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SOMATIC CHROMOSOME NUMBERS OF SOME AFRICAN SAPOTACEAE

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SUMMARY

Somatic chromosome numbers of nineteen species of African *Sapotaceae* have been determined. Species of the genera *Pachystela*, *Donella*, *Gambeya* and *Omphalocarpum* have 2n = 28, those of *Synsepalum* (except for the polyploid species *S. subcordatum*, 2n = 112), *Tridesmostemon*, *Autranella*, *Tieghemella* have 2n = 26, whereas *Baillonella toxisperma* has 2n = 24 chromosomes. It is concluded that 2n = 28 presents the most primitive number, whereas 2n = 26 and 2n = 24, like the polyploid numbers, are secundary.

1. INTRODUCTION

This paper presents the results of a karyological analysis of twenty-four seedling samples, comprising nineteen species of African Sapotaceae. The analysis was carried out simultaneously with a study of the seedling morphology of various African taxa of that family by BOKDAM (1977). Previously MIEGE (1954) suggested already that knowledge of the chromosome numbers of the Sapotaceae might contribute to the delimitation of the genera, a subject on which students of the family have often expressed conflicting opinions. An opportunity to establish the somatic chromosome numbers of taxa not yet cytologically investigated was offered, when recently seedlings became available at the Department of Plant Taxonomy and Geography. These seedlings were produced from seeds collected since the beginning of 1974 in Zaire and Cameroon.

2. MATERIALS AND METHODS

The taxa investigated are presented in *table 1*, column I, and arranged according to the system of AUBRÉVILLE (1964), whereas the determination and nomenclature corresponds with BOKDAM'S (1977) paper. For some taxa two or more seed samples have been collected at different times and locations. For proper identification of the seedlings, herbarium samples of the mother plants were taken also. These specimens are conserved in the Wageningen herbarium (WAG); their numbers are shown in column IV of the table. Depending on the number of seeds collected per sample and on their germination, root tips of one to five seedlings per sample were analyzed. After analysis the seedlings were harvested at various stages of development and subsequent-

ly conserved. The herbarium numbers (WAG) of the conserved seedlings are shown in column III.

Root tips were collected at about 10.00 to 11.00 p.m. and pretreated in 0,002 M aqueous solution of 8-hydroxyquinoline during five hours at about 20°C, fixed in a 3:1 mixture of 96% ethanol and 100% acetic acid respectively, and stored in a refrigerator at 7°C. After maceration at 60°C in 1 N HCl for three minutes, the root tips were kept for at least two hours in a small test tube containing 2% orcein in 45% acetic acid, and transferred to a microscope slide. The minute meristematic parts were carefully chopped up in a drop of 0,5% orcein in 45% acetic acid, and covered with a glass slip. The slide was then kept in a petri-dish saturated with 45% acetic acid vapour for two hours, slightly heated, firmly pressed and blotted, and made semi-permanent by sealing with paraffine wax. The karyotypes were studied using a Carl Zeiss microscope equipped with a 100 × oil-immersion apochromatic phase-contrast objective (N.A. = 1,32). Photographs were made om 9 × 12 cm photographic plates.

