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Triploid breeding in *Populus*

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1 Background

1.1 Triploid *Populus* species

P. tremula

P. tremuloides

P. tomentosa

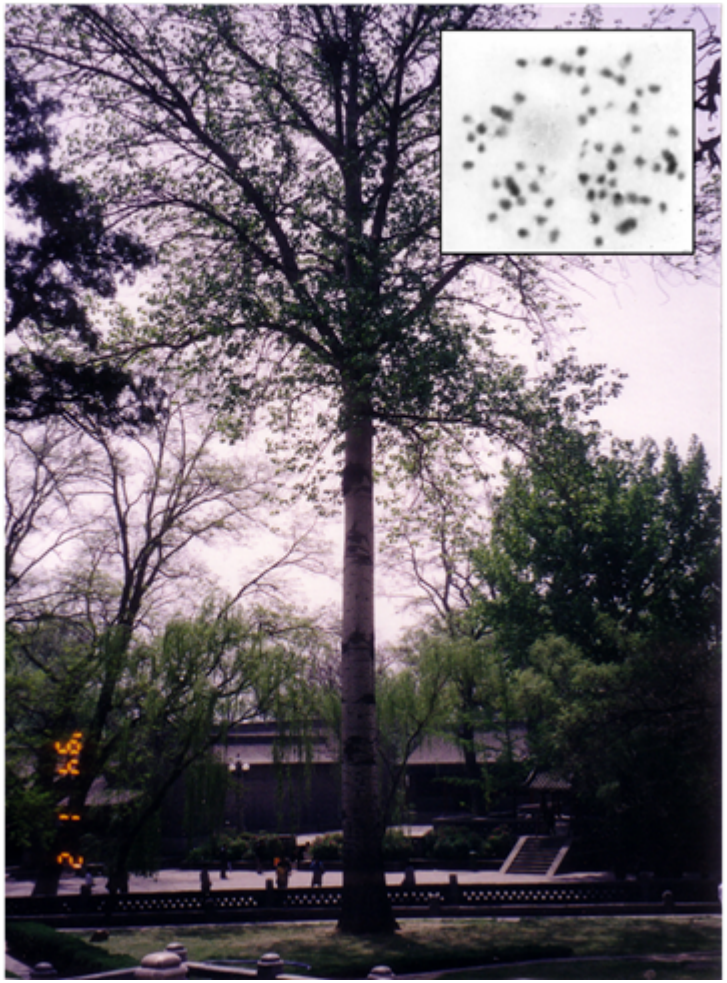
P. × *canadensis* cv. "Sacrau"

