CHROMOSOME NUMBERS OF TRIGONOBALANUS VERTICILLATA FORMAN (FAGACEAE)

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SUMMARY

Chromosome numbers of *T. verticillata* Forman have been preliminarily found to be n = *c.* 21 and 2n = 40, 42, 44. They require future verification based on the study of more collections from its geographical range of distribution.

The haploid chromosome numbers in *Fagaceae* so far known are 12 in most of the genera and 13 in *Nothofagus*. The number of *c.* 21 for *Trigonobalanus* is unique and new in this family.

Irregularities of chromosome behaviour during meiosis, such as secondary association, lagging, precocious separation of chromosomes, etc. have been observed.

1. INTRODUCTION

The family Fagaceae, according to FORMAN (1964, 1966), consists of eight genera: *Fagus*, *Nothofagus*, *Castanea*, *Lithocarpus*, *Castanopsis*, *Chrysolepis*, *Quercus*, and *Trigonobalanus*. Normal chromosome numbers were reported mostly for species or sometimes interspecific hybrids of *Quercus*, *Castanea*, and *Fagus* in the northern temperate zone, which are uniformly known as n = 12 or 2n = 24 (DARLINGTON & WYLIE 1955). Various deviating numbers were recorded or published in the past, for example, 2n = 8, 12, 22 and 48 in *Quercus*; however, such erroneous numbers have been corrected or eliminated by later studies (SAX 1930; DUFFIELD 1940; JAYNES 1962; SANTAMOUR 1962). Recently, in 1968, *Quercus castaneifolia* C. A. Mey. was reported to have 2n = 28 (*fide* MOORE 1970); I have not seen any confirmation of it.

In the southern region, ARMSTRONG & WYLIE (1965) found that four New Zealand species of *Nothofagus* have the same chromosome numbers, *i.e.* n = 13.

In Malesia, SOEPADMO (1968) stated that Mr. Kwiton Jong "made some preliminary counts in several species of *Castanopsis*, *Lithocarpus*, *Quercus* (subgenus *Cyclobalanopsis*), and *Trigonobalanus*, and found that the diploid number in the first three genera is also 24, whilst that of *Trigonobalanus verticillata* is not yet fully understood".

As for the occurrence of polyploidy, JOHNSON (1940) found that triploidy (2n = 36) occurred in plants of *Quercus robur* with a frequency of about 0.4%. In *Castanea*, JAYNES (1962) recorded that Schad *et al.* observed a few natural polyploids among three species and their hybrids with 2n = 31–48 and that he himself found a triploid hybrid with 2n = 36 ± 1.

From the above concise review it appears that in Fagaceae chromosome num-

bers have been reported for representatives of the genera accepted by Forman except for *Chrysolepis* and *Trigonobalanus*.

2. MATERIALS AND METHODS

2.1. For mitotic chromosomes

One seedling of *T. verticillata* Forman was collected from Fraser's Hill, Malaya, in 1968 and was ingeniously brought back by my colleague Dr. M. Jacobs. It has been kept in a rather "cool" greenhouse of the Botanic Garden, Leiden, under the number of 15768; it is growing well there and its whorled leaves show a characteristic feature of the species (Pl. I: A & B). I may mention here that the leaf scars are alternate in the basal part of the plant (*cf.* FORMAN 1967). Dr. Jacobs encouraged me to study the chromosome number of this interesting plant.

Since September, 1968, root tips were occasionally removed from the seedling. By trial-and-error the material was usually fixed at 11:00–11:30 a.m. In order to have the chromosomes well contracted to facilitate counting, the material was treated first in a saturated aqueous solution of paradichlorobenzene for about 4 hours at room temperature or stored in a refrigerator (*c.* 5°C) for 4–24 hours (*cf.* KUROSAWA 1966; BURDET 1967). One should be aware that in such pretreated material the number of cell divisions will tend to be diminished (*cf.* BURDET 1967). For squash preparations with aceto-carmine or -orcein, the general procedure was followed (JOHANSEN 1940; DARLINGTON & LA COUR 1962).

2.2. For meiotic chromosomes

Flower-buds of *T. verticillata* were collected and fixed in Carnoy's solution by my colleague Mr. H. P. Nooteboom (voucher specimen: Nooteboom & Chai 1669, L) in the early evening of March 25, 1970, at Kelabit Highlands, alt. 1000 m, 4th Division, Sarawak. As soon as the material arrived at Leiden by air on April 7, 1970, it was transfered to alcohol (70%). The fixed material was stored in a refrigerator (*c.* 5°C) until needed for examination. For preparation of microscopic slides the squash technique for roottips with aceto-carmine or -orcein was applied. As far as stain ability is concerned, good result was obtained when both stains were applied. After hydrolysis the material was kept in aceto-carmine for a few hours or stored overnight in a refrigerator and then squashed in aceto-orcein. The material seemed rather fragile. The pollen mother cells were easily broken if high pressure was applied and then both the chromosomes and cytoplasm received intense stain. In order to have good contrast for the chromosomes, the cells should be kept entire by not applying high pressure during squashing.

