

several cases, for example *A. auriculiformis* in Thailand (LUANGVIRIYASAENG and PINYOPUSARERK, 1998). Build-up of inbreeding through self-fertilization occurring in isolated trees or poorly-flowering stands used for seed collection over successive generations, and the associated depression of vigour that we have demonstrated in this study, could explain this phenomenon.

Acknowledgments

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References

BUTCHER, P. A., MORAN, G. F. and PERKINS, H. D.: RFLP diversity in the nuclear genome of *Acacia mangium*. *Heredity* **81**: 205–213 (1998). — BUTCHER, P. A., GLAUBITZ, J. C. and MORAN, G. F.: Applications for microsatellite markers in the domestication and conservation of forest trees. *Forest Genetic Resources Information* **27**: 34–42 (1999). — BUTCHER, P. A., DECROOQ, S., GRAY, Y. and MORAN, G. F.: Development, inheritance and cross-species amplification of microsatellite markers from *Acacia mangium*. *Theoretical and Applied Genetics* **101**: 1282–1290 (2000). — BUTCHER, P. A., HARWOOD, C. E. and TRAN HO QUANG: Studies of mating systems in seed stands suggest possible causes of variable outcrossing rates in natural populations of *Acacia mangium*. IUFRO Symposium 'Population and Evolutionary Genetics of Forest Trees'. Stará Lesná, Slovakia, August 25–29, 2002 (2002). — ELDRIDGE, K. G., DAVIDSON, J., HARWOOD, C. E. and VAN WYK, G.: *Eucalypt Domestication and Breeding*. Oxford University Press (1993). — GRIFFIN, A. R. and COTTERILL, P. P.: Genetic variation in growth of outcrossed, selfed and open-pollinated progenies of *Eucalyptus regnans* and some implications for breeding strategy. *Silvae Genetica* **37**: 124–131 (1988). — HARWOOD, C. E. and WILLIAMS, E. R.: A review of provenance variation in the growth of *Acacia mangium*. Pp. 22–30 in CARRON, L. T. and AKEN, K. eds.: *Breeding Technologies for Tropical Acacias*. ACIAR Proceedings No. 37. Canberra: ACIAR (1992). — HODGSON, L. M.: Some

aspects of flowering and reproductive behaviour of *Eucalyptus grandis* (Hill) Maiden at J.D.M. Keet Research Station 2. The fruit, seed, seedlings, self-fertility, selfing and inbreeding effects. *South African Forestry Journal* **98**: 32–43 (1976). — LUANGVIRIYASAENG, L. and PINYOPUSARERK, K.: Genetic variation in a second-generation progeny trial of *Acacia auriculiformis* in Thailand. *Journal of Tropical Forest Science* **14**: 131–144 (2001). — MOFFATT, A. A. and NIXON, K. M.: The effects of self-fertilization on green wattle (*Acacia decurrens* Willd.) and black wattle (*Acacia mearnsii* De Wild.). Pp 66–84 Report for 1973 to 1974, Wattle Research Institute of South Africa (1974). — MUONA, O., MORAN, G. F. and BELL, J. C.: Hierarchical patterns of correlated mating in *Acacia melanoxylon*. *Genetics* **127**: 619–626 (1991). — NIRSATMANTO, A., KURINOBU, S. and HARDIYANTO, E. B.: A projected increase in stand volume of introduced provenances of *Acacia mangium* in seedling seed orchards in south Sumatra, Indonesia. *Journal of Forest Research* **8**: 127–131 (2003). — SEDGLEY, M. and HARBARD, J.: Pollen storage and breeding system in relation to controlled pollination of four species of *Acacia* (Leguminosae: Mimosoideae). *Australian Journal of Botany* **41**: 601–609 (1993). — SEDGLEY, M., WONG CHIN YONG, NEWMAN, V., HARBARD, J. SMITH, R., KOH KIAN GHAN and TAJUDDIN, A.: Phenology of *Acacia mangium* and *A. auriculiformis* in Australia and Malaysia. Pp. 36–44 in CARRON, L. T. and AKEN, K. eds.: *Breeding Technologies for Tropical Acacias*. ACIAR Proceedings No. 37. Canberra, ACIAR (1992). — SIM BOON LIANG: The genetic base of *Acacia mangium* Willd. in Sabah. Pp 597–603 in BARNES, R. D. and GIBSON, G. L. eds.: *Provenance and Genetic Improvement Strategies in Tropical Forest Trees*. Oxford: Commonwealth Forestry Institute (1984). — TURNBULL, J. W., MIDGLEY, S. J. and COSSALTER, C.: Tropical acacias planted in Asia: an overview. Pp. 14–28 in TURNBULL, J. W., CROMPTON, H. and PINYOPUSARERK, K.: *Recent Developments in Acacia Planting*. ACIAR Proceedings No. 82. Canberra: ACIAR (1998). — TURVEY, N. D.: Growth at age 30 months of *Acacia* and *Eucalyptus* species planted in *Imperata* grasslands in Kalimantan Selatan, Indonesia. *Forest Ecology and Management* **82**: 185–195 (1996). — WANG TONGLI, HAGQVIST, R., TIGERSTEDT, P. M. A. and WANG, T. L.: Inbreeding depression in three generations of selfed families of silver birch (*Betula pendula*). *Canadian Journal of Forest Research* **29**: 662–668 (1999). — WILLIAMS, C. G. and SAVOLAINEN, O.: Inbreeding depression in conifers: implications for breeding strategy. *Forest Science* **42**: 102–117 (1996). — WU, H. X., MATHESON, A. C. and SPENCER, D.: Inbreeding in *Pinus radiata*. 1. The effect of inbreeding on growth, survival and variance. *Theoretical and Applied Genetics* **97**: 1256–1268 (1997).

