Taylor & Francis
Taylor & Francis Group

Caryologia

International Journal of Cytology, Cytosystematics and Cytogenetics

ISSN: 0008-7114 (Print) 2165-5391 (Online) Journal homepage: https://www.tandfonline.com/loi/tcar20

The Somatic and Meiotic Chromosomes of *Kalanhoe Pinnata* (Lamark) Persoon (Syn. *Bryophyllum Calycinum*, Salisbury)

(with 13 figures)

I. Banerji & S. Sen

To cite this article: I. Banerji & S. Sen (1956) The Somatic and Meiotic Chromosomes of *Kalanhoe Pinnata* (Lamark) Persoon (Syn. *Bryophyllum Calycinum*, Salisbury), Caryologia, 8:2, 195-204, DOI: 10.1080/00087114.1956.10797558

To link to this article: https://doi.org/10.1080/00087114.1956.10797558

THE SOMATIC AND MEIOTIC CHROMOSOMES OF *KALANHOE PINNATA* (LAMARK) PERSOON (SYN. *BRYOPHYLLUM CALYCINUM*, SALISBURY)

by

I. BANERJI and S. SEN

Department of Botany, Calcutta University, India.

(with 13 figures)

Received: 1st September 1955.

There is considerable diversity of opinion regarding the nomenclature of the genus *Bryophyllum* belonging to the family *Crassulaceae*. SALISBURY (1806) first established this genus and gave the name *Bryophyllum calycinum* to *Kalanchoe pinnata* of PERSOON (1805) (*Sedum madagascericum* of CLUSIUS 1605) on account of the specialized character of the calyx. BERGER (1930) retains the genus *Bryophyllum* with about twenty species, placing it together with *Kalanchoe* and *Kitchingia* under the subfamily *Kalanchoideae*. HAMET (1907, 1908, 1915, 1916) on the other hand, included the genera *Bryophyllum* and *Kitchingia* within the genus *Kalanchoe*. This view has since been supported by many other taxonomists. Embryological and cytological studies by MAURITZON (1933) and BALDWIN (1938) have added evidence in support of the regrouping of the three genera into a single genus *Kalanchoe*.

In *Crassulaceae* TAYLOR (1926) first gave an account of the somatic chromosomes of *Bryophyllum calycinum*. This was followed by the work of TOYOHUKU (1935), BALDWIN (1938) and UHL (1948). Baldwin determined the chromosome numbers of a large number of species of the genus *Kalanchoe*, but gives no information regarding the morphology of the somatic chromosomes.

The importance of the study of chromosome morphology in elucidating phylogenetic relationship in plants has been clearly indicated in the classical works of TAYLOR (1924, 1925*a*, *b*, *c*) on *Gasteria*, *Howorthia* and *Aloe*, followed by LONGLEY (1941), GATES (1950), LÖVE (1953) and many others. The present investigation was undertaken primarily with the hope that a detailed study of the chromosome morphology and chromosome behaviour of the different members of the large aggregated genus *Kalanchoe* might indicate its relationship with other members of the family.

Materials and Methods

The material has been obtained from plants grown in the College garden. Root tips for the study of mitosis were obtained from plants, especially grown in pots containing a high percentage of sand. For the study of meiosis and mitosis both sectional and smear methods were employed. Flower buds and anther smears were fixed in a mixture of Belling's modified Navaschin solution and 0.002M oxyquinoline in the proportion 1 : 1 : 1 (Sharma and Ghosh 1951). For the study of somatic chromosomes Platinic chloride and Formalin 1 : 1 gave the best result. Sections were cut 12μ thick and crystal violet-Iodine was used for staining. A premordanting in Chromic acid (1% aqueous) was necessary to bring out the best result. Clear metaphase plates were obtained when a pretreatment in paradichlorobenzene (Meyer 1945; Conagain 1951; Sharma and Mookerjea 1955) at about 34° C for twenty-five minutes, and hydrolysis in a mixture of 2% Aceto orcein : Normal HCl in the proportion 9 : 1 was followed by preparation of squash of root tip meristem in 1% Aceto orcein.

