

The 23rd Session of the International Poplar Commission

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

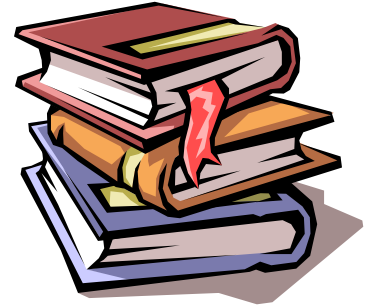


Speaker Jin-feng Zhang (Jennifer)

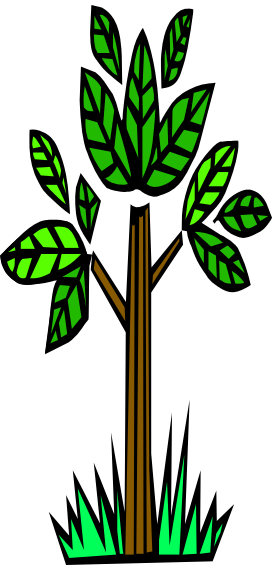
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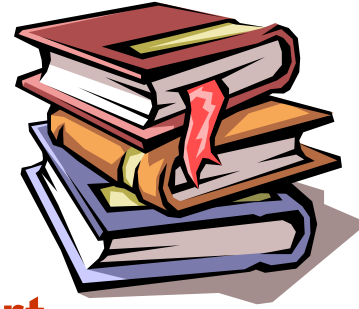
OUTLINE



- 1 Introduction**
- 2 Materials and Methods**
- 3 Results and Analysis**
- 4 Discussion**

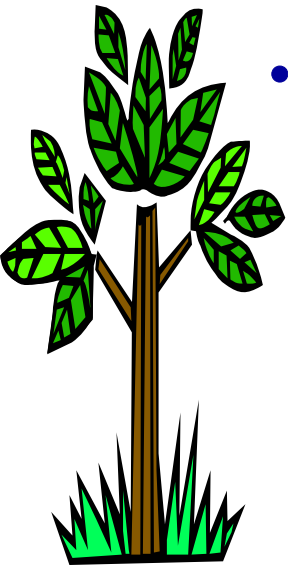


1 Introduction

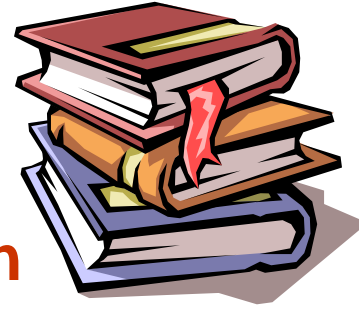


1.1 Polyploid breeding is an important part in poplar breeding

- 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

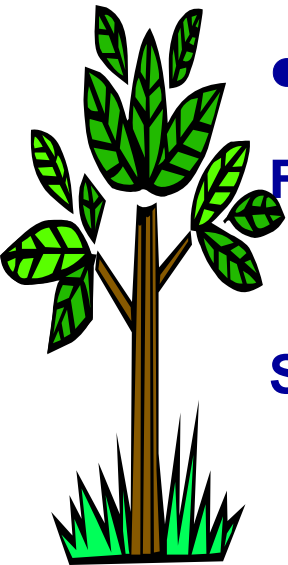
- Premeiotic doubling
- Omission of the first or second meiotic division
- Abnormal spindle
- Abnormal cytokinesis
- Nuclear fusion

FDR (first division restitution) :

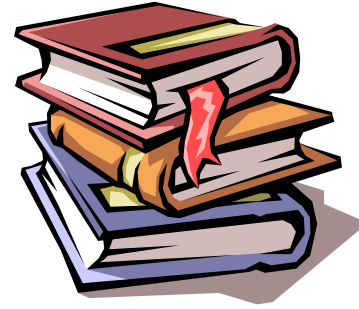
Contains non-sister chromatids

SDR (second division restitution):