A Report of Triploid *Populus* of the Section *Aigeiros*

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Abstract

This screening study analyzed ploidy levels by counting the chromosome number of 61 *Aigeiros* cultivars grown in China. Triploid *Aigeiros* has been found in four of these cultivars: *Populus x euramericana* (Dode) Guiner cv. Wuhei-1, *P. x Liaohenica*, *P. Langfangensis*-3 Wang (*P. deltoides* Barry cv. "Shan-

haiguan" x *P. simonii* x *P. pyramidalis*-12+ *Ulmus pumila* Linn.), and *P. x euramericana* (Dode) Guinier cv. "Zhonglin-46". The karyotype analysis indicates that triploid *Aigeiros* might be derived from original allotriploid. Because growth of the triploid trees was faster than their respective diploid hybrids or clones in the plantations where we collected the materials, we expect that they will play a significant role in breeding, reforestation and fiber production in China.

Key words: *Aigeiros*, triploid.

Introduction

The genus *Populus* is widely distributed and cultivated in the world. More than 20 million hectares of natural forests occur across the world, of which about three million hectares

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Table 1. – Chromosome number of *Aigeiros* species and varieties (lines or hybrids) examined in the study.

No.	Specific name	Voucher	Chromosome number (2n)	Locality
1	<i>P. euramericana</i> (Dode) Guinier. cv. Wuhei-1	Chen RY 03-1	57	Wuqin, Tianjin
2	<i>P.×beijingensis</i> W. Y. Hsu-255	Qi LW 03-71	38	Datong, Shanxi
3	<i>P.×beijingensis</i> W. Y. Hsu-2	Qi LW 03-72	38	Changping, Beijing
4	<i>P.×canadensis</i> Moench	Qi LW 03-73	38	Gu'an, Hebei
5	<i>P.×euramericana</i>	Qi LW 03-74	38	Fengning, Hebei
6	<i>P.×euramericana</i> CL 13	Qi LW 03-75	38	Fengning, Hebei
7	<i>P.×euramericana</i> CL 14	Qi LW 03-76	38	Fengning, Hebei
8	<i>P.×euramericana</i> CL 33	Qi LW 03-77	38	Fengning, Hebei
9	<i>P.×euramericana</i> CL 34	Qi LW 03-78	38	Fengning, Hebei
10	<i>P.×euramericana</i> Cv. 'Guarienco'	Qi LW 03-79	38	Shunyi, Beijing
11	<i>P.×euramericana</i> Zhonglin- 2025	Qi LW 03-80	38	Shunyi, Beijing
12	<i>P.×euramericana</i> cv. '74/76'	Qi LW 03-81	38	Shunyi, Beijing
13	<i>P.×euramericana</i> cv. '74/76'-1	Qi LW 03-82	38	Shunyi, Beijing
14	<i>P.×xiaozhuanica</i> W. Y. Hsu et Liang cv. <i>Opera</i>	Qi LW 03-83	38	Datong, Shanxi
15	<i>P. nigra</i> L. × <i>P. Simonii</i> Carr	Qi LW 03-84	38	Datong, Shanxi
16	<i>P. deltoides</i> CL 'Beikang-1' (16-27/92)	Qi LW 03-85	38	Huairou, Beijing
17	<i>P. nigra</i> CL 12(GMO)	Qi LW 03-86	38	Huairou, Beijing
18	<i>P. deltoides</i> Bartr. CL. 'Naking-3'	Qi LW 03-87	38	Huairou, Beijing
19	<i>P. tacamahace</i> × <i>P. del.</i> DJack	Qi LW 03-88	38	Datong, Shanxi
20	<i>P.×euramericana</i> Cl. '35'	Qi LW 03-89	38	Datong, Shanxi