Observations

I. *The somatic chromosomes.* The somatic number of chromosomes was found to be 40 (Fig. 1). This confirms the previous determinations of Toyohuku (1935), Baldwin (1938) and Uhl (1948). It also incidentally indicates that there are possibly no ecotypes in *K. pinnata* as the materials previously studied were from widely separated regions.

The chromosomes fall under four categories on the basis of their relative lengths :

1) Four pairs of long chromosomes varying in length from 3.3 to 2.4 microns.

2) Nine pairs of medium sized chromosomes varying in length from 2.2 to 1.7 microns.

3) Four pairs of short chromosomes varying in length from 1.5 to 1.2 microns.

4) Three pairs of very short chromosomes varying in length from 1 to 0.8 micron.

An idiogramatic representation of the chromosomes reveals the following types (Fig. 2) :

Type A — One pair of long chromosomes with subterminal primary constrictions and secondary constriction on the longer arm.

Type B — One pair of long chromosomes with subterminal primary constrictions.

Types C and D — Two pairs of long chromosome with slight size difference and submedian primary constrictions.

Type E — One pair of medium-sized chromosomes with subterminal primary constrictions.

Type F — One pair of medium-sized chromosomes with submedian primary constrictions.

Type G — One pair of medium-sized chromosomes with subterminal primary constrictions.

Type H — Three pairs of medium-sized chromosome, equal in length, with submedian primary constrictions. Length of the longer and shorter arm identical in the three pairs.

Types I and J — Two pairs of medium-sized chromosome with slight size difference of types I, J and median primary constrictions.

Type K — One pair of medium-sized chromosomes with subterminal primary constrictions.

Type L — Two pairs of short chromosome, identical in morphology with median primary constrictions.

Type M — One pair of short chromosomes with median primary constrictions.

Type N — One pair of short chromosomes with submedian primary constrictions.

Types O, P and Q — Three pairs of very short chromosome with slight size difference and median primary constrictions.

II. *The meiotic chromosomes.* The behaviour of the meiotic chromosomes has been followed from diakinesis onwards. At diakinesis the twenty bivalent (Fig. 3) chromosomes are more or less globular in form and their bivalent nature is apparent. Interbivalent connections are observed during diakinesis (Fig. 4). The presence of interbivalent connections or secondary association of groups of bivalents is also evident during prometaphase stage of meiosis (Fig. 5).

During metaphase I, twenty clear bivalents have been observed (Fig. 9). The bivalents align themselves regularly in the equatorial region (Fig. 8). Occurrence of secondary association between groups of bivalent has been observed in clear metaphase plates (Figs. 6, 7, 10 and 11). An analysis of the different types of secondary association as observed in metaphase I is presented in Table I below :

TABLE I

No. of times observed out of 100 clear plates	No. of bivalents in association				Configurations	Units of groupings
	1	2	3	4		
5	17	—	1	—	1 (3) + 17 (1)	18
4	15	1	1	—	1 (3) + 1 (2) + 15 (1)	17
8	18	1	—	—	1 (2) + 18 (1)	19
6	16	2	—	—	2 (2) + 16 (1)	18
3	14	3	—	—	3 (2) + 14 (1)	17

The maximum association in this case is 1 (3) + 1 (2) + 15 (1) of bivalents (Fig. 10). The association of bivalents that occurs in maximum frequency is 1 (2) + 18 (1) of bivalents (Fig. 11).

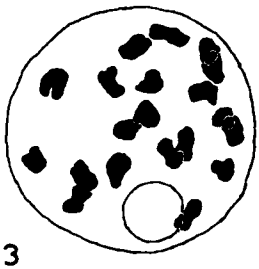
Segregation of the bivalents during anaphase is generally normal. During the interkinetic stage the chromosomes retain their globular form (Fig. 12), the nucleolus reappears and nuclear membranes delimits the two daughter nuclei.

