>
</tr>
<tr>
<td>Sterile-a (n = 9)</td>
<td>2,7 – 14,8</td>
<td>7,3- 42,6</td>
</tr>
<tr>
<td>Sterile-b (n = 6)</td>
<td>0,0</td>
<td>20,0-56,0</td>
</tr>
</tbody>
</table>
This finding suggests that degradation of the ES occurred very early and that hybrid sterility affects both male and female gametophytes in ADL2 plants.

The sterility of the amphidiploid plants could be the result of negative interaction between \textit{Eml-A1} wheat and \textit{Eml-R1b} incompatible alleles during gametogenesis.

or other parental genes leading to sterility of the amphidiploid plants.
A model system (working scheme) for studying the differences in the interaction and expression of incompatible wheat *Eml*-A1 and rye *Eml*-R1b alleles that cause embryo lethality.

1. **Double fertilisation**
   - **CS x L2**, *Eml*-A1 *Eml*-R1b
   - **Embryo lethality**

2. **Callus induction**
   - **Plant regeneration**
   - **Amphihaploid plants (sterile)**

3. **Colchicine treatment**
   - **Plant regeneration**
   - **96.5% amphidiploid plants sterile**
   - **3.5% amphidiploid plants fertile**

4. **Double fertilisation**
   - **CS 6AL-8 x L2**, *Eml*-R1b
   - **SAM**
   - **Viable hybrid embryos**
Conclusion:

1. Postzygotic barrier in crosses common wheat with rye, which is the result of interaction of *Eml-A1* wheat and *Eml-R1b* incompatible alleles can be easily overcome by embryo rescue of abnormal embryos in age 14-16 DAP. High regenerative capacity in callus culture of abnormal embryos with or without application of exogenous cytokinin leads to the conclusion that the reproductive barrier between hexaploid wheat and certain rye inbred lines has epigenetic nature.

2. Double fertilization is a key biological event which activates the interaction between incompatible wheat *Eml-A1* and rye *Eml-R1b* alleles and leads to embryo lethality via shoot apical meristem abortion.

3. Regeneration in callus culture goes by two ways: somatic embryogenesis (embryoids) and organogenesis (adventive buds formation) and results in development of normal amphihaploid plants.

4. Two types of amphidiploid plants: fertile (3.5%) and sterile (96.5%) were produced by colchicine application in callus culture.

5. A model involving differential expression of the wheat *Eml-A1* and rye *Eml-R1b* alleles in an identical genetic background was developed. This model includes four states of incompatible alleles from the parental forms.

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