21	<i>P.×euramericana</i> (Dode) Guiner CL. 'Zhonglin46'	Qi LW 03-90	57	Daxing, Beijing
22	<i>P.</i> 'Zhongsui-12'	Qi LW 03-91	38	Fengning, Hebei
23	<i>P. deltoides</i> × <i>cathayana</i> 'Zhongheifang'	Qi LW 03-92	38	Gu'an, Hebei
24	<i>P. maximowiczii</i> Henry× <i>P. del.</i> var.ssp	Qi LW 03-93	38	Jianshi, Hubei
25	<i>P.×eur.</i> cv.San Martino× <i>P.×canadensis</i> cv. Shanhaiguanensis	Qi LW 03-94	38	Dalian, Liaoning
26	<i>P. del.</i> cv Lux× <i>P.×canadensis</i> cv. Shanhaiguanensis	Qi LW 03-95	38	Dalian, Liaoning
27	<i>P. nigra</i> var. <i>thevestina</i> Bean. 'Shanyin'	Qi LW 03-96	38	Datong, Shanxi
28	<i>P. nigra</i> var. <i>thevestina</i> Bean. 'Yanmenguan'	Qi LW 03-97	38	Datong, Shanxi
29	<i>P.×euramericana</i> cv. <i>serotina</i>	Qi LW 03-98	38	Datong, Shanxi
30	<i>P.×Zhongjinnensis</i> 2	Qi LW 03-99	38	Datong, Shanxi
31	<i>P.×Zhongjinnensis</i> 3	Qi LW 03-100	38	Datong, Shanxi
32	<i>P.×Zhongjinnensis</i> 7	Qi LW 03-101	38	Datong, Shanxi
33	<i>P.×Zhongjinnensis</i> 10	Qi LW 03-102	38	Datong, Shanxi
34	<i>P.×Zhongshangnensis</i> 8	Qi LW 03-103	38	Datong, Shanxi
35	<i>P.×qungainensis</i> 2	Qi LW 03-104	38	Datong, Shanxi
36	<i>P.×qungainensis</i> 3	Qi LW 03-105	38	Datong, Shanxi
37	<i>P.×qungainensis</i> 4	Qi LW 03-106	38	Datong, Shanxi
38	<i>P. pseudo-simonii</i> × <i>P. nigra</i>	Qi LW 03-107	38	Gu'an, Hebei
39	<i>P.×Liaohenica</i>	Qi LW 03-108	57	Gu'an, Hebei
40	<i>P.×Liaoningensis</i>	Qi LW 03-109	38	Gu'an, Hebei

Continuation Table 1.

41	<i>P. × gaixianesis</i>	Qi LW 03-110	38	Fengning, Hebei
42	<i>P. Langfangensis</i> -1 Wang	Qi LW 03-111	38	Langfang, Hebei
43	<i>P. Langfangensis</i> -2 Wang	Qi LW 03-112	38	Langfang, Hebei
44	<i>P. Langfangensis</i> -3 Wang	Qi LW 03-113	57	Langfang, Hebei
45	<i>P. Langfangensis</i> -4 Wang	Qi LW 03-114	38	Langfang, Hebei
46	<i>P. nigra</i>	Qi LW 03-115	38	Xinjiang
47	<i>P. deltoids</i> CL 1	Qi LW 03-116	38	Datong, Shanxi
48	<i>P. deltoids</i> CL 2	Qi LW 03-117	38	Datong, Shanxi
49	<i>P. deltoids</i> CL 3	Qi LW 03-118	38	Datong, Shanxi
50	<i>P. deltoids</i> CL 4	Qi LW 03-119	38	Datong, Shanxi
51	<i>P. deltoids</i> CL 5	Qi LW 03-120	38	Datong, Shanxi
52	<i>P. deltoids</i> CL 6	Qi LW 03-121	38	Datong, Shanxi
53	<i>P. deltoids</i> CL 7	Qi LW 03-122	38	Datong, Shanxi
54	<i>P. deltoids</i> CL 8	Qi LW 03-123	38	Datong, Shanxi
55	<i>P. deltoids</i> CL 9	Qi LW 03-124	38	Datong, Shanxi
56	<i>P. deltoids</i> CL 10	Qi LW 03-125	38	Datong, Shanxi
57	<i>P. deltoids</i> CL 11	Qi LW 03-126	38	Datong, Shanxi
58	<i>P. deltoids</i> CL 12	Qi LW 03-127	38	Datong, Shanxi
59	<i>P. deltoids</i> CL 13	Qi LW 03-128	38	Datong, Shanxi
60	<i>P. deltoids</i> CL 14	Qi LW 03-129	38	Datong, Shanxi
61	<i>P. deltoids</i> CL 15	Qi LW 03-130	38	Datong, Shanxi

