Wheat Chromosome Engineering and Breeding

Jianli Chen
Chromosome Engineering

- A process to transfer favorable alleles through inter-specific hybridization and interchange of chromatin using aneupoloids
Aneuploids?

- Individuals having chromosome numbers other than an exact multiple of the basic chromosome set.
- A basic chromosome set contains all chromosomes in a genome.
- A genome is defined as the basic chromosome set that contains all the genetic information needed to produce an organism, denoted by a x.
Review Concepts

\( n \): gametic (haploid) chromosome number. \( n = 3x = 21 \) (wheat)

\( 2n \): disomic (somatic) chromosome number.

wheat: \( 2n = 6x = 42 \); Barley: \( 2n = 2x = 14 \); Soybean: \( 2n = 2x = 20 \); Maize: \( 2n = 2x = 20 \)
Outline of current lecture

• Types of aneuploids in common wheat
• Application of aneuploids in wheat genetic and mapping studies
• Application of aneuploids in wheat breeding
Genetic Features

- Hexaploid wheat has homoeologous genomes A, B, D derived from diploid species (AA- T. urartu, BB- Ae. Speltoides, DD- Ae. Squarrosa)
- Most of the genes have three homoeologous loci, which can functionally compensate for one another.
- The homoeologous genomes can tolerate loss or addition of chromosomes.
- Complete sets of aneuploids of Chinese Spring and other wheat varieties are available.
Wheat Aneuploid Series

- Monosomic lines (Sears, 1954)
- Nullisomic lines (Sears, 1954; Xue et al., 1990)
- Nulli-tetrasomic lines (Sears, 1954).
- Ditelosomic lines (Sears and Sears, 1978).
- Deletion stocks (Endo, 1978)
Monosomics (2n-1): a set of 21

- An individual **lacks** one of a pair of chromosomes from the normal diploid (disomic) complement (20 II +1I or 41).
- Plants look similar to the disomics and fertility is close to normal.
- Gametes are transmitted at a different rate through male and female.
Monosomy 4A (2n-1) - 4AM
Monosomic
Expected transmission of the monosomics in *Triticum aestivum*

<table>
<thead>
<tr>
<th>Female Gamete</th>
<th>Male Gamete</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (21)</td>
<td>n-1 (20)</td>
</tr>
<tr>
<td>96%</td>
<td>4%</td>
</tr>
<tr>
<td>Disomic</td>
<td>Monosomic</td>
</tr>
<tr>
<td>2n (42) 24%</td>
<td>2n-1 (41) 1%</td>
</tr>
<tr>
<td>Monosomic</td>
<td>Nullisomic</td>
</tr>
<tr>
<td>2n-1 (41) 72%</td>
<td>2n-2 (40) 3%</td>
</tr>
</tbody>
</table>
Nullisomics (2n-2): a set of 21

- An individual lacks of one pair of chromosomes from the normal diploid complement (20II or 40)
- Plants distinguishable by morphological features (vigor and size)
- Gametes transmitted via same rate in female and male
Nullisomy 4A (2n-2) – 4AN

Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | Group 7
-------|--------|--------|--------|--------|--------|--------
A       |        |        |        |        |        |        

homologous chromosomes (homologues)

B       |        |        |        |        |        |        

related chromosomes (homoeologues)

D       |        |        |        |        |        |        

non-homologous chromosomes
Nullisomic
Nulli-tetrasomics: a set of 42

• An individual lacks one pair of chromosomes but have a doubled pair of chromosomes from the homoeologous group (19 Ⅱ + 1Ⅳ, or 42)

• Plants look similar to disomics and fertility is close to normal

• Gametes transmitted via similar rate in female and male
Nullisomy 4A Tetrasomy 4B - N4AT4B
Ditelosomics: a set of 42

• An individual lacks a pair of chromosome arms from the normal diploid complement
• Plants look similar to disomics and fertility is close to normal
• Gametes transmitted via same rate in female and male
Ditelosomy 4AL – 4AL

(A) homologous chromosomes (homologues)

(B) related chromosomes (homoeologues)

(D) non-homologous chromosomes
Ditelosomy 4AS – 4AS
Deletion stocks: unlimited

• An individual lacking a segment of a chromosome from the normal diploid complement
• Fertility is based on the location of deletions
• Mainly used in physical mapping
5B Deletion stocks

Genetic Study

Locate genes of interest:
on specific chromosome
on specific chromosome arm
on specific chromosome segment
# Monosomic analysis – F1

<table>
<thead>
<tr>
<th></th>
<th>Male gamete</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female gamete</td>
<td>n (21)</td>
</tr>
<tr>
<td>n (21 chr., 25%)</td>
<td>Disomic (2n = 42, 25%)</td>
</tr>
<tr>
<td>n-1 (20 chr., 75%)</td>
<td>Monosomic (2n-1 = 41, 75%)</td>
</tr>
</tbody>
</table>
Expected transmission of the monosomics - F2

<table>
<thead>
<tr>
<th>Female Gamete</th>
<th>Male Gamete</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (21) 96%</td>
<td>n-1 (20) 4%</td>
</tr>
<tr>
<td>Disomic</td>
<td>Monosomic</td>
</tr>
<tr>
<td>2n (42) 24%</td>
<td>2n-1 (41) 1%</td>
</tr>
<tr>
<td>25%</td>
<td>Nullisomic</td>
</tr>
<tr>
<td>Monosomic</td>
<td>2n-2 (40) 3%</td>
</tr>
<tr>
<td>n-1 (20) 75%</td>
<td>Nullisomic</td>
</tr>
<tr>
<td>2n-1 (41) 72%</td>
<td>Nullisomic</td>
</tr>
</tbody>
</table>
Locating Recessive Genes
(1A is gene carrier)