P. × *canadensis* cv. "I-214"

P. × *liaohenica*

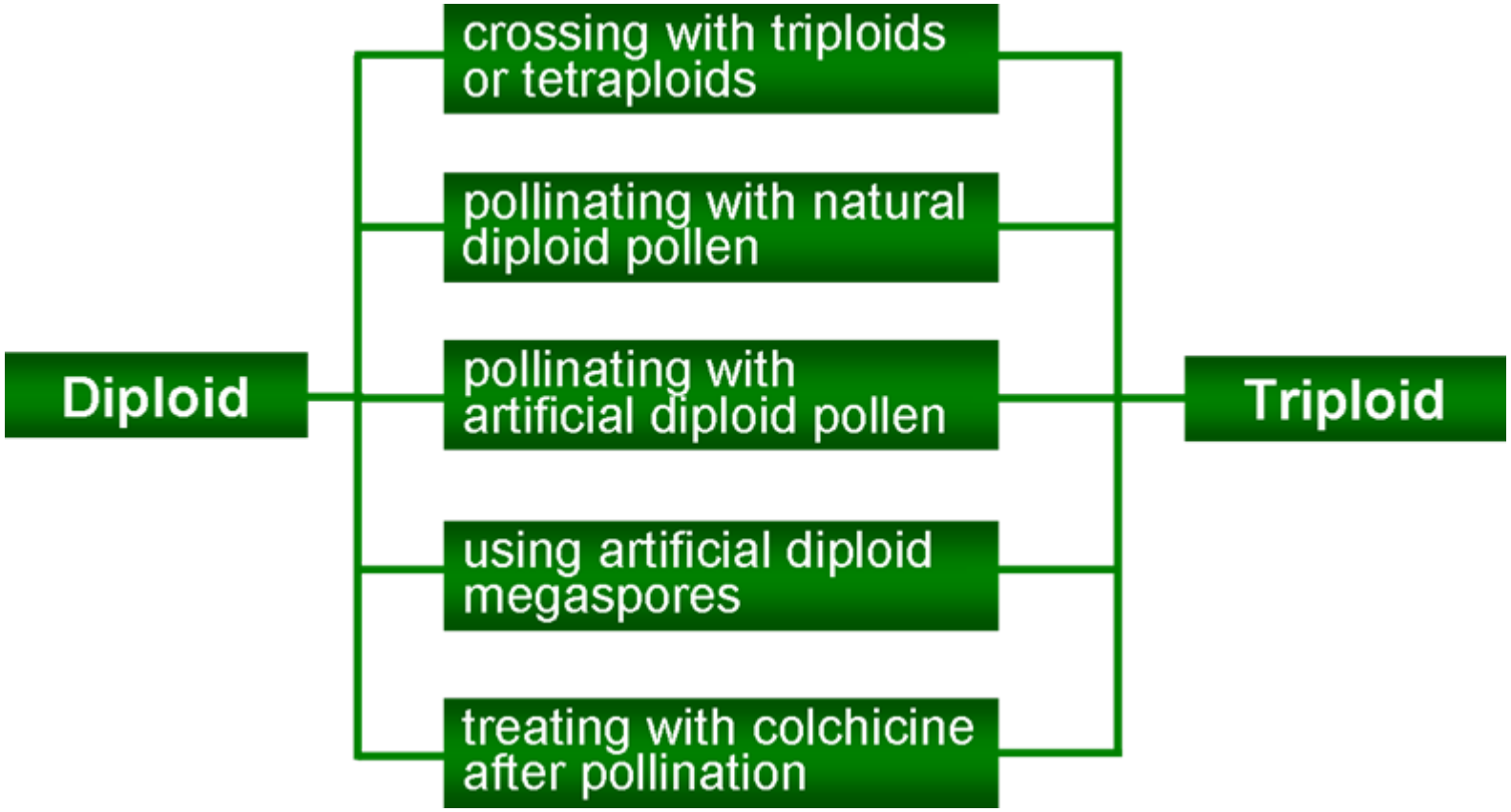
P. × *langfangensis*-3

P. × *euramericana* cv. "Zhonglin-46"



1.2 Triploid breeding in *Populus*

Five approaches have been applied to sexual triploid breeding.



2n pollen

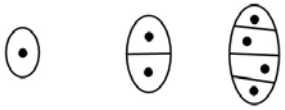
The usage of 2n pollen is restricted, because it does not compete well with n pollen

2n female gametes

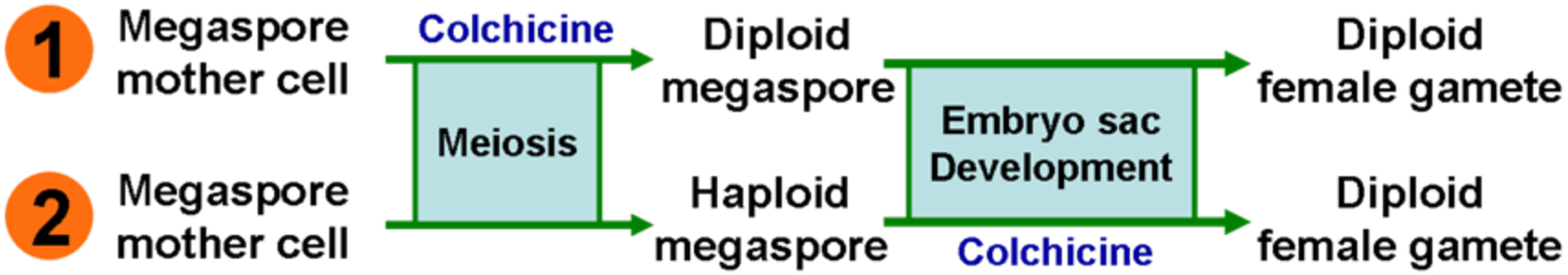
Once fertilization with n pollen occurs, triploids can be produced

1.3 Female gametophyte development

In angiosperms, female gametophyte development includes two phases referred to as megasporogenesis and megagametogenesis.