are in China. Since the 1990's, China has initiated an enormous reforestation program with *Populus* species for conservation and fiber supply purposes. Today, 70% of these plantations are *Aigeiros*, which is not surprising because more than 90% of poplar cultivation throughout the world is concentrated on the species and hybrids in the section of *Aigeiros* (THIELGES, 1985). Although triploid *Aigeiros* has been reported in the past (DARLINGTON and WYLIE, 1956; BRADSHAW and STETTLER, 1993), they have not been used as extensively as *P. tomentosa*. Because *Aigeiros* has more advantages in reproduction and growth than *P. tomentosa*, such as easier rooting with cuttings, faster juvenile growth, better tree form, and more adaptability to drought and poor soils, triploid *Aigeiros* may have great potential in poplar breeding and production programs in China as well as in the world.

In this study, we collected 61 species, hybrids, or clones from the section of *Aigeiros* cultivated and planted in China. The purpose is to identify triploids in these artificial plantations.

Materials and Methods

The branch materials from the section of *Aigeiros* were collected in the plantations in Beijing, Hebei, Liaoning, Shanxi, and Xinjing of China (Table 1). Triploid *P. tomentosa* as a check was collected from Shanxi, China.

After these branches were brought back from the field, they were stored at 15 °C in a refrigerator. When the experiment started, we cultured branches in tap water until the roots grew

about 0.5–1 cm long. Chromosome samples were prepared by wall degradation hypotonic method (CHEN et al., 1979, 1982). Briefly, root tips were removed and treated with saturated P-Dichlorobenzene solution for three hours before they were immersed in 0.075M KCl solution at 25 °C for 30 min for pre-hypotonic treatment. After that, they were treated with fixation solution (methanol: glacial acetic acid = 3:1) for 30 min and washed with distilled water. Then, materials were treated with 2.5% cellulase and pectinase enzyme mixture at 25 °C for about 40–60 min, washed with 0.075M KCl solution twice and incubated at 25 °C for 15 min. Fresh prepared fixation solution was added a drop of fixation solution on the slide, and heated the slide over an alcohol burner. The dried slide was stained with a 1:20 Giemsa solution for 15 min, rinsed in distilled water, air-dried, and mounted in a dammar balsam. Twenty slides were produced and counted per tree. Each genotype was validated at least three times. Karyotype analysis followed LI and CHEN (1985). The "M" is median point, "m" is median region, "sm" is submedian region, "st" is subterminal region and "t" is terminal region, "SAT" is satellite.

Results and Discussion

Of 61 *Aigeiros* genotypes, we found that 57 materials were diploid ($2n = 2x = 38$) (Figure 1). Four triploid genotypes were found: *Populus × euramericana* (Dode) Guiner cv. Wuhei-1, *P. × euramericana* (Dode) Guinier. cv. "Zhonglin-46", *P. × Liaoheni-ca*, and *P. Langfangensis*-3 Wang (*P. deltoides* Barry cv. "Shan-

