

# Studies on the induced polyploids of the North American Pawpaws (I)

## Chromosome number and mixoploids in *Asimina triloba* DUNAL

By

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North American Pawpaws which are in cultivation in Japan have little differences of vegetative and floral characteristics among them, and their propagation is always dependent upon seedling, because the breeding of this fruit tree is far behind the others in research. The important points for breeding, in regard to this fruit, are the problems concerning the increase in size and yield, improvement of the fragrance and taste, ability for preservation and seedless forms etc. The research of polyploidy in Annonaceae should probably be useful in the breeding of the pawpaw. Concerning the chromosome number, LOCKE (1936) reported that the haploid number is apparently 9. By BOWDEN (1940) thereafter, the facts that the haploid number is 9 and that the diploid is 18 were ascertained. The same author (1948) reported that 5 triploid ( $2n=27$ ) mutants were found among 200 diploid ( $2n=18$ ) seedlings.

### Materials and methods

For the determination of the chromosome number and for the observation of the chromosome complement of *Asimina triloba* DUNAL, many adult plants and 15 seedlings (two years old) which are growing in the garden of the Saikyo University were served. Two polyploid plants examined were obtained in 1949 by one of the present authors through a colchicine treatment, by application by drops of a 0.4 % solution on a lateral bud of the seedlings for two days. This colchicine treatment was carried on immediately after germination, when a few young

leaves of the seedlings grew 0.5 to 2.0 cm in length and all the little leaves except a basic leaf were cut off. Thereafter, lateral buds at the cotyledon and adventitious buds on the hypocotyl were scraped away. As a consequence of the treatment, about a dozen thick seedlings were obtained, but most of them died, and at the present time only two plants are viable and on the whole these are growing slowly. This spring, after a lapse of 7 years from the treatment, both trees are one meter or so in height. The upper ground parts of one plant (A) withered in June or July of this year. But, fortunately, each grafting upon two other adult stocks succeeded in early April. Moreover, some shoots which seem to be tetraploid are beginning to develop from the base of the withered stem.

For the cytological studies, the following squash method (MUTSUURA, 1956) was used. Leaf tips or young anthers were treated successively with three solutions as shown in Table 1, in which each treatment hour was stated, then they were squashed in aceto-carmin. Good chromosome preparations were obtained by this method.

For the observation of stomata, 10 leaves were taken up from the material plant and a round piece of lamina, 8 mm in diameter, was excised from the center region between the main nerve and the margin at the widest part of each leaf. These fragments were boiled for three minutes in 99 % ethyl alcohol and were then immersed into the chloralhydrate solution (5g : 3cc distilled water) for 20 hours, thereafter these were immersed in glycerine on slides and

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**Table 1. Solutions used for treatment and treatment time**

	treatment time		
	I 1.2 N HCl	II chromoacetic mixture <sup>1</sup>	III ammonia alcohol <sup>2</sup>
young leaf	13-15 min.	10 sec.	10-15 sec.
P. M. C.	15 min.	10 sec.	10 sec.

1) CrO<sub>3</sub> 1g, glacial acetic acid 3cc., H<sub>2</sub>O 100cc.

2) 30% alcohol 100cc, ammonia 6 drops.

After each treatment the material is rinsed in water for 30 sec.

were observed microscopically.

### Observation and conclusion

Meiotic division in PMC was regular and 8 bivalents were counted at the first metaphase (Plate 1 : A<sub>1</sub>, A<sub>2</sub>), while in mitosis 16 homologous chromosomes were observed (Plate 1 : B<sub>1</sub>, B<sub>2</sub>). It is therefore obvious that the chromosome numbers of *Asimina triloba* are n=8 and 2n=16. Each pair of the chromosomes are characterized by its length and shape, and the chromosome complement is classified into the following 5 types :

a type, two chromosomes of one pair, the largest (ca. 4 $\mu$  long) median type with a secondary constriction in one of the arms and two constrictions in the other arm. (The chromosome of this type is apt to be miscounted as two single chromosomes)

b type, two chromosomes of one pair, submedian type with a secondary constriction in the long arm, and with a large satellite on the short arm.

c type, four chromosomes of two pairs, submedian, with secondary constrictions in both arms.

d type, four chromosomes of two pairs, submedian type with a secondary constriction in the long arm.

e type, four chromosomes of two pairs, the shortest submedian type (ca. 2 $\mu$  long) with a secondary constriction in the long arm.