Type	Megasporogenesis			Megagametogenesis				
	Megaspore mather cell	Meiosis I	Meiosis II	Functional megaspore	Mitosis I	Mitosis II	Mitosis III	Mature female gametophyte
Monosporic (*Polygonum*)								
Bisporic (*Allium*)								
Tetrasporic (*Adoxa*)								
Tetrasporic (*Plumbago*)								
Tetrasporic (*Drusa*)								

Two ways to induce 2n female gametes:

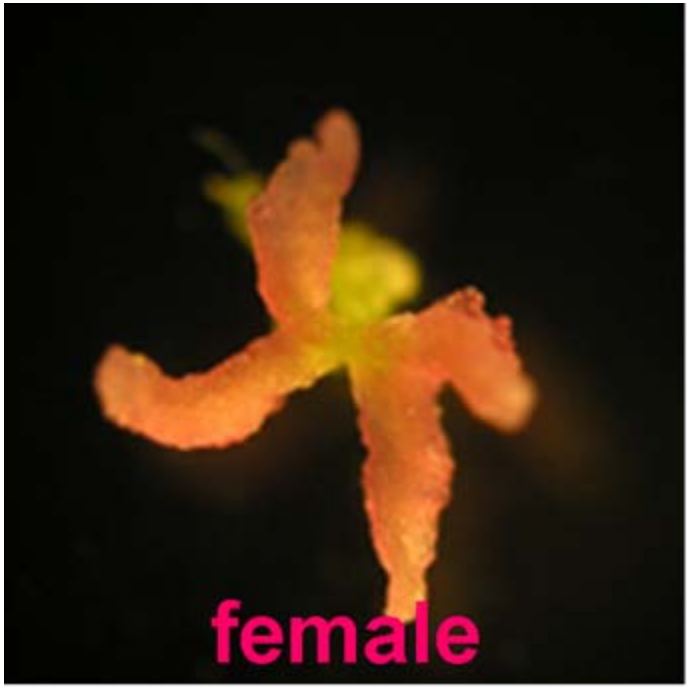


2 Diploid megaspore induction

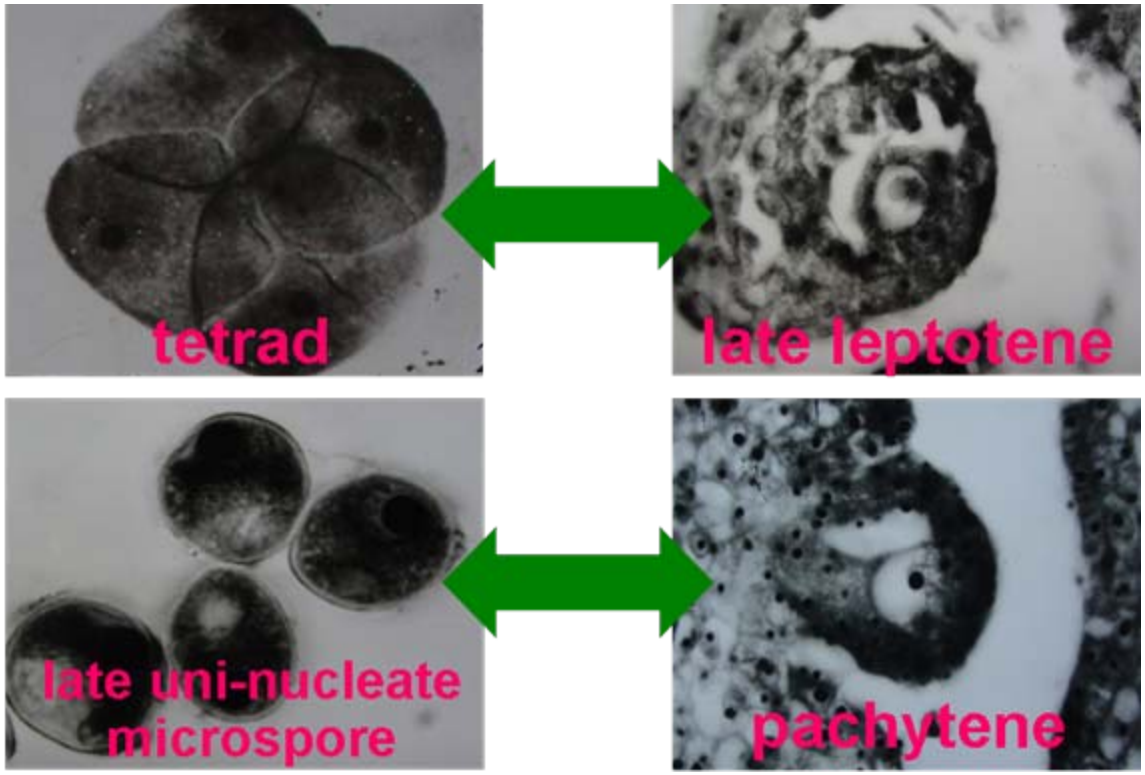
2.1 Quick determination of MMC meiotic stage