3. OBSERVATIONS

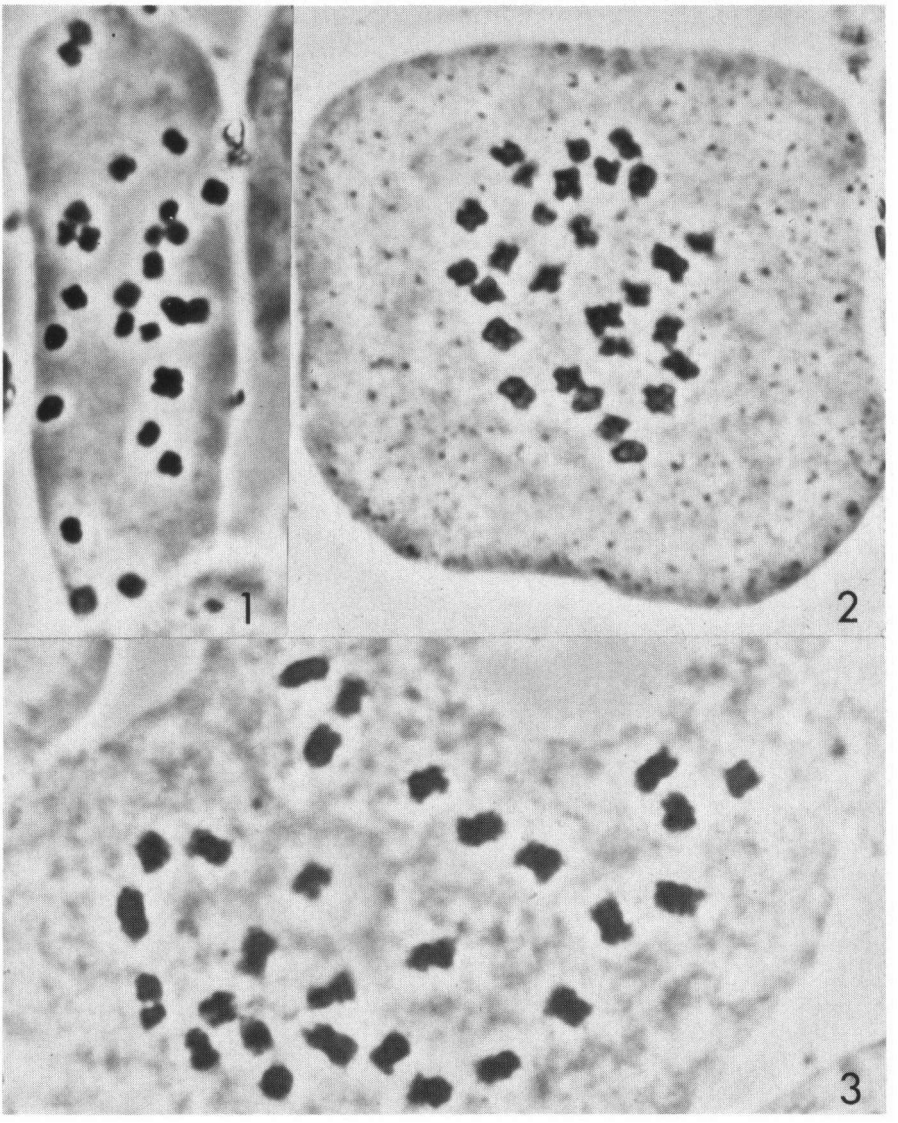
The method employed yielded a fair number of analysable cells, with, in general, satisfactorily stained chromosomes. Chromosomes not having reached the ultimate stage of metaphase contraction often showed the best spreading. The chromosomes, however, were most distinct when contracted completely, but at that stage the occurrence of varying degrees of stickiness was a common phenomenon. Since the observed chromosome numbers are generally fairly low, a slight stickiness did not hamper the analysis seriously; nevertheless for one of the species observed, *Synsepalum subcordatum*, which turned out to be a high polyploid, the chromosome number is given with some reserve. In analysing a polyploid species TJIO (1948) also noticed the occurrence of stickiness. It is felt that, in spite of satisfactory staining and spreading of the chromosomes, the use of phase-contrast microscopy highly aids in determining the correct number of chromosomes. Several of the karyotypes could be recorded photographically, from which some examples are presented here (*fig. 1* to *5*).

The somatic chromosome numbers found in this investigation vary from $2n = 24$, through $2n = 26$ and $2n = 28$ to $2n = 112$ (column II, *table 1*). The lowest number $2n = 24$ is found in the species *Baillonella toxisperma*. A somatic number of $2n = 26$ is found for in total five species, comprising one species of respectively *Tieghemella*, *Autranella* and *Tridesmostemon*, and two species of *Synsepalum*. Another twelve species have $2n = 28$ chromosomes: three species (one of which is most likely undescribed) of *Omphalocarpum*; four species of *Gambeya*; three species of *Donella* and finally two species of *Pachystela*. A somatic number of $2n = 112$ finally, is found for *Synsepalum subcordatum*, while the other two species of *Synsepalum* investigated here have $2n = 26$ chromosomes.