The permanent microscopic slides (72 pieces) mounted with Euparal have been deposited in the collection of the Rijksherbarium, Leiden.

3. OBSERVATION AND DISCUSSION

3.1. Somatic chromosomes

Somatic chromosome counts were made from roottip cells of the seedling growing in the greenhouse as mentioned before. The somatic chromosome number has been preliminarily found to be 2n = 40, 42, 44 (*fig. 1–3*; *pl. I: C–E*), which requires future verification based on the study of more material.

It was surprising at first to observe such a chromosome number in a woody family with rather uniform diploid number, 2n = 24. Since chromosome numbers of some plants may vary due to enviromental causes (*cf.* LEWIS 1970) and various ploidy levels of chromosomes may occur in cells of the same roottip (*cf.* TURNER & FEARING 1959), material was occasionally collected and examined cytologically in the last two and half years.

The chromosomes are rather slender and crowded and sometimes resemble a tangle. They appear to have various forms and sizes and a few of them bear satellites. When the material was pretreated with saturated aqueous paradichlorobenzene solution, the contracted and well spread chromosomes are easier to count, but the very short ones with subterminal or obscure centromeres may be misleading. Various numbers of chromosome complements may possibly occur here, or such counts may be due to incorrect observation. Cells with higher ploidy were occassionally observed, but a definite chromosome number of such a cell could not be established (*pl. I:F*).

It is a pleasure to record here the information given me by Dr. E. Soepadmo, University of Malaya, Kuala Lumpur. He told me that last year his student, Miss Goh Yee See, also worked on the chromosomes of *T. verticillata* by using roottips of seedlings and that her finding of the somatic chromosome number was 2n = 43. I saw a photomicrograph of it which showed that number.

3.2. Meiotic chromosomes

Fixed material of flower-buds as mentioned before was examined cytologically. One might find most of the stages of microsporogenesis sometimes in one slide prepared with two or three flowers. No attempt was made to study the details of development.

Although there were many cells in early prophase, hardly one in distinct diakinesis with well spread bivalents was observed. Cells showing first or second metaphase were numerous and most of them were in side view. Chromosomes appeared frequently in irregular groups in many of them; it is not clear whether this phenomenon is due to fixation or to occurrence of secondary or irregular associations of bivalents. Some of the cells of first and second metaphase in polar view showed the chromosome number to be n = *c.* 21 (*fig. 4*; *pl. II: A*). A definite number of such counts could not be obtained because of the condition of the material and the occurrence of irregularities of chromosome behaviour during meiosis.

A certain amount of secondary association (*pl. II: E*) was frequently observed here, which had been already found in *Quercus* and *Castanea* (*cf.* SANTAMOUR

1962; STAIRS 1964). SMITH-WHITE (1948) stated that the occurrence of such an association "has been used, with or without supporting evidence, to establish the secondarily derived nature of the chromosome complements in many groups of plants ..." (*cf.* also SMITH-WHITE 1950).

In addition to secondary association, other irregularities of chromosome behaviour sometimes also occurred during microsporogenesis. Lagging was observed at first anaphase and telophase (*pl. II: C & D*); such lagging was simultaneous in the two spindles at second anaphase or telophase (*pl. II: F*). Precocious separation of chromosomes was found (*pl. II: B*), which "may be purely accidental, or due to a certain lack of homology between the chromosomes concerned" (SMITH-WHITE 1942).

There were tetrads with abnormal number of microspores; usually one to three extra small "microspores" occurred in such a tetrad (*pl. II: G*).

The rather low frequency of irregularities seemed not to cause abnormality in pollen formation (*pl. II: H*). My colleague Mr. J. Muller examined the acetolyzed pollen preparations of specimens collected on Mt Kinabalu (Chew, Corner & Stainton 2645, L) and Fraser's Hill, Malaya (Poore 1337, L), and found that the grains appeared normal.