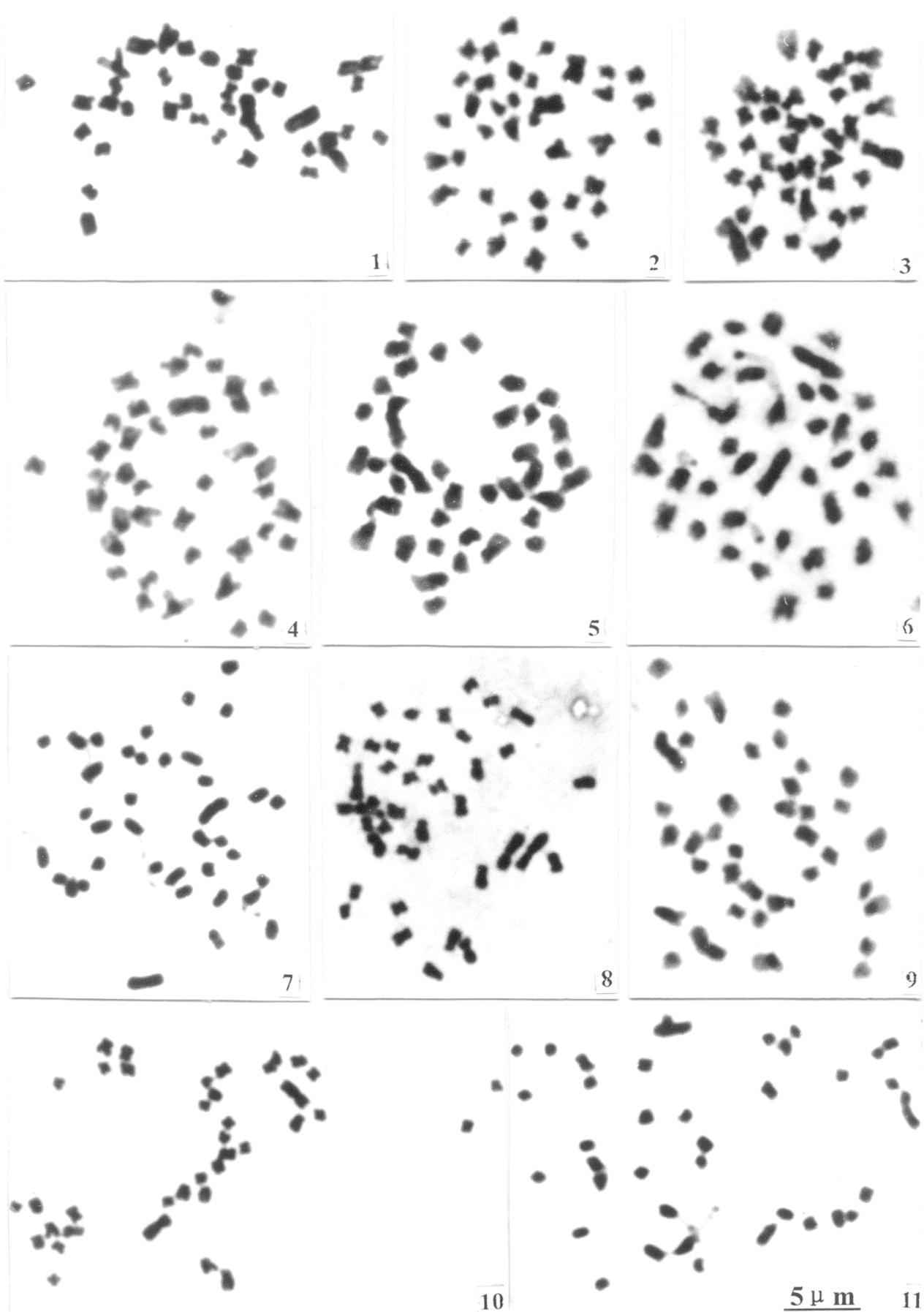


Figure 1. – The Chromosomes of some species and varieties (lines and hybrids) of the section *Aigeiros*. 1. *P. x qungainensis* 2; 2. *P. x Liaoninensis*; 3. *P. deltoides* CL 2; 4. *P. deltoides* Bartr.CL. 'Naking-3'; 5. *P. Langfangensis*-2; 6. *P. x euramericana* cv. '74/76'; 7. *P. x Zhongjinnensis* 2; 8. *P. nigra* var. *thevestina* Bean. 'Shanyin'; 9. *P. x euramericana* Cl. '35'; 10. *P. x beijingensis* W. Y. Hsu-2; 11. *P. x canadensis* Moench.