Both artificially induced polyploid plants (A and B) which were obtained by the colchicine treat-

ment were shown to be mixoploids, although their tissues have not yet been observed in detail. The tissue of the leaves of A plant are a mixture of different cells which contain either 16 or 32 chromosomes. The ratio appears ca. 1 (2x) : 4 (4x). In the B plant, the ratio is ca. 2 (4x) : 3 (8x). It is interesting that chimera in a polyploidy condition has been maintained even after a lapse of 7 years.

**Table 2. Size of the stomata of the auto-octaploid, tetraploid and diploid plants in *Asimina triloba***

	size of stomata ( $\mu$ ) <sup>1</sup>		number of stomata per unit area <sup>2</sup>
	length	width	
octaploid	215 $\pm$ 26	110 $\pm$ 17	23.6 $\pm$ 10.1
octaploid (grafted)	205 $\pm$ 37	111 $\pm$ 20	24.7 $\pm$ 11.3
tetraploid (grafted)	173 $\pm$ 18	87 $\pm$ 12	39.4 $\pm$ 13.4
diploid	117 $\pm$ 9	65 $\pm$ 8	72.0 $\pm$ 18.0

1) average of 200 stomata

2) average of 100 fields of vision

**Table 3. Size of the leaves of auto-octaploid, tetraploid and diploid plants in *Asimina triloba***

	size of leaf		thickness of leaf ( $\mu$ )
	length(cm)	width(cm)	
octaploid*	15.3 $\pm$ 3.4	8.6 $\pm$ 1.7	308 $\pm$ 46
octaploid** (grafted)	29.8 $\pm$ 5.7	14.5 $\pm$ 2.7	377 $\pm$ 52
tetraploid** (grafted)	28.4 $\pm$ 1.9	16.3 $\pm$ 2.8	329 $\pm$ 32
diploid*	27.2 $\pm$ 4.8	11.9 $\pm$ 2.2	154 $\pm$ 24

\* average of 150 leaves

\*\* average of 20 leaves

In the observation concerning the stomata of diploid, tetraploid and octaploid plants, the size and number are significantly different in each of them (Table 2, Plate 2: G-K). A shoot of the octaploid plant was also grafted on the diploid stock, and the grafted octaploid, in comparison with the original one, has much more vigorous leaves, though there is no difference in size of the stomata between the grafted and the original one (Table 2 & 3, Plate 2: K).

### Summary

1. The chromosome numbers of *Asimina triloba* were determined as  $n=8$ ,  $2n=16$ . The chromosome complement was classified into 5 types. The long arms of each type have a constriction and that of a type has two constrictions. The chromosomes of b type have a satellite.

2. By the colchicine treatment autotetraploid ( $2n=32$ ) and auto-octaploid ( $2n=64$ ) plants were obtained. These plants were shown to be mixoploids; in the leaf of tetraploid plant diploid numbered cells were found, and similarly, in the octaploid plant tetraploid numbered cells were found. The size of the stomata in the diploid, tetraploid and octaploid plants show significant differences from each other.

### Literature cited

- 1) BOWDEN W. M, 1945. Amer. Jour. Bot. 32 : 81-92.
- 2) ——. 1948. Amer. Jour. Bot. 35 : 377-381.
- 3) LOCKE J. F. 1936. Bot. Gaz. 98 : 159-168.
- 4) MUTSUURA O. 1956. Sci. Rep. Saikyo Univ., Nat. Sci. & Liv. Sci. 2 : 187-191. (in Japanese with English summary)

## 摘 要

伊藤五彦・六浦修：ポポウに関する人為倍数体の研究 (I)