Determination of MMC meiotic stages by paraffin sectioning is slow and destructive.

How to quickly confirm the MMC meiotic stage?



A close association between MMC meiosis and male gametophyte development under the same cultured condition was found.



2.2 Production of triploids

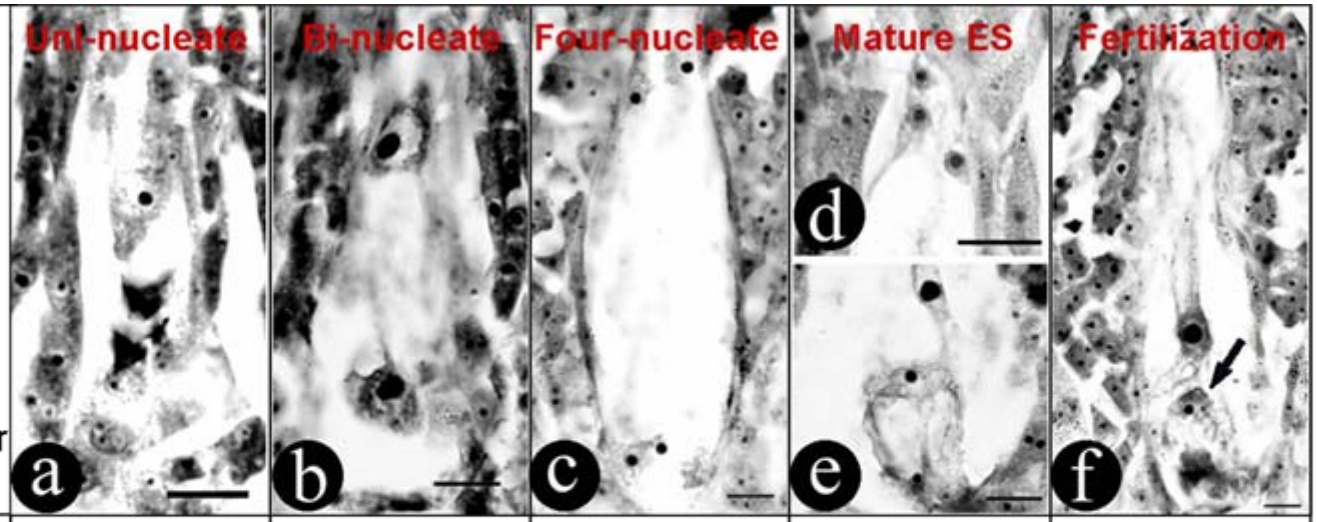
A total of 12 triploids were produced.

