The Mechanism of 2n Pollen Formation in *Populus × euramericauna* and *P. × popularis*

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1 Introduction

1.1 Polyploid breeding is an important part in poplar breeding

- Triploid white poplar trees
- Triploid and aneuploid hybrids in *Populus trichocarpa × P. deltoides*
- Triploidy were found in the cultivar poplar clones in section *Aigeiros*
1 Introduction

1.2 Mechanisms of 2n gamete formation in plant

- Pre-meiotic doubling
- Omission of the first or second meiotic division
- Abnormal spindle
- Abnormal cytokinesis
- Nuclear fusion

FDR (first division restitution):
Contains non-sister chromatids

SDR (second division restitution):
Contains two sister chromatids
1 Introduction

1.3 The objective of this study

- Poplar can produce 2n gamete naturally or by artificial induction
- Elucidation of the cytological mechanisms of 2n gamete formation has been seldom in poplar.
- To detect and elucidate the mechanisms of 2n pollen formation in diploid poplar
- Results from this research may offer a more effective method for polyploid breeding in poplar in section Aigeiros.
2 Materials and Methods

2.1 Plant materials

- *Populus × euramericana*
  
  Four male: EA1, EA2, EA3 and EA4
  One female: A

- *P. × popularis*  
The offspring of  
(P. simonii × (P.nigra var pyramidalis + Salix matsudana mixed pollen)

One Male: P

- The crosses
  
  A × EA1,  
  A × EA2,  
  A × EA3,  
  A × EA4,
2 Materials and Methods

2.2 Microsporogenesis observation

2.3 Flow cytometry analyses

2.4. Chromosome counting

2.5. SSR analysis
3. Results and Analysis

3.1 Cytological determination on 2n pollen formation
Table 1 The expected and observed rate of 2n pollen grains

<table>
<thead>
<tr>
<th>Code of poplar</th>
<th>Sporads</th>
<th>Expected rate of 2n pollen %</th>
<th>Observed rate of 2n pollen %</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dyad</td>
<td>Triad</td>
<td>Tetrad</td>
<td>Total</td>
</tr>
<tr>
<td>EA1</td>
<td>539</td>
<td>341</td>
<td>6629</td>
<td>7509</td>
</tr>
<tr>
<td>EA2</td>
<td>163</td>
<td>1818</td>
<td>5528</td>
<td>7509</td>
</tr>
<tr>
<td>EA3</td>
<td>689</td>
<td>682</td>
<td>6354</td>
<td>7725</td>
</tr>
<tr>
<td>EA4</td>
<td>2189</td>
<td>4093</td>
<td>1691</td>
<td>7973</td>
</tr>
<tr>
<td>P</td>
<td>308</td>
<td>484</td>
<td>7308</td>
<td>8100</td>
</tr>
</tbody>
</table>

** Indicated significant difference between expected rate of 2n pollen from sporads sample and the observed rate of 2n pollen from pollen sample at $P < 0.01$. The percentage were converted to arcsine data before $\chi^2$ test.
3. Results and Analysis

3.2. Detection of polyploid offspring of 2n pollen

Diploid 61#

Triploid 65#
Diploid 61#

Triploid 73#
Diploid 61#

Tetraploid 75#

Channels (FL2-A- 1.27)

Number 500

0 50 100 150 200 250

Diploid 61#

Tetraploid 75#
3. Results and Analysis

3.3. SSR determination on mechanism of 2n pollen formation

(a) Primer: 14
(b) Primer: 41
(c) Primer: 47
(d) Primer: 68
(e) Primer: 105
Table 2 Segregation of alleles at loci where the male *Populus ×euramericana.* (Dode) Guinier parent EA4 is heterozygous

<table>
<thead>
<tr>
<th>Code</th>
<th>SSR primer</th>
<th>locus</th>
<th>EA4(♂)</th>
<th>A(♀)</th>
<th>75#(4x)</th>
<th>421(2x)</th>
<th>422(2x)</th>
<th>423(2x)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>GCPM_2453-1</td>
<td>1</td>
<td>AB</td>
<td>CDE</td>
<td>ABCDE</td>
<td>ADE</td>
<td>ADE</td>
<td>ADE</td>
</tr>
<tr>
<td>41</td>
<td>GCPM_3345-1</td>
<td>2</td>
<td>AB</td>
<td>AC</td>
<td>ABC</td>
<td>AC</td>
<td>AC</td>
<td>AC</td>
</tr>
<tr>
<td>47</td>
<td>GCPM_3559-1</td>
<td>3</td>
<td>AB</td>
<td>B</td>
<td>AB</td>
<td>B</td>
<td>AB</td>
<td>AB</td>
</tr>
<tr>
<td>68</td>
<td>GCPM_432-1</td>
<td>4</td>
<td>AB</td>
<td>A</td>
<td>AB</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>105</td>
<td>ORPM_29</td>
<td>5</td>
<td>A0</td>
<td>C0</td>
<td>A0C0</td>
<td>C0</td>
<td>C0</td>
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<tr>
<td></td>
<td></td>
<td>6</td>
<td>B0</td>
<td>DE</td>
<td>B0DE</td>
<td>BE</td>
<td>BE</td>
<td>BE</td>
</tr>
</tbody>
</table>

These letters do not necessarily correspond to discrete alleles (e.g. the “E” band for GCPM_2453-1 may be the non specific amplification) and ORPM_29 primer detects two loci, 0 means a null allele.
3. Results and Analysis
3.3. SSR determination on mechanism of 2n pollen formation

(a) Primer: 13

(b) Primer: 68

(c) Primer: 105
Table 3  Segregation of alleles at loci where the male
*P. × popularis* parent P is heterozygous

<table>
<thead>
<tr>
<th>Code</th>
<th>SSR primer</th>
<th>locus</th>
<th>P(♂)</th>
<th>A(♀)</th>
<th>65#(3x)</th>
<th>73#(3x)</th>
<th>321(2x)</th>
<th>322(2x)</th>
<th>323(2x)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>GCPM_2453-1</td>
<td>1</td>
<td>AB</td>
<td>CAD</td>
<td>CAB</td>
<td>CAB</td>
<td>AD</td>
<td>AD</td>
<td>AD</td>
</tr>
<tr>
<td>68</td>
<td>GCPM_432-1</td>
<td>2</td>
<td>AB</td>
<td>C</td>
<td>AC</td>
<td>BC</td>
<td>AC</td>
<td>AC</td>
<td>AC</td>
</tr>
<tr>
<td>105</td>
<td>ORPM_29</td>
<td>3</td>
<td>00</td>
<td>C0</td>
<td>C0</td>
<td>C0</td>
<td>00</td>
<td>C0</td>
<td>00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>AB</td>
<td>BD</td>
<td>AD</td>
<td>AD</td>
<td>BB</td>
<td>AB</td>
<td>AB</td>
</tr>
</tbody>
</table>

These letters do not necessarily correspond to discrete alleles (e.g. the “C” band for GCPM_2453-1 may be the non-specific amplification) and the ORPM_29 primer detects two loci, 0 means a null allele.
4. Discussion

4.1 Mechanisms of 2n pollen formation

4.2 The biological reason for high percentage of 2n pollen

4.3 The formation of 2n female gametes in poplars of section *Aigeiros*

4.4 Polyploidy identification using molecular markers

4.5 Implications of polyploidy for genetic research and tree breeding
Thank you!