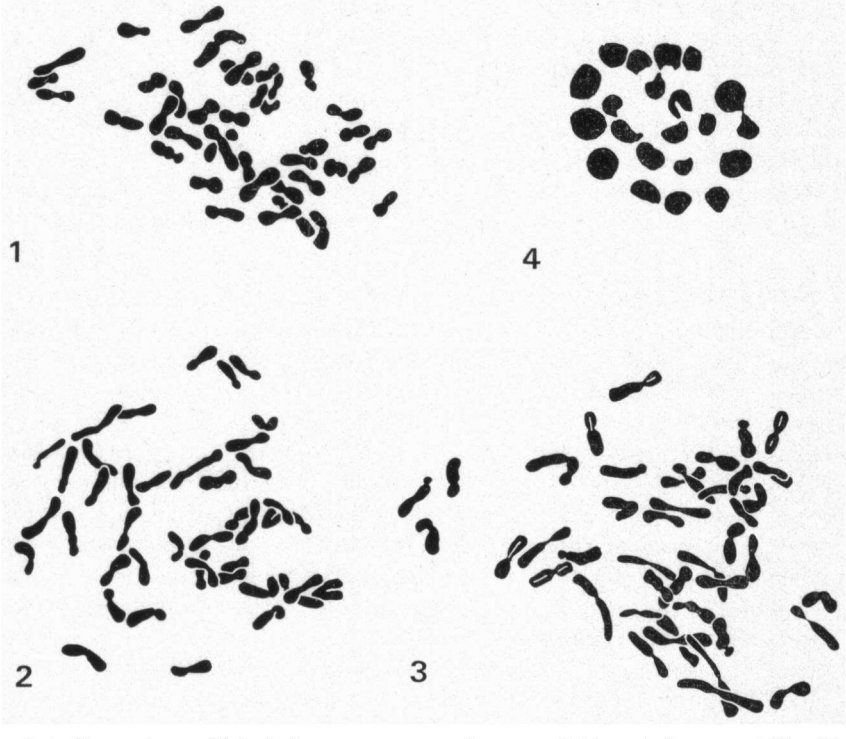

Fig. 1-4. Illustrations of inked chromosome complements of *Trigonobalanus verticillata* Forman-1-3. Somatic chromosomes with 2n = 40, 42, 44, respectively, × 1800, *cf.* Pl. I: C–E; 4. meiotic chromosomes with n = 21, × 3000, *cf. Pl. II: A.*