Contains two sister chromatids

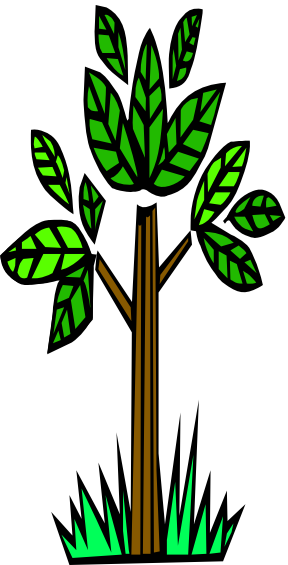


1 Introduction

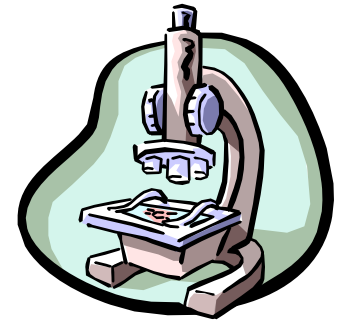


1.3 The objective of this study

- 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

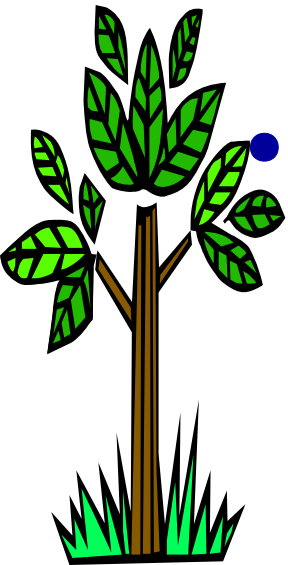
A × EA1,

A × EA2,

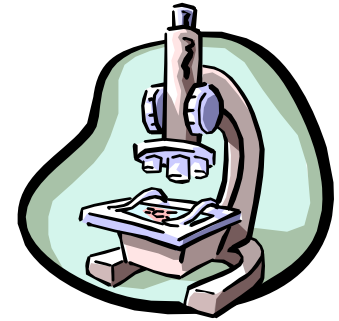
A × EA3,

A × EA4,

A × P



2 Materials and Methods

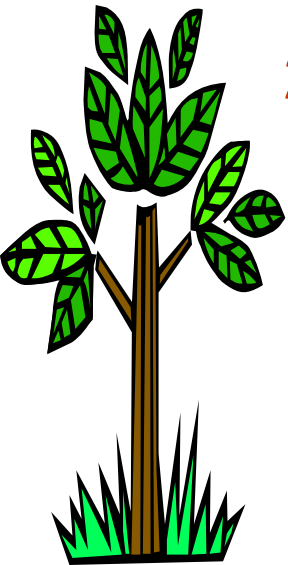


2.2 Microsporogenesis observation

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

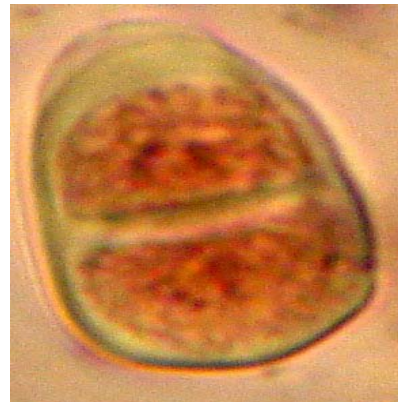
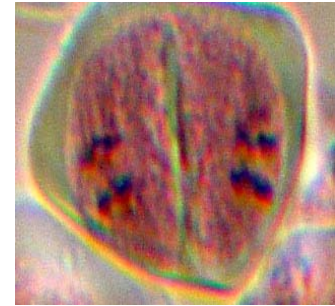
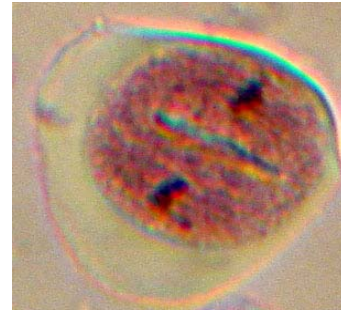
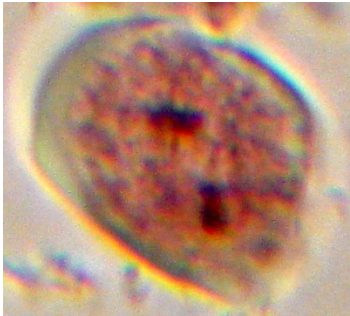
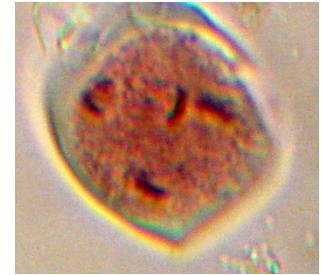
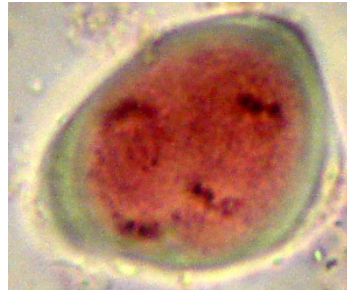
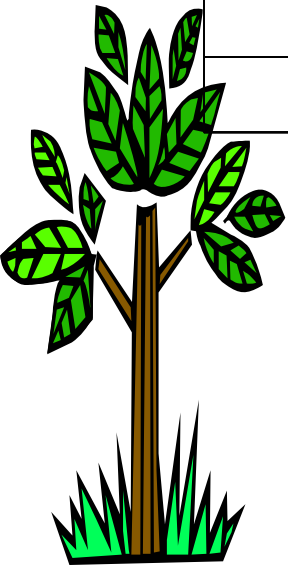