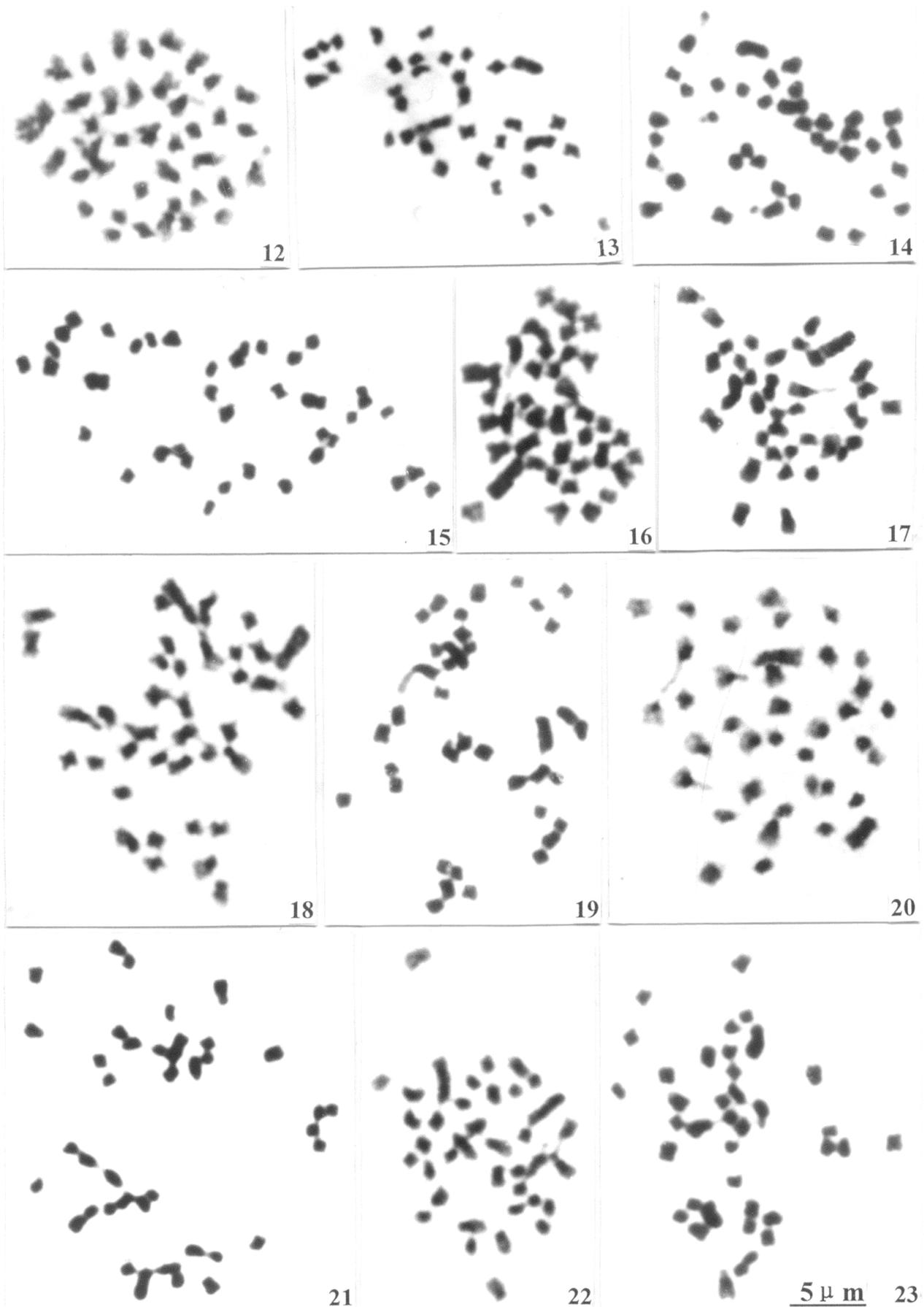


Figure 1 (continues). – *P. deltooides* CL 13; 13. *P. x gaixianensis*; 14. *P. deltooides* CL 9; 15. *P. deltooides* CL 10; 16. *P. x Zhongshangnensis* 8; 17. *P. x Zhongjinnensis* 10; 18. *P. deltooides* CL 6; 19. *P. deltooides. x cathayana* 'Zhongheifang'; 20. *P. x euramericana* Zhonglin-2025; 21. *P. deltooides* CL 15; 22. *P. deltooides* CL 12; 23. *P. deltooides* CL 7.

haiguan" \times *P. simonii* \times *P. pyramidalis*-12+*Ulmus pumila* Linn.). Their chromosome numbers are: $2n=3x=57$ (Figure 1). Aneuploids and tetraploids were not found.

The karyotype analysis indicates that the karyotype of triploid *Aigeiros* differed significantly from that of triploid *P. tomentosa*. The karyotype of triploid *euramericana* (Dode) Guiner cv. Wuhei-1 is $2n=3x=57=2M+38m(4SAT)+14sm(2SAT)+3st$, while the karyotype of triploid *P. tomentosa* is $2n=3x=57=M+31m+10sm+15st$ (Figure 2). The chromosome pairs of triploid *P. tomentosa* are the same, while the chromosome pairs of triploid *Aigeiros* are remarkably different. This indicates that triploid *Aigeiros* might be derived from original allotriploid.

There are generally two paths to form a triploid. One is hybridization between diploids and tetraploid. However, as far as we know, tetraploid poplar has been not found in China. Therefore, a triploid tree is unlikely formed through this path. Another path is for the germcells to double in one parent (a $2n$ pollen grain or oocyte), which is then fertilized by a $1n$ gamete from the other parent forming a triploid seed. This has been proven for triploid *P. tomentosa* (ZHU et al., 1995). The formation of triploid *Aigeiros* most likely followed this path. However, this hypothesis needs to be tested with further experiments.

Of the triploid that we identified, *P. x euramericana* (Dode) Guiner CL. "Zhonglin-46" is a third generation poplar variety