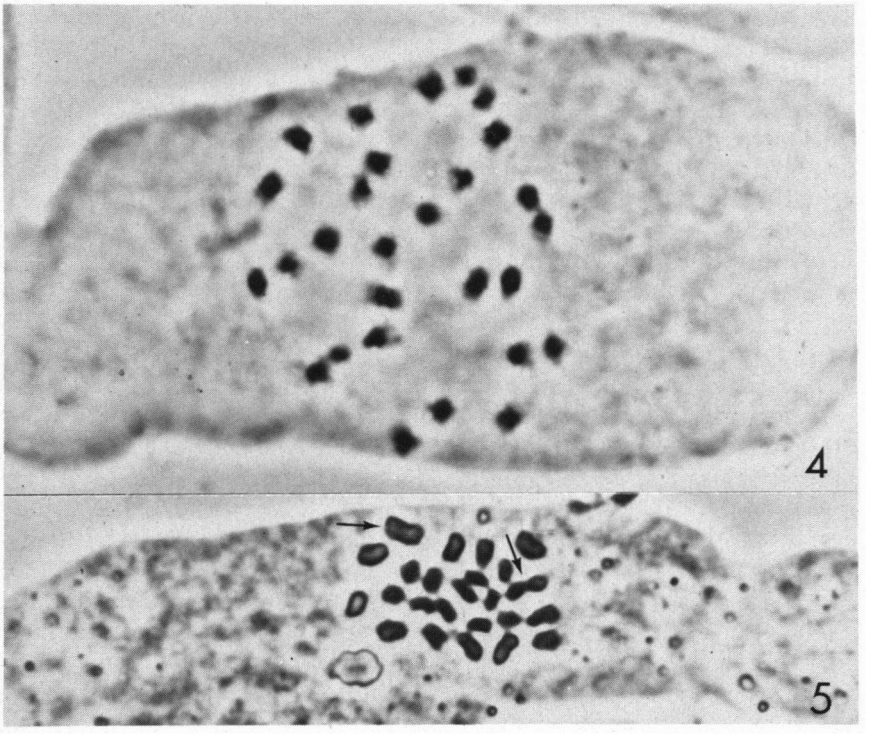
Chromosome morphology is demonstrated in the *figs. 1* to *5*. The overall size

Table 1. Somatic chromosome numbers in several species of African Sapotaceae.

I. Taxon	II. 2n	III. Seedling specimen herbarium WAG	IV. Mother plant herbarium WAG	V. Provenance
Sideroxyloideae				
Synsepalum stipulatum (Radlk.) Engl.	26	Bokdam 4612	Bokdam 4459	Zaire
Synsepalum stipulatum (Radlk.) Engl.	26	Bokdam 4591	Bokdam 4541	Zaire
Synsepalum dulcificum (Schum.) Baillon	26	Bokdam 4595	Bokdam 4586	Cameroon
Synsepalum subcordatum De Wild.	112	Teulings 16	Bokdam 4550	Zaire
Pachystela brevipes (Bak.) Engl.	28	Bokdam 4630	Bokdam 4507	Zaire
Pachystela bequartii De Wild.	28	Teulings 18	Bokdam 4565	Zaire
Donella pruniformis (Pierre ex Engl.) Aubrév. et Pellegr.	28	Bokdam 4611	Bokdam 4566	Zaire
Donella ubangiensis (De Wild.) Aubrév.	28	Bokdam 4618	J. J. F. E. de Wilde 8229-A	Cameroon
Donella spec.	28	Bokdam 4616	J. J. F. E. de Wilde 8220-C	Cameroon
Gambeya beguei (Aubrév. et Pellegr.) Aubrév. et Pellegr.	28	Teulings 15	Bokdam 4402	Zaire
Gambeya lacourtiana (De Wild.) Aubrév. et Pellegr.	28	Bokdam 4608	Bokdam 4549	Zaire
Gambeya perpulchra (Mildbr.) Aubrév. et Pellegr.	28	Bokdam 4610	Bokdam 4553	Zaire
Gambeya spec.	28	Bokdam 4617	J. J. F. E. de Wilde 8223	Cameroon
Omphalocarpiodeae				
Omphalocarpum procerum P. Beauv.	28	Bokdam 4577, Teulings 11 & 17	Bokdam 4421	Zaire
Omphalocarpum procerum P. Beauv.	28	Teulings 7	Bokdam 4382-A	Zaire
Omphalocarpum procerum P. Beauv.	28	Bokdam 4613	Bokdam 4564	Zaire
Omphalocarpum procerum P. Beauv.	28	Teulings 10, Bokdam 4575	Bokdam 4392	Zaire
Omphalocarpum lecomteanum Pierre ex Engler	28	Teulings 8 & 12	Bokdam 4312	Zaire
Omphalocarpum lecomteanum Pierre ex Engler	28	Bokdam 4590	Bokdam 4567	Zaire
Omphalocarpum spec. A	28	Bokdam 4589	J. J. F. E. de Wilde 7835	Cameroon
Tridesmostemon omphalocarpoides Engl.	26	Bokdam 4620 & 4631	J. J. F. E. de Wilde s.n.	Cameroon
Mimusopoideae				
Autranella congolensis (De Wild.) A. Chev.	26	Bokdam 4619	Bokdam 4457	Zaire
Tieghemella africana Pierre	26	Teulings 6, Bokdam 4578	Bokdam 4420	Zaire
Baillonella toxisperma Pierre –	24	Bokdam 4629	J. J. F. E. de Wilde 8373-A	Cameroon