Table 1 The expected and observed rate of 2n pollen grains

Code of poplar	Sporads				Expected rate of 2n pollen %	Observed rate of 2n pollen %	χ^2
	Dyad	Triad	Tetrad	Total			
EA1	539	341	6629	7509	4.96	0.03	26.056**
EA2	163	1818	5528	7509	7.69	0.09	
EA3	689	682	6354	7725	7.14	10.08	
EA4	2189	4093	1691	7973	36.17	29.41	
P	308	484	7308	8100	3.15	2.35	

**** 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 χ^2 test.**



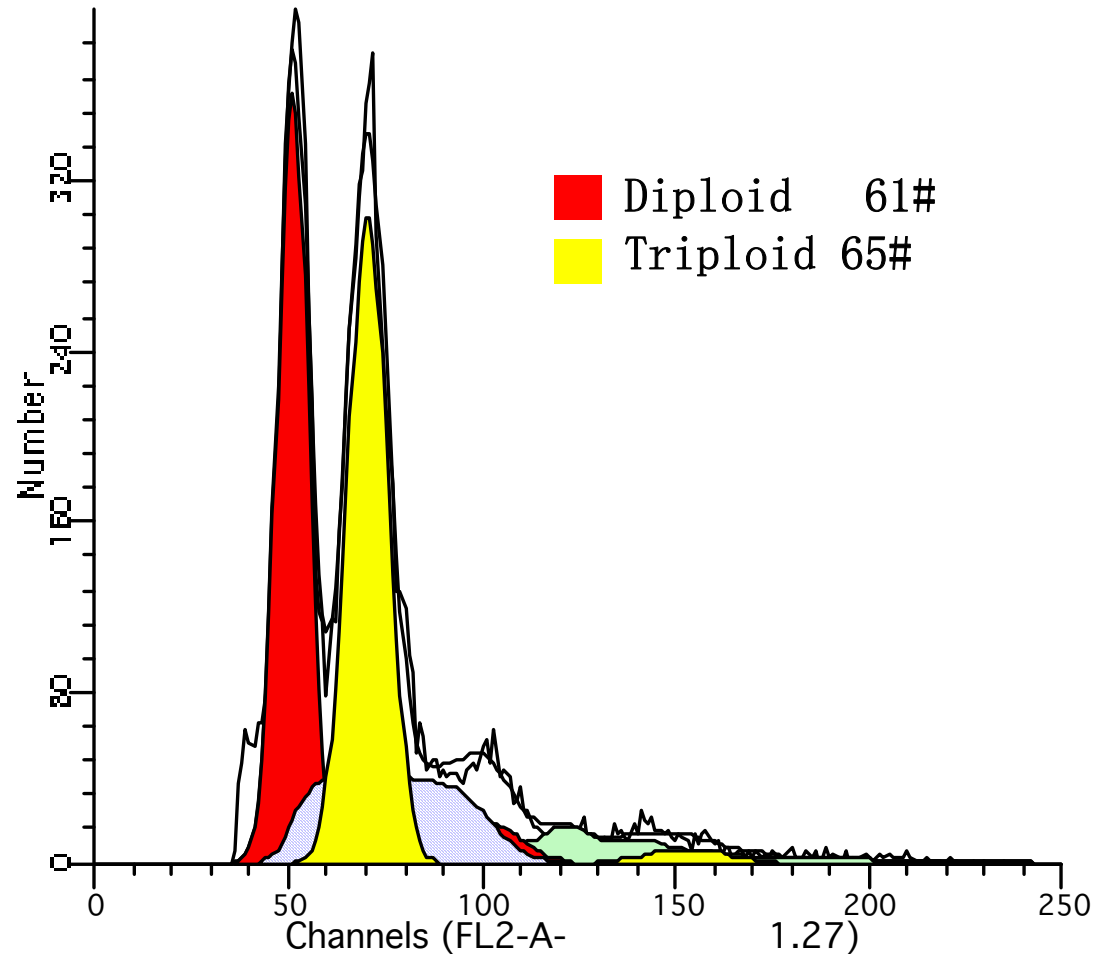
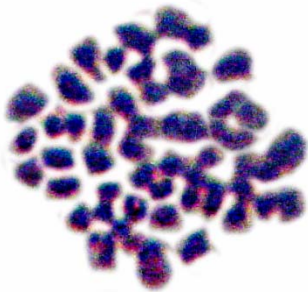
3. Results and Analysis