Hours after cultured	Stage of male gametophyte development	Meiotic stage of megaspore mother cells	No. of triploids	Rate of triploids (%)
140	Tetrad	Leptotene, late leptotene	1	0.7
152	Tetrad, early uni-nucleate microspore	Late leptotene	2	**16.7**
164	Late uni-nucleate microspore	Late leptotene, pachytene	2	2.3
176	Late uni-nucleate microspore	Pachytene, diplotene	3	12.0
188	Bi-nucleate microspore	Pachytene ~ metaphase	4	5.0
Total			12	

3 Embryo sac chromosome doubling

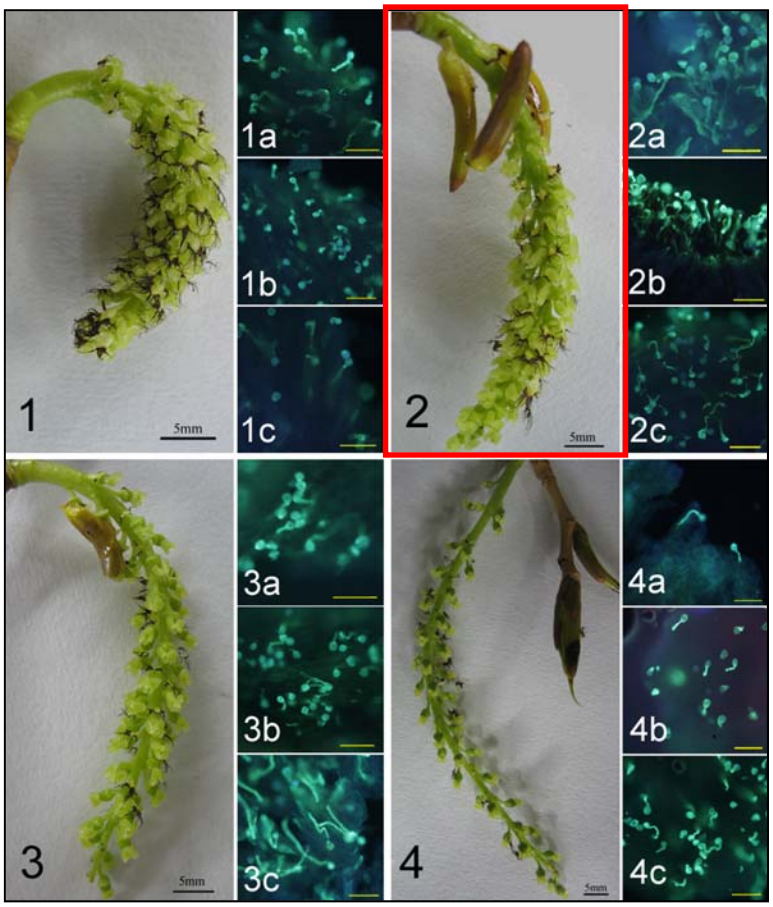
3.1 Development of embryo sacs

The embryo sac development was of typical *Polygonum* type. It was a successive and asynchronous process.



Hours after pollination	Uni-nucleate	Bi-nucleate	Four-nucleate	Mature ES	Fertilization
-12	28.21%				
12	68.63%	15.69%	5.88%		
42	13.04%	31.88%	26.09%	28.99%	
66	3.57%	8.93%	41.07%	46.43%	
84		10.00%	21.67%	63.33%	5.00%
120			10.81%	27.03%	62.16%

3.2 The optimal pollination state



The stigma receptivity lasted 3-4 d and began at the base of catkins and proceeded toward the tip