Twenty univalents are found to be present during metaphase II in each of the two nuclei produced as a result of the first division (Fig. 13). On the completion of the second division, which is normal, four daughter nuclei are organized which are arranged mostly in a tetrahedral form. Cytokinesis takes place by furrowing.

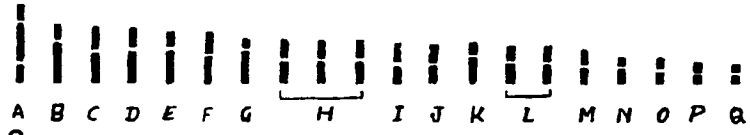
It should be mentioned in this connection that small spherical bodies, distributed at random in the cytoplasm, appear during metaphase II (Fig. 13) persisting upto the telophase. They stain brightly with crystal-violet, but are Feulgen negative.

Fig. 1. Somatic metaphase plate of *Kalanchoe pinnata* showing 40 chromosomes; × 2,900. — Fig. 2. Idiogram of *Kalanchoe pinnata*; × 2,900. — Fig. 3. Diakinesis showing 20 clear bivalents; × 3,900. — Fig. 4. Diakinesis showing interbivalent connection; × 3,900. — Fig. 5. Prometaphase showing secondary association of bivalents, 3 (2) + 14 (1); × 3,900. — Figs. 6, 7, 10 and 11 Metaphase I showing types of secondary association of bivalents. — Fig. 6. 1 (3) + 17 (1); (× 3,400). — Fig. 7. 2 (2) + 16 (1); (× 3,400). — Fig. 10. 1 (3) + 1 (2) + 15 (1); (× 3,900) and Fig. 11. 1 (2) + 18 (1); (× 3,400). — Fig. 8. Side view metaphase I. Three pairs of very short chromosomes shaded. × 3,400. — Fig. 9. Metaphase I showing 20 clear bivalents; × 3,400. — Fig. 12. Interkinesis showing the two daughter nuclei with 20 univalents and a nucleolus in one of the nucleus; × 3,400. — Fig. 13. Metaphase II showing 20 univalents. Extranuclear bodies (not shaded) distributed at random in the cytoplasm; × 3,400.

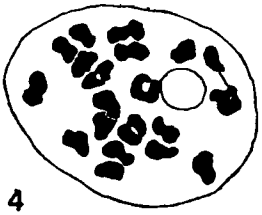

1


3


5

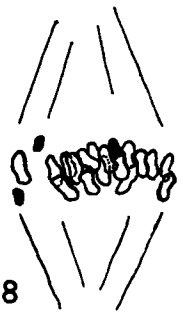


2


4


6

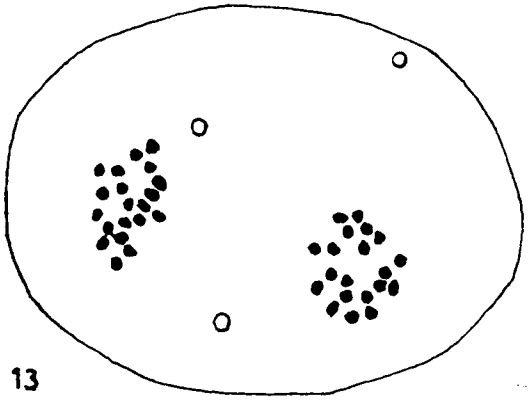

7

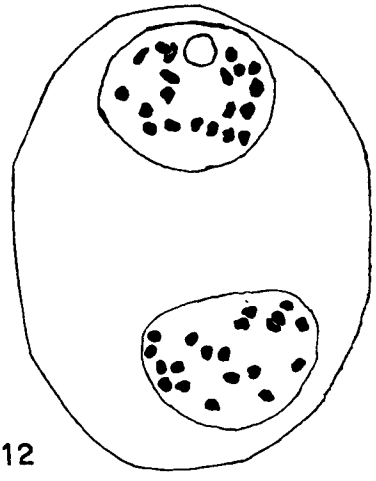

8


9


10


11


13


12

Discussion

Study of meiosis reveals the presence of secondary association between the bivalents. Secondary association has also been noted by Baldwin (1938) to occur at first and second division metaphase in such a way that only 18 to 19 units could be counted. Present writers, however, have observed 17, 18 and 19 as units of groupings. Maximum association in this case being 1 (3) + 1 (2) + 15 (1), the basic number of chromosomes should thus be 17.