3.2. Detection of polyploid offspring of 2n pollen

Diploid 61#



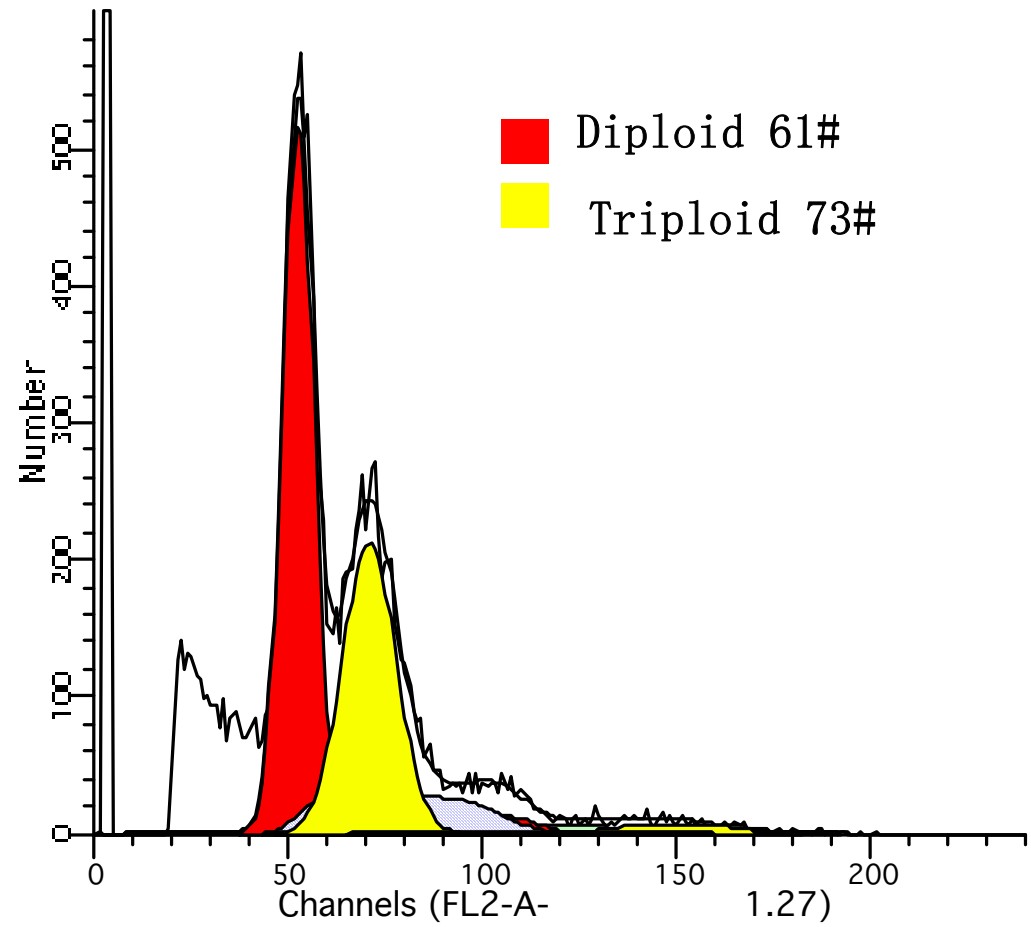
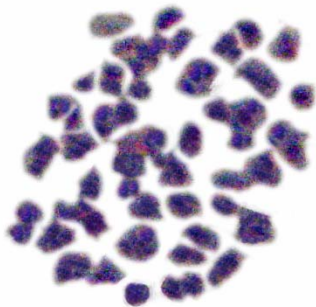
Triploid 65#