Female                Male
(Awnless)             (Awn)
20II + 1I<sub>1A</sub> X  20 II + 1II<sub>1aa</sub>

F<sub>1</sub>    75%   20 II + 1I<sub>1a</sub>
25%  20 II + 1II<sub>1Aa</sub>

3(Awn and monosomic)  
1(Awnless and disomic)

Female                Male
(Awnless)             (Awn)
19II + 1II<sub>1AA</sub> + 1I<sub>1b</sub> X  20 II + II<sub>1aa</sub>

F<sub>1</sub>    75%   19II + 1II<sub>1Aa</sub> + 1I<sub>1b</sub>
25%  19II + 1II<sub>1Aa</sub> + 1II<sub>1bb</sub>

All plants are awnless
Locating Dominant Genes
(Monosomic Analysis)

Female (S)  Male (R)
20II + 1I_{1a} X 20 II + 1II_{1AA}

F_1  25%  20 II + 1II_{1Aa}
75%  20 II + 1II_{1A}

F_2  4%  S -- nullisomic
73%  R -- monosomic
24%  R -- disomic

Female (S)  Male (R)
19II + 1II_{1aa} + 1I_{1b} X 19 II + 1II_{1AA} + 1II_{1bb}

F_1  25%  19II + 1II_{1Aa} + 1II_{1bb}
75%  19II + 1II_{1Aa} + 1II_{1b}

F_2  75%  R to 25% S
Locating Dominant Genes
(Nullisomic Analysis)

Female                Male
(S)                    (R)
20II                  20 II + 1II_{1AA}

F_{1}  20 II + 1II_{1A}

F_{2}  4%  S -- nullisomic
       73%  R -- monosomic
       24%  R -- disomic

Female                Male
(S)                    (R)
19II + 1II_{1aa}      19II + 1II_{1AA} + 1II_{1B}

F_{1}  19II + 1II_{1Aa} + 1II_{1B}

F_{2}  75%  R to 25%  S

No cytogenetic work in F_{1}!
Genome Mapping

Locate genes of interest or gene tightly linked markers

– on specific chromosome
– on specific chromosome arm
– on specific chromosome segment
Nulli-tetrasomic analysis - RFLP

( Devos KM and Gale MD. 1993. Theme:93-99)
Nulli-tetrasomic and ditelosomic analysis - RFLP

(J.A. Anderson et al. 1992. TAG 83:1035 - 1043)
Deletion analysis – locate markers on chromosome segment

| CS  | 7A | 7B | 7D | 7AL | 7BL | 7DL | 8 | 9 | .4 | .2 | .7 | .5 | .1 | .6 | .1 | .4 | .3 |
|-----|----|----|----|-----|-----|-----|---|---|----|----|----|----|----|----|----|----|
|     |    |    |    |     |     | Nullisomic | 7AL Deletions | 7BL Deletions | 7DL Deletions |    |    |    |    |    |    |    |
Variety Improvement: interspecific hybridization

- Introgression of useful traits from alien species to common wheat
- Issues on interspecific crosses
  - sterile or partially sterile in $F_1$
  - effective way to tag introduced chromosome or chromosome segments
  - Chromosome banding, genomic in situ hybridization, and molecular marker assisted selection
Procedures for transferring useful genes

- Screening of donor populations
- Producing hybrids
- Chromosome doubling/backcrossing
- Production and identification of alien addition/substitution lines
- Induction of recombination
- Screening/stabilizing recombinants/translocations
- Gene tagging/Newer technologies
Useful agronomic traits in alien species

Disease resistance

• Powdery mildew – *Ae. Markgrafii, Ae. Comosa, D. villosum, T. spelta, T. dicoccoides, T. macha, Ae. kotschii*

• Leaf rust – *Ae. Caudata, T. monococcum, T. tauschii, Th. Distinchnum*

• Stem rust – *T. diccoides, T. tauschii*

• Yellow rust – *T. spelta, synthetic hexaploids*

• Karnal bunt – *triticale (4R, 6R), T. monococcum*
Method of Translocation Induction

• Tissue culture (BYDV) (Bank et al., 1995)
• Radiation (Lukaszewski, 1995)
• The 5B system – induction of homeoeologous paring (Sears, 1977)
The 5-B system in wheat

• The 5B system, a Ph gene (homoeologous pairing suppressor) is a genetic control which restricts chromosome pairing to homologs.

• When Ph is removed, or its activity is suppressed, not only do homoeologous chromosome pair but they also pair with the chromosome of related species and genera, making alien gene transfer possible (Sears, 1975, 1976).
Chromosome pairing in polyhaploids of bread wheat (2n = 21) with (a) and without (b) Ph gene (from Jauhar et al., 1991)
Procedures for inducing homoeologous pairing

1. Monosomic or Nullisomic 5B x Alien species, $F_1$ x adapted lines or variety;
2. Monosomic or Nullisomic 5B x Alien addition or substitution lines, $F_1$ x adapted lines or variety.
Identification wheat-alien translocation lines
Identification wheat-alien substitution lines
Identification wheat-alien translocation lines
Identification wheat-alien addition lines

Figure 3   RAPD analysis in 10 individuals of two addition lines from JC1050. RAPD was performed by primer OPZ11(C), and the product was hybridized with OPZ11-350 (C1).
Conclusion

• Aneuploids are unique in hexaploid wheat.
• Genome analysis in wheat has served as a model in other plant systems, and has made tremendous advances.
• Chromosome engineering will continue to make contributions to wheat improvement as new techniques become available.
Aneuploid Analysis

Practical considerations
• Availability of aneuploid stocks in your crop species
• Nature of genes: qualitative or quantitative; dominant or recessive