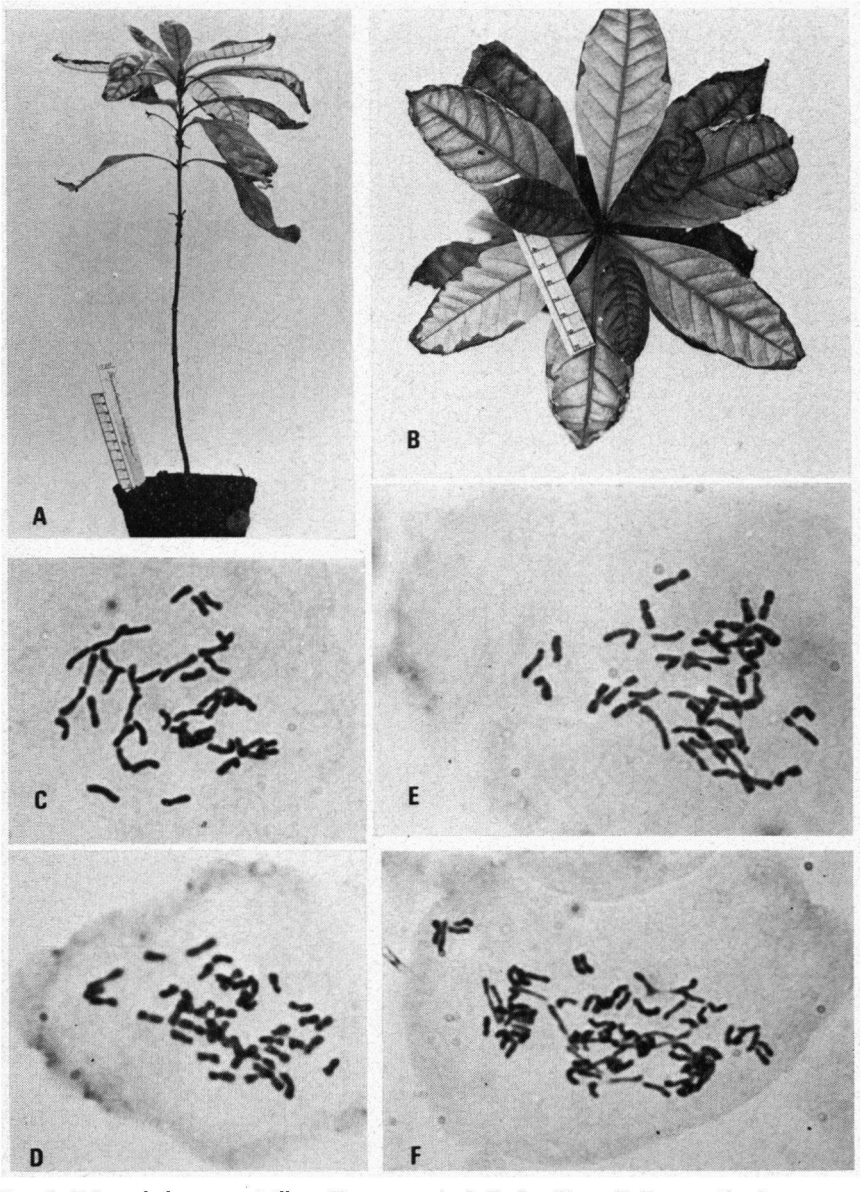

Plate I. *Trigonobalanus verticillata* Forman – A & B. Seedling; C–E. somatic chromosome complements showing various numbers (2n = 42, 40, 44, respectively) as well as different degrees of contraction of chromosomes, × 1250, *cf. Fig. 1–3*; F. somatic chromosome complement showing higher ploidy than 2n = 44, × 1250.

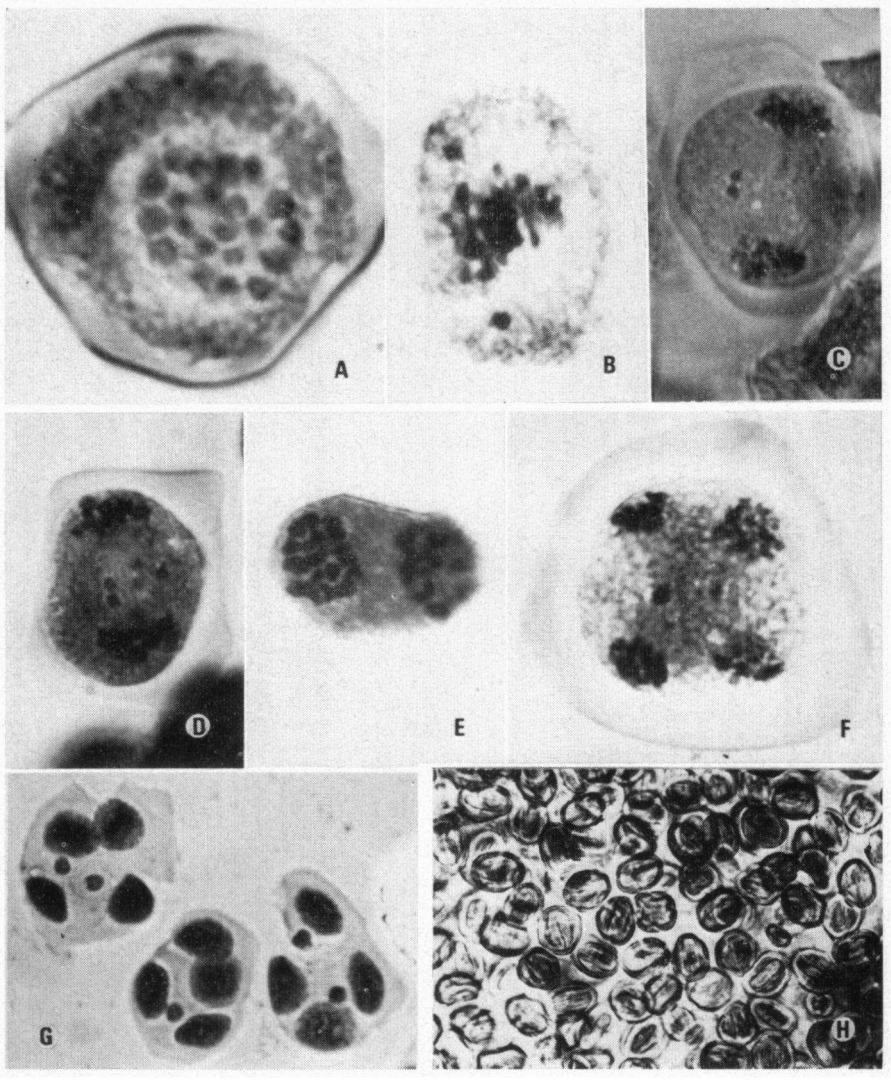

Plate II. Meiotic behaviour in *Trigonobalanus verticillata* Forman – A. Metaphase I with n = 21, × 3000, *cf. fig. 4*; B. early anaphase I, showing precocious separation of chromosomes, × 1500; C & D. telophase I, showing various numbers of lagging chromosomes, × 1500; E. metaphase II, showing secondary and irregular associations of bivalents, × 1500; F. telophase II, showing simultaneous lagging in the two spindles, × 1500; G. tetrads with abnormal numbers of microspores, × 575; H. a group of young pollen grains, showing almost all of them to be in normal development.

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