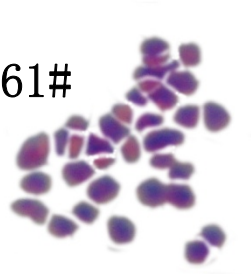
Diploid 61#



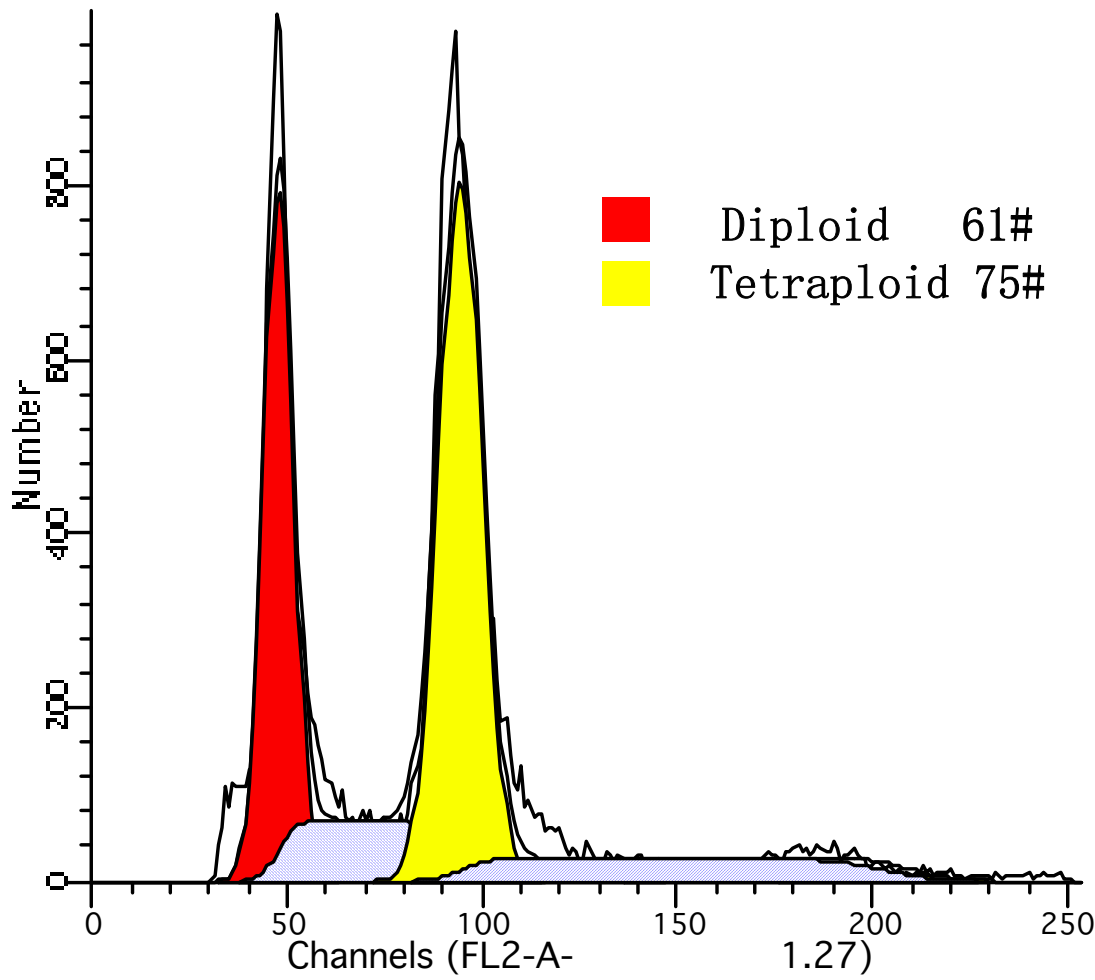
Triploid 73#



Diploid 61#

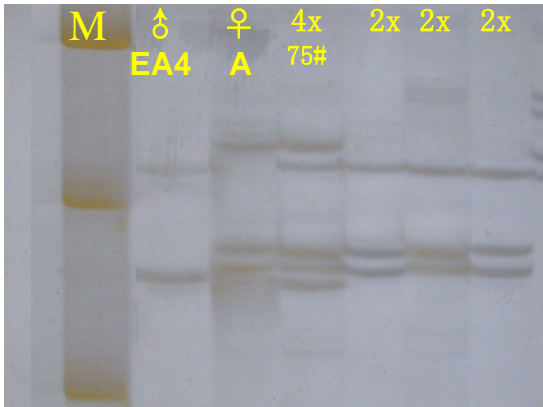


Tetraploid 75#

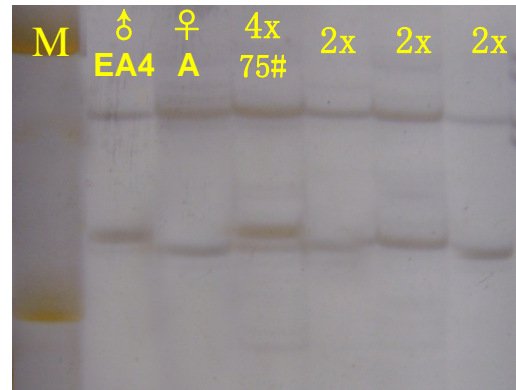