The optimal pollination state

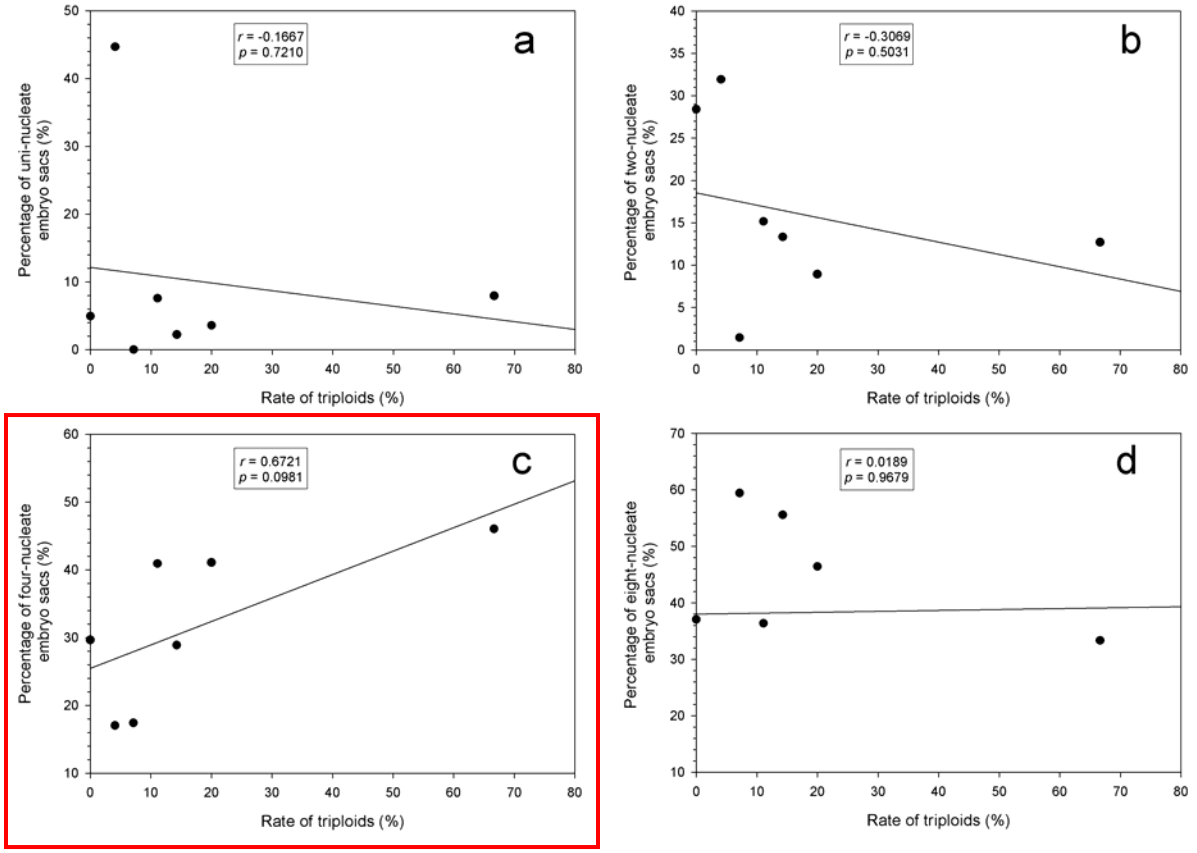
The catkins elongated to 5.62±0.47 cm long 84 h after they emerged from their bract scales. All bracts turned inside out and all stigmas were exposed (Fig. 2).

3.3 Production of triploids

A total of 23 triploids were obtained.

No.	Hours after pollination	Concentration of colchicine (%)	Duration of treatment (h)	Number of Seeds	Number of Seedlings	Number of Triploids	Rate of triploids (%)
1	18	0.3	24	23	-	-	-
2	24	0.3	24	208	49	2	4.08
3	30	0.5	24	-	-	-	-
4	36	0.3	24	27	3	0	0
5	42	0.5	24	4	-	-	-
6	48	0.5	24	13	-	-	-
7	54	0.5	18	19	9	1	11.11
8	54	0.5	30	137	74	7	9.46
9	60	0.5	18	78	6	4	66.67
10	66	0.5	30	80	35	7	20.00
11	72	0.5	24	41	7	1	14.29
12	84	0.5	24	-	-	-	-
13	96	0.5	24	83	14	1	7.14
Control				345	118	0	0
Total				1058	315	23	