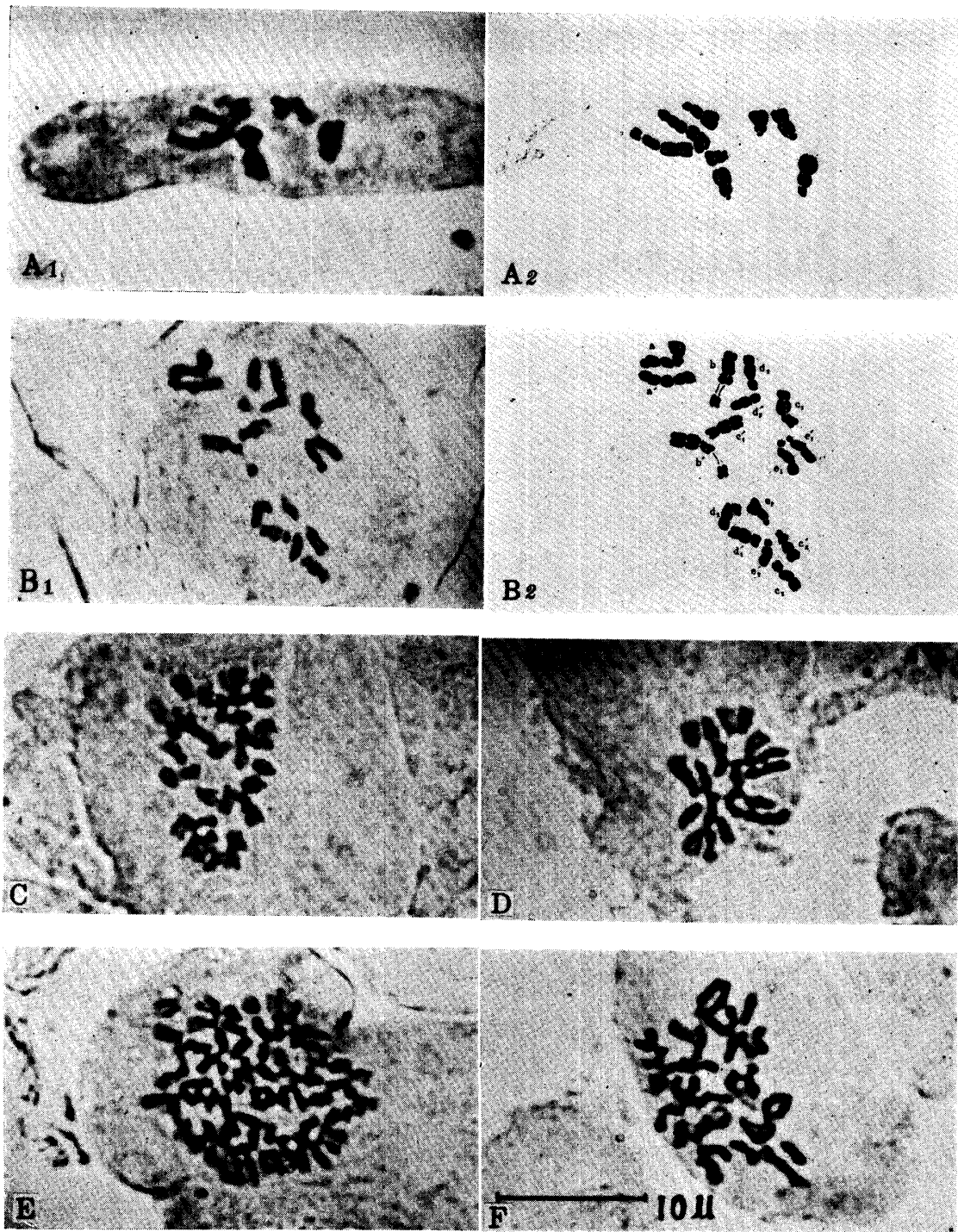
*Asimina triloba* DUNAL の染色体とミクソプロイド

1. *Asimina triloba* DUNAL の染色体は、 $n=8$ ,  $2n=16$  と決定された。染色体は5型 (a-e) に分類され、各染色体の長い腕上には、1個の狭窄が存在し、a型上にはのみ、2個の狭窄が見られる。b型には、1個の附随体が存在する。

2. 実生の腋芽に対するコルヒチン滴下処理により、

4倍体 ( $2n=32$ ) 及び8倍体 ( $2n=64$ ) 体夫々1個が得られた。この2個体は、ミクソプロイドである事が認められた。2倍体、4倍体及び8倍体個体の気孔の大きさは、互に、有意な差異を示し、漸次、8倍体に向つて、巨大性を示してゐる。