Figures 1–3. Somatic chromosomes in root tip cells, 8-hydroxyquinoline pretreatment, orcein staining, phase-contrast, 2700 ×.
Figure 1. *Synsepalum stipulatum* 2n = 26 (Seedling Bokdam 4612 WAG).
Figure 2. *Gambeya beguei* 2n = 28 (Seedling Teulings 15 WAG).
Figure 3. *Gambeya lacourtiana* 2n = 28 (Seedling Bokdam 4608 WAG).



Figures 4–5. Somatic chromosomes in root tip cells, 8-hydroxyquinoline pretreatment, orcein staining, phase-contrast, 2700 ×.
FIGURE 4. *Omphalocarpum procerum* 2n = 28 (Seedling Teulings 11 WAG).
Figure 5. *Baillonella toxisperma* 2n = 24 (Seedling Bokdam 4629 WAG). The arrows indicate a pair of longer chromosomes.

of the chromosomes varies from about 0,7 to 2.5 μm. The relative length of the chromosomes within a given karyotype of a species appears to be fairly uniform and to vary gradually. One of the species, *Baillonella toxisperma*, however, seems to have within its chromosome complement a pair of larger chromosomes (*fig. 5*, arrows). As can be noted from the photographs, primary constrictions are generally not distinct. In those cases where these constrictions could be observed they appeared to be median to submedian. *Fig. 2* for example shows the karyotype of *Gambeya beguei* having (sub)-metacentric chromosomes.

4. DISCUSSION

It appears from literature that chromosome numbers of the *Sapotaceae* have been mainly determined so far from root tip materials. Two investigators (MEHRA & BAWA 1969 and MEHRA 1972), however, published some meiotic chromosome counts. Further it can be concluded that in only one (GADELLA

1970) out of thirteen publications, reference is made to herbarium specimens of the materials studied. Since there is much disagreement on the taxonomy of the *Sapotaceae* and hence nomenclatural confusion, the lack of reliable reference materials will seriously hamper the comparison of the cytological data of the earlier authors with those presented here, or published in the future. In respect to the somatic chromosome numbers found here, the numbers of 2n = 24, 26 and 28 have all been recorded in previous reports. The somatic number of 2n = 112 for *Synsepalum subcordatum* on the other hand is a new one. Within the first subfamily, the Sideroxyloideae, as distinguished by Aubréville, several species belonging to four genera have been analyzed. The first genus, *Synsepalum*, is represented by three species, *S. stipulatum*, *S. dulcificum* and *S. subcordatum*. The first two have 2n = 26 chromosomes, the latter 2n = 112, this number not being an exact multiple of 26. The next genus, *Pachystela*, is represented by two species, *P. brevipes* and *P. bequartii* for which in this investigation 2n = 28 was found. This number does not corroborate the number of 2n = 26 as given by MIÈGE(1954) for *P. brevipes*.