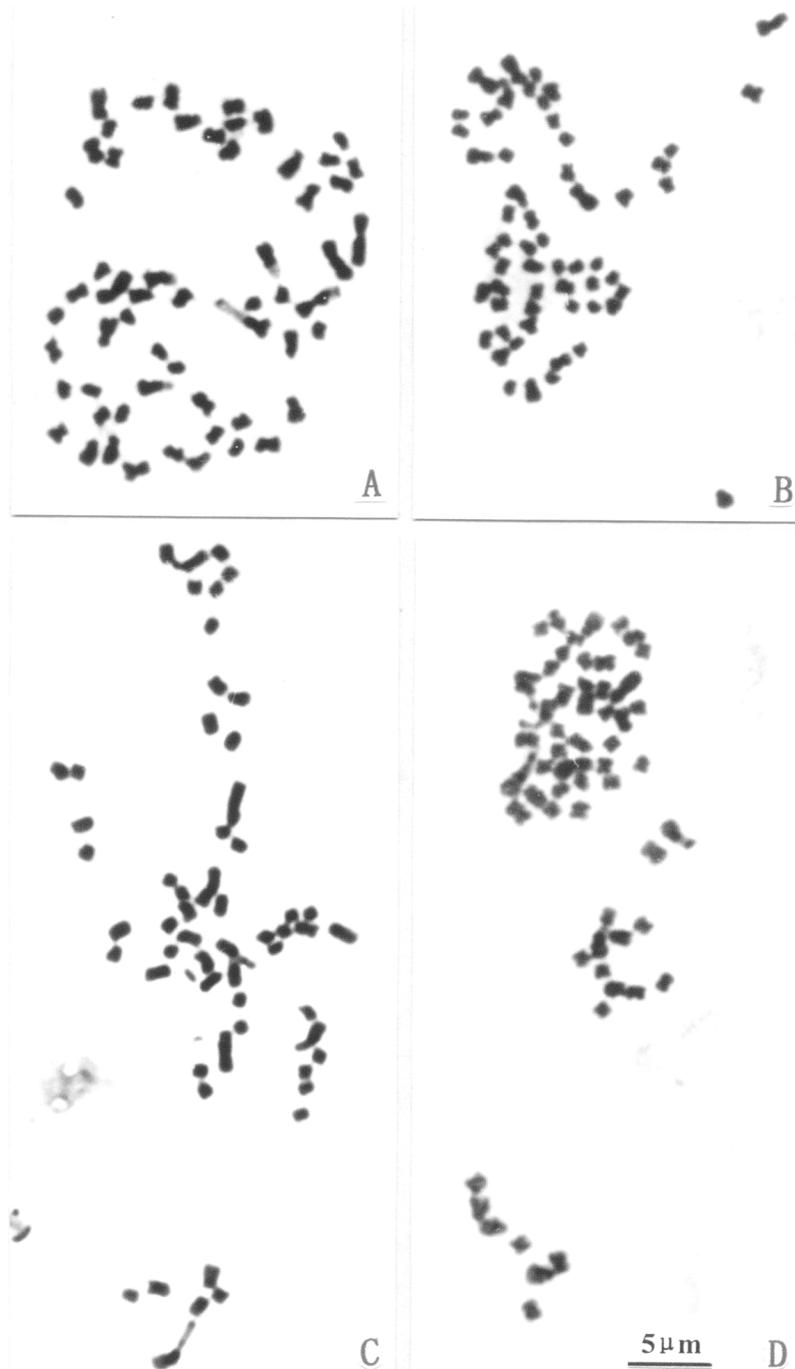


Figure 2. – The chromosomes from triploid *Populus*: (A) *Populus x euramericana* (Dode) Guiner. cv. Wuhei-1; (B) *P. x Liaohenica*; (C) *P. Langfangensis*-3; (D) *P. x euramericana* (Dode) Guiner. CL. "Zhonglin-46".