3. Results and Analysis

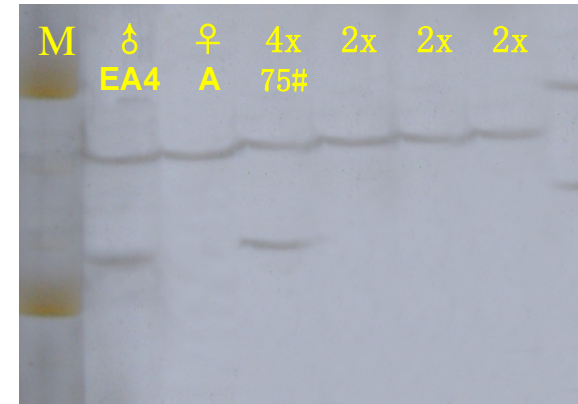
3.3. SSR determination on mechanism of 2n pollen formation



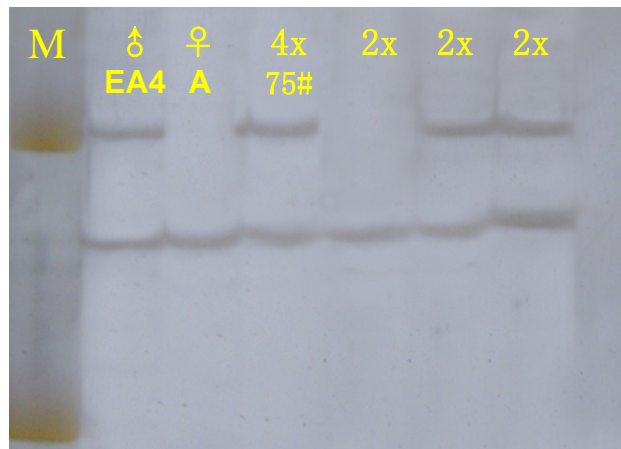
(a) Primer: 14:



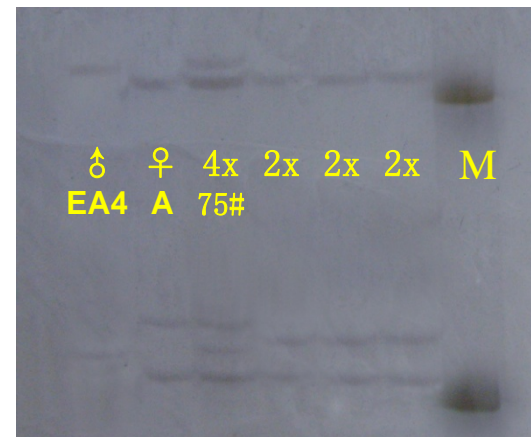
(b) Primer: 41



(c) Primer: 47



(d) Primer: 68



(e) Primer: 105

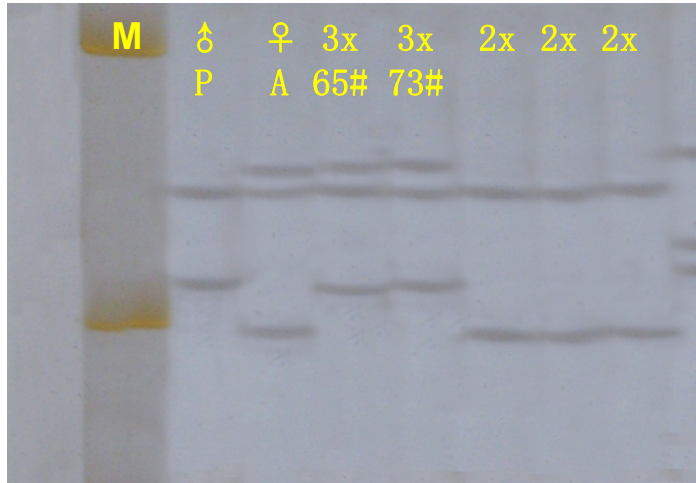
Table 2 Segregation of alleles at loci where the male *Populus xeuramericana*.(Dode) Guinier parent EA4 is heterozygous

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

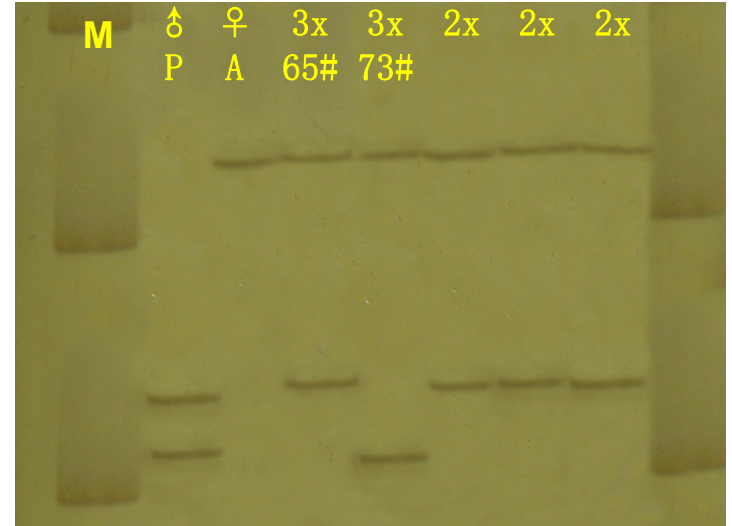
These letters do not necessarily correspond to discrete alleles(e.g. the “E”band for GCPM_2453-1 may be the non specific amplification) and ORPM_29 primer detects two loci, 0 means a null allele.

3. Results and Analysis

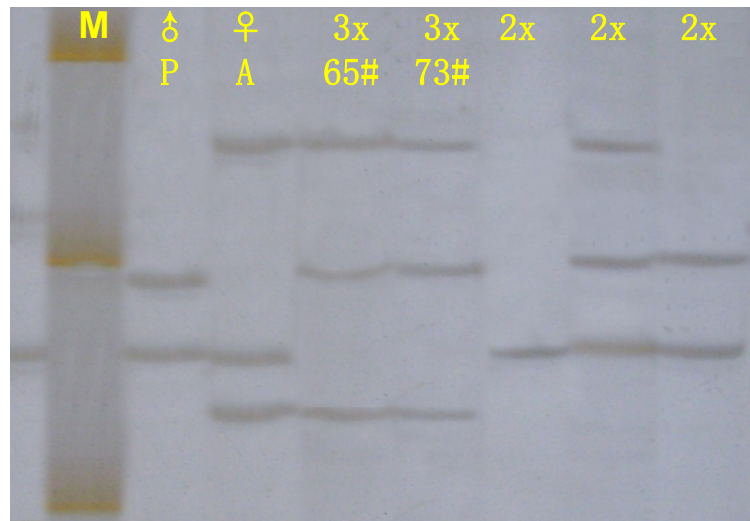
3.3. SSR determination on mechanism of 2n pollen formation



(a) Primer: 13



(b) Primer: 68



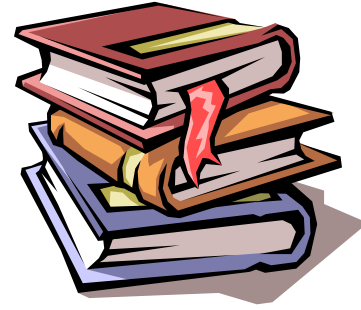
(c) Primer: 105

Table 3 Segregation of alleles at loci where the male *P. × popularis* parent P is heterozygous

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

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