According to AUBRÉVILLE (1964), the next two genera, *Donella* and *Gambeya*, are distinct from *Chrysophyllum*, the latter being confined to the new world. Considering the epithets used by MIÈGE (1954) and MANGENOT & MANGENOT (1962) three species of *Donella* and *Gambeya*, which were analyzed here, have also been investigated by them. These authors assigned the three species correctly to *Chrysophyllum*, since *Donella* and *Gambeya* had not yet been proposed at that time. During the present investigation a chromosome number of 2n = 28 was found for the three species concerned, *Donella pruniformis*, *Gambeya beguei* (see *fig. 2*) and *Gambeya perpulchra*, whereas the aforecited authors found 2n = 26. The remaining two species of this group, *Donella ubangiensis* and *Gambeya lacourtiana (fig. 3)* also have 2n = 28 chromosomes. Earlier TJIO (1948) and also MIÈGE (1954) found 2n = 26 chromosomes for *Chrysophyllum cainito*, an American species. Although the evidence is scanty it appears that species of *Chrysophyllum* have 2n = 26 chromosomes (or multiples of that number, as 2n = 52 was recorded for *C. oliviforme* by Tjio too), whereas the species of *Donella* and *Gambeya* have 2n = 28 chromosomes. The cytological findings presented here seem to confirm the opinion of Aubréville that African species formerly considered to belong to *Chrysophyllum* should be placed in separate genera. For the seven seedling samples, comprising four species of *Omphalocarpum*, a chromosome number of 2n = 28 was found consistently. This observation is again not in accordance with the results of earlier authors who published 2n = 26 for some other species of *Omphalocarpum* (MIÈGE 1954, MANGENOT & MANGENOT 1957 & 1962 and GADELLA 1970). The present finding of 2n = 28 chromosomes is nevertheless clearly demonstrated by the example in *fig. 4* presenting the karyotype of *Omphalocarpum procerum*. However, the species *Tridesmostemon omphalocarpoides* which in the system of AUBRÉVILLE (1964) is assigned to the same subfamily has 2n = 26 chromosomes. The remaining three species investigated here all belong to the subfamily Mimusopoideae. *Autranella congolensis* as well as

Tieghemella africana have both 2n = 26 chromosomes. Chromosome records for these genera have not been published so far. The last species *Baillonella toxisperma* has 2n = 24 chromosomes and is related to *Butyrospermum parkii* (AUBRÉVILLE 1964). For the latter species MIÈGE (1954) also recorded 2n = 24 chromosomes, so it can be concluded that the relationship as assumed by Aubréville is supported by the finding of similar chromosome numbers.

Thus it can be concluded that the chromosome numbers as given in the present publication do not justify drawing conclusions as to whether the arrangement of the various taxa into the subfamilies as recognized by Aubréville is correct or not. Moreover, when the cytological data of the earlier authors are taken into consideration as well, it would be even more hazardous to draw any conclusions. Further it is evident that reliable conclusions in respect to karyotype morphology cannot be drawn either. Some cautious and preliminary conclusions concerning the evolution of chromosome numbers in the African Sapotaceae, however, can be made.

BOKDAM (1977) points towards the importance of using characters which provide a reliable base for drawing valid conclusions concerning the phylogeny of a plant group. He therefore distinguishes within the Sapotaceae several types of seedlings based on the stage of endosperm and cotyledon development within the seeds and the presence and functioning of these organs during subsequent seedling development. The species which have been analyzed here can be divided into two groups according to seedling type. The first group, having a more primitive seedling-type comprises the genera *Donella*, *Gambeya* and *Omphalocarpum*; the second group, having a more advanced seedling-type, includes the genera *Synsepalum*, *Autranella*, *Tieghemella* and *Baillonella*. The species of the first group all have 2n = 28 chromosomes, the species of the latter group have lower chromosome numbers of 2n = 26 and 2n = 24 respectively, irrespective of the polyploid species *Synsepalum subcordatum* (2n = 112).