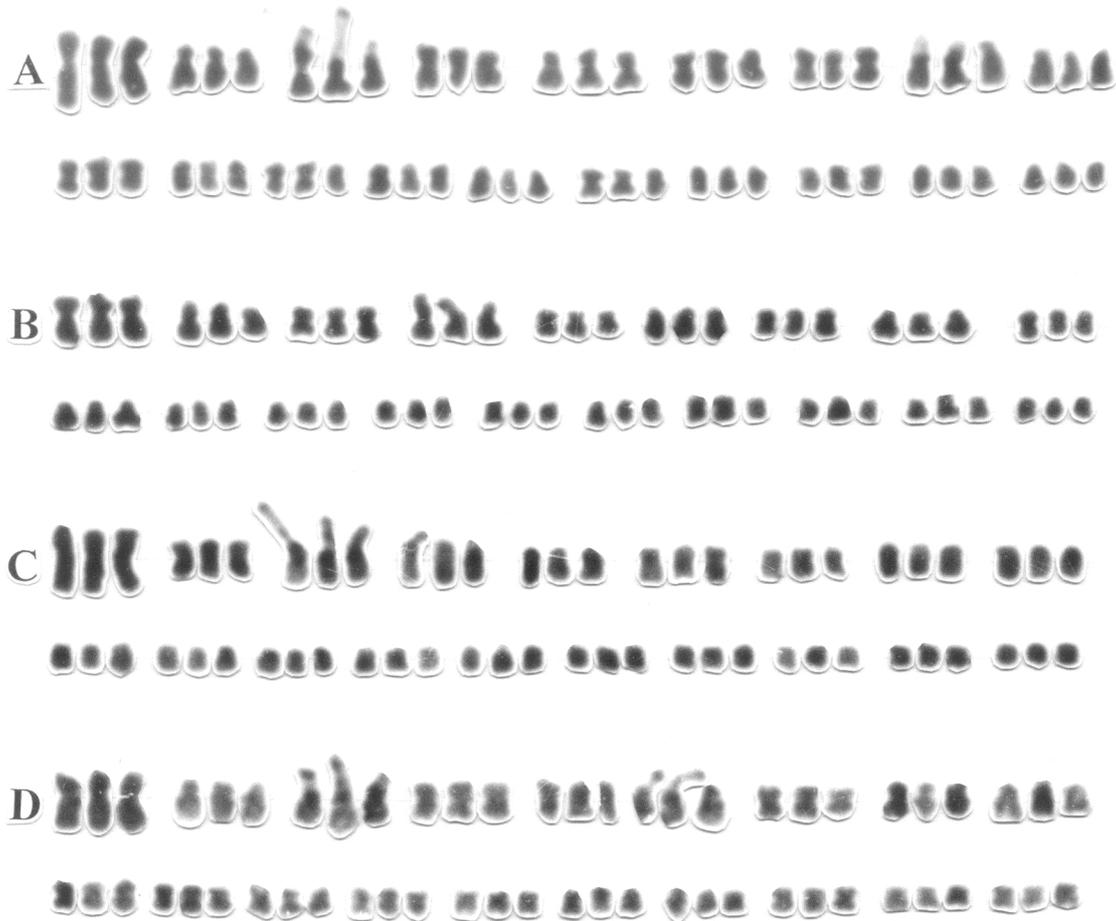


Figure 3. – The karyotypes of (A) *Populus euramericana* (Dode) Guinier. cv. Wuhei, (B) *P. x Liaohenica*, (C) *P. Langfangensis-3* and (D) *P. x euramericana* (Dode) Guinier CL. “Zhonglin-46”.

created by The Chinese Academy of Forestry in the 1970's and 1980's. *P. x Liaohenica* is a hybrid of *P. deltoides* cv. *Shanhaiguanensis* and *Populus deltoides* cv. Harvard. *P. “Langfangensis-3”* is a new cultivated material, yet the *P. “Langfangensis-1”*, *P. “Langfangensis-2”*, and *P. “Langfangensis-4”* are all diploids. *P. x euramericana* (Dode) Guinier. Wuhei-1 was collected in Wuqing of Tianjin and some of its characters are similar with *P. x euramericana* CL. Zhonglin-46. We speculate that both could be from the same origin or a contamination occurred when these plantations were established.

A common garden study will be established to compare growth among these triploids as well as to compare them with diploids. From the plantations where we collected the samples, we observed that the triploids grew about 5 cm in DBH and 4 to 5 m in height. After two years, their DBH could reach more than 6 to 9 cm and height could be 9 m. The fast growing characteristics could have tremendous potential in plantation forests in China.

From this screening process, we found that 6.8% of 61 *Aigeiros* materials are triploids, which is high for percentage of triploids. It is not clear whether these triploids were formed through natural fertilization or if they were triploids originally. However, what can be affirmed is that the finding of triploid *Aigeiros* will have significant effect on poplar breeding in the future.

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References

- BRADSHAW, H. D. and STETTLER, R. F.: Molecular genetics of growth and development in *Populus*. I. Triploidy in hybrid poplars. *Theoretical and Applied Genetics* **86**: 301–307 (1993). — CHEN, R. Y., SONG, W. Q. and LI, X. L.: A new method for preparing mitotic chromosomes from plant. *Acta Botanica Sinica* **2**(3): 297–298 (1979). — CHEN, R. Y., SONG, W. Q. and LI, X. L.: Wall degradation hypotonic method of preparing chromosome samples in plant and its significance in the cytogenetics. *Acta Genetica Sinica* **9**(2): 151–159 (1982). — DARLINGTON, C. D. and WYLIE, A. P.: *Chromosome atlas of flowering plants*. Macmillan, New York, 519pp. (1956). — Li, M. X. and CHEN, R. Y.: A suggestion on the standardization of karyotype analysis in plants. *Journal of Wuhan Botanical Research* **3**(4): 291–302 (1985). — THIELGES, B. A.: Breeding poplars for disease resistance. *Forestry Paper 56*, FAO, Rome. 66pp. (1985). — ZHU, Z. T., LIN, H. B. and KANG, X. Y.: Studies on allotriploid breeding of *Populus tomentosa* B301 clones. *Scientia Silvae Sinicae* **31**(6): 499–505 (1995).