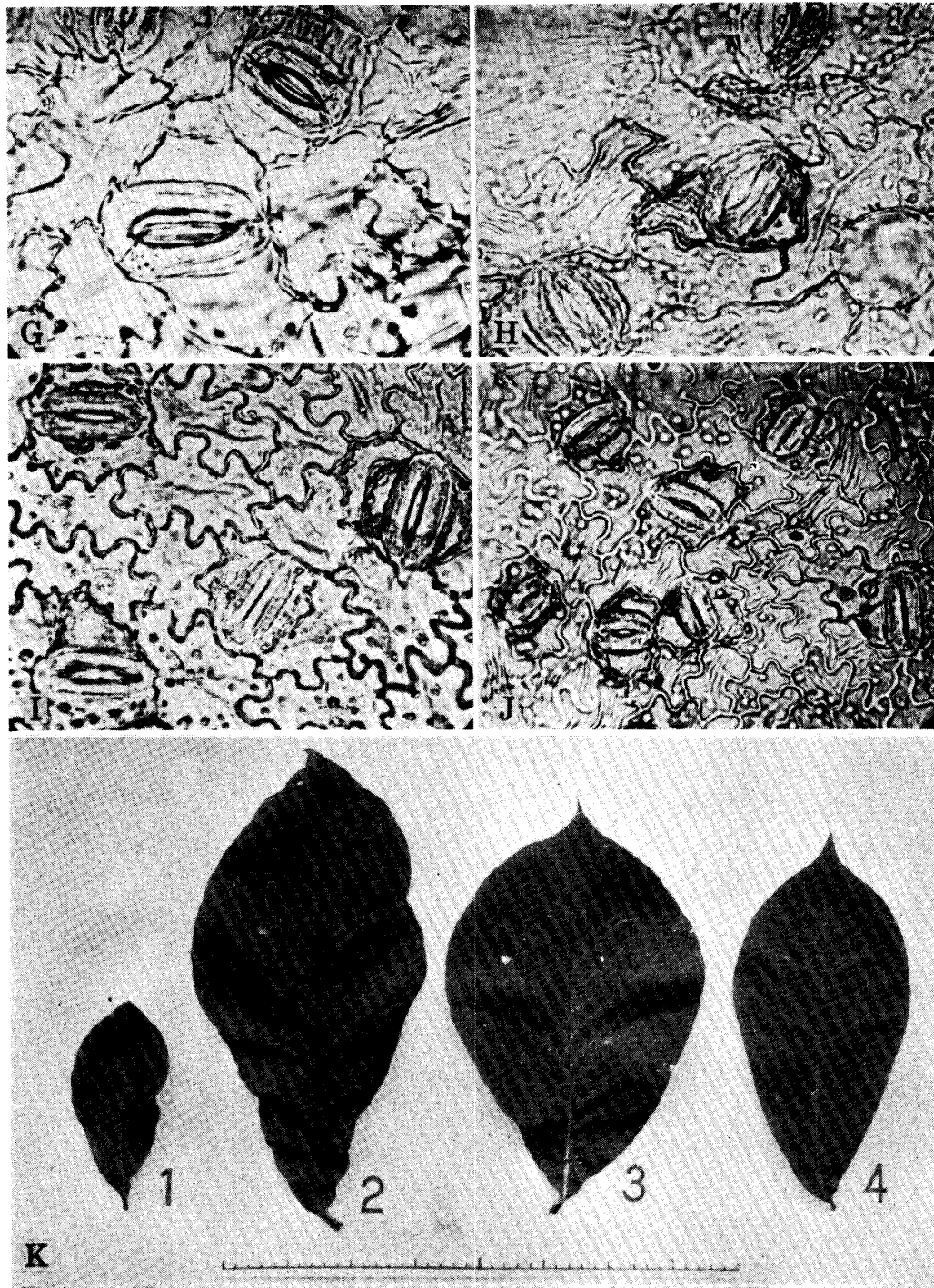
Plate 1



Explanation of Plate 1

Photomicrographs and explanatory diagrams of chromosome in *Asimina triloba* (ca.  $\times 2000$ ). A<sub>1</sub> : PMC meiosis, A<sub>2</sub> : its explanatory Diagram, B<sub>1</sub> : mitosis in young leaves, B<sub>2</sub> : its explanatory diagram, C—F : mitosis, C : in tetraploid, D : of diploid cell in tetraploid plant, E : in octaploid, F : of tetraploid cell in octaploid plant.

Plate 2



**Explanation of Plate 2**

Photomicrographs of stomata and photograph of leaves of polyploid plants in *Asimina triloba*. G : stomata of octaploid plant(original), H : octaploid plant(grafted), I : tetraploid plant(grafted), J : diploid plant(control) (ca.×380), K : leaves of polyploid plants. 1. octaploid(original), 2. octaploid(grafted), 3. tetraploid (grafted), 4. diploid(control). Scale indicates 40cm.