Assuming that there exists a correlation between primitiveness of seedling type and chromosome number, the hypothesis can be forwarded that 2n = 28 presents a primitive condition and the lower numbers 2n = 26 and 2n = 24 a more advanced condition in the Sapotaceae. Indeed *Baillonella toxisperma* having 2n = 24 chromosomes possesses a particularly advanced type of seedling. As was shown earlier, this species has two relatively long chromosomes within its karyotype. These long chromosomes may represent the product of translocation processes leading to the reduction of chromosome number which may have occurred during evolution. Similar evolutionary processes have been analyzed by BABCOCK (1947) in the well known example of *Crepis* as referred to by STEBBINS (1971). TAKHTAJAN (1969) and HUTCHINSON (1969) agree in their respective works that the Sapotaceae should be considered as an advanced group closely related to the Ebenaceae. Regarding the latter family it appears from the chromosome numbers enumerated by FEDEROV (1969) that the basic chromosome number of the Ebenaceae *(Diospyros)* is x = 15, the majority of the species having 2n = 30 chromosomes and some having 2n = 60 or

2n = 90 chromosomes. As far as can be gathered from literature (DE LA MENSBRUGE 1966) the seedlings of *Diospyros* can be classified as primitive, having more or less foliaceous cotyledons. From the preceding evidence it thus may be concluded that within the evolutionary branch Ebenaceae-Sapotaceae of the Ebenales there appears to be a general tendency of decreasing chromosome numbers which is correlated with increasing specialisation of certain morphological characters. It should be remarked though that already one exception with respect to the forementioned correlation is known: the lowest chromosome number found within the Sapotaceae is 2n = 20, for *Argania spinosa* (MIÈGE 1954), a species having a less advanced seedling type. Apart from its seedling type, however, this species is distributed in arid regions which are ecologically different from the area in which the other Sapotaceae occur. It exhibits many advanced characters. Further the two investigated species of *Pachystela* for which the putative primitive chromosome number 2n = 28 was found, both have advanced seedling types. According to Bokdam (personal communication) these rainforest trees nevertheless show many primitive characters. Also *Tridesmostemon omphalocarpoides* (2n = 26) does not meet the supposed correlation, since its characters, including seedling type, are, as far as known, primitive.

As far as the findings up to the present indicate, an increase in chromosome number by polyploidisation seems to have occurred only in groups having advanced seedling types. This pertains to the species *Chrysophyllum oliviforme* (2n = 52, TJIO 1948), *Gluena ivorensis* (2n = 48, MANGENOT & MANGENOT 1962) and *Planchonella sandvicensis* (2n = 48, SKOTTSBERG 1955). The species *Synsepalum subcordatum* analyzed here and found to have 2n = 112 chromosomes *(table 1)* is not only an exceptional one regarding its degree of ploidy but also of its growth habit. Whereas the other species of *Synsepalum* are shrubs, *S. subcordatum* is a dominated rainforest tree reaching heights of about 20 meters. A solution to the problem why this species has 2n = 112 instead of 2n = 104 chromosomes (the exact multiple of 2n = 26), the number found so far for the other species of *Synsepalum*, could be found in the supposition that some primitive ancester of *Synsepalum* had 2n = 28 chromosomes. One line of evolution could have followed the reduction of chromosome number, while another line developed by polyploidisation.

According to MANGENOT & MANGENOT (1962) the flora of the dense tropical African forest in which the species here analyzed occur, is of cretaceous, hence ancient origin. These authors therefore designate the species having for example 2n = 28 chromosomes as palaeopolyploids. This term was introduced by FAVARGER (1960) in order to distinguish a group of plants with apparently polyploid chromosome numbers, from which the diploid ancestors are extinct. Considering the forementioned phylogenetic link of the Ebenaceae and the Sapotaceae, the frequent finding of 2n = 30 in the former family and the observation of chromosome numbers of a similar level in the Sapotaceae, it appears more appropriate to characterize these species as, possibly secundary, diploids. The species having higher chromosome levels, such as *Synsepalum*

subcordatum (2n = 112), could be designated as palaeopolyploids. The possibility that some of these polyploid species might have to be designated as mesopolyploids appears to be unlikely, but may not be ruled out, at least not before all taxa have been thoroughly analyzed. Such an analysis which should include several samples of each species in its distributional area, may again affect some of the conclusions presented here.

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