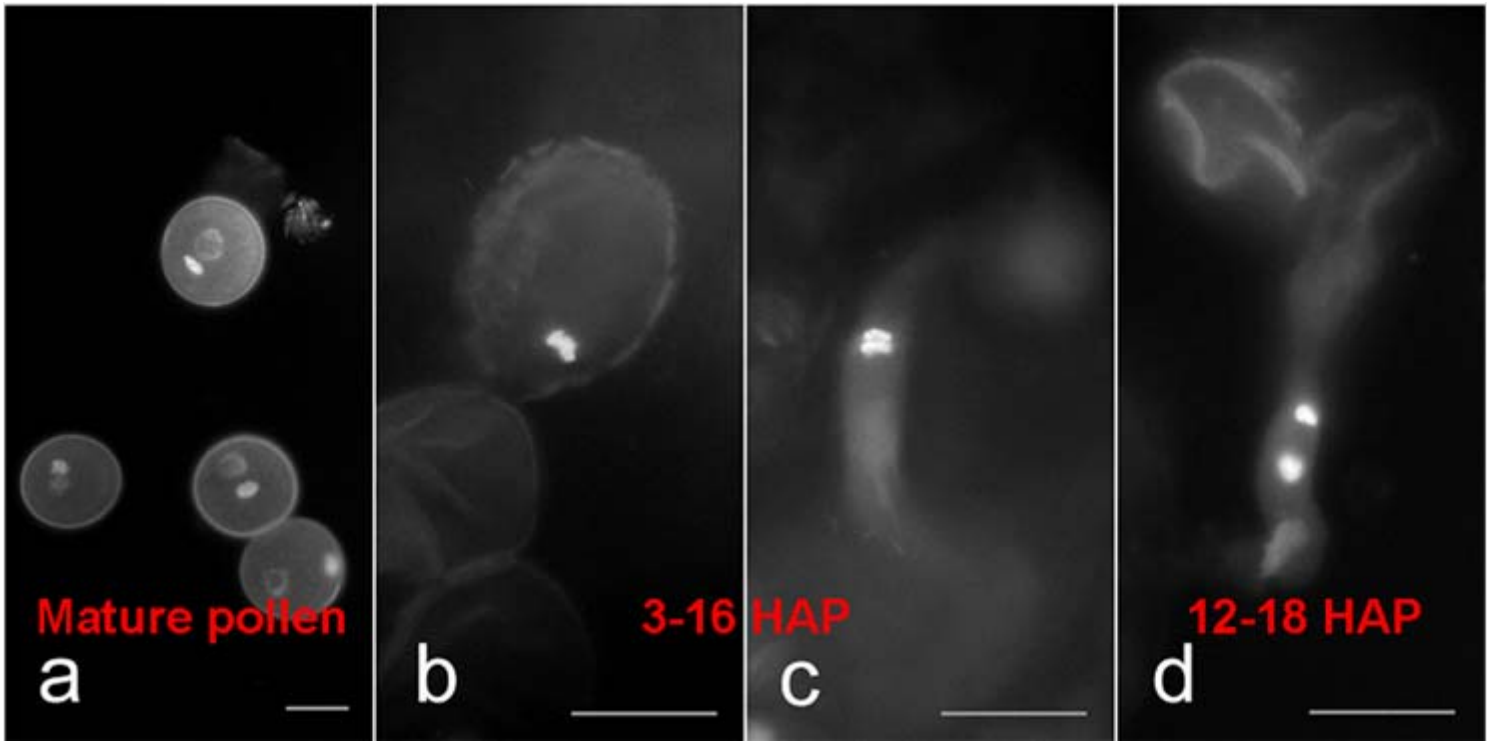
3.4 Determination of the optimal treating period



Significantly positive correlation

The third mitotic division during embryo sac development may be the optimal treating period for 2n egg induction.

3.5 Effect of sperm cell in triploid production



DAPI staining showed the generative cell division of male parent occurred in pollen tube 3-16 h after pollination, which did not coincide with the effective period of triploid production.

4 Conclusion

Efficiency of triploid production:

Embryo sac chromosome doubling		2n megaspore		2n pollen
66.7%	>	16.7%	>	12.9%

Kang et al., 2000

Conspicuously, triploid production via 2n female gametes is more efficient than that via 2n pollen.

Embryo sac chromosome doubling is a novel approach for triploid production.

Kang et al. (2004) obtained 57.1% allotriploids of white poplar by treating female catkins with colchicine 24-36h after pollination. But where were the triploids from?

Embryo sac chromosome doubling

Diploid female gamete induction, especially embryo sac chromosome doubling is a promising approach for polyploid breeding in *Populus* and other plants.

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