This coroborates the observation of Baldwin for the genus *Kalanchoe*. According to him, « Each of the chromosome numbers precisely determined for *Kalanchoe* is divisible by 17, 18 or 20 and of these "basic numbers" 17 is taken to be primary ». But Uhl (1948) concludes 18 to be the basic number in *Kalanchoidae*, suggesting that this represents tetraploidy from *Cotyledon*-like ancestors with a basic number of 9. However, the possibility of 17 as the basic number of this group was not overlooked by him.

It may be mentioned here that though the validity of the theory of secondary association as a means of finding out the basic number of chromosomes has been questioned by Thomas and Revell (1946), it has been shown by Sharma (1955) that the fusion of heterochromatic segments, which is responsible for interbivalent connections and secondary groupings are specific in nature, occurring between distantly related chromosomes. Fusion of ancestral type of heterochromatin has been suggested to occur on account of the least change undergone by these regions of the chromosomes during evolution, as they are to some extent genetically inert. Due to the operation of several factors within the nucleus the chances of all these distantly related chromosomes in a complement to associate specifically are remote, yet the maximum association whenever encountered should be taken into consideration.

Spherical bodies have been noted to be irregularly distributed in the cytoplasm during the second division of meiosis. The negative reaction of these bodies to Feulgen stain strongly indicates that they do not contain chromatin or chromatin-like substances (Sparrow and Hammond 1947). Similar Feulgen negative bodies were found by Pollister and Lavin (1944) in *Viviparus malleatus*. These bodies were considered to be conversion products of chromatin. The chromonucleic acid of the chromosomes have been changed to plasmonucleic acid in the cytoplasmic bodies leaving the nucleotide groupings intact. This step of conversion

forms the basis of the concept of gene action proposed by SPIEGELMAN and KAMEN (1946) and as such, these bodies may be considered to play a useful role in cellular metabolism. However, it may be pointed out that virus and other infections may cause such unusual cellular inclusions (SCHEFFIELD, 1941 and LUDFORD 1942). In the present investigation fresh and healthy materials were taken to avoid the influence of any type of infection as far as it was practicable by macroscopic examination.

Working on the somatic chromosomes of *Bryophyllum calycinum*, TAYLOR (1926) states as follows: « the chromosomes are very small, somewhat pointed curved and unlike in size at the equatorial stage ». BALDWIN (1938) in his account of the chromosome numbers of a large number of species of *Kalanchoe* states that « ... the chromosomes are very small throughout the genus. For this reason, although morphological differences are often comparatively great within a specific complement, such characteristics were not constantly recognized ».

Though the chromosomes are very small, nevertheless, it has been possible to study their morphology from clear somatic metaphase plates. Karyotype analysis reveals that the chromosome complement of *Kalanchoe pinnata* can be classified into 17 types (Fig. 2), taking size and location of primary and secondary constrictions into account. This indicates that the plant is a secondarily balanced polyploid.

UHL (1948) states that in the karyotype of *Kalanchoe pinnata*, « .... a few of the chromosomes are markedly smaller than the rest and a better interpretation of their number may be n = 17 + 3, 2n = 34 + 6, the smallest chromosomes possibly being merely heterochromatic fragments ». However, there is no evidence in his paper or in the present investigation on the behaviour of the chromosomes, supporting the fragmentary nature of these smallest chromosomes (Types O, P and Q, Fig. 2). The fragment-like appearance of these chromosomes in the somatic plate of his figure (Fig. 23, p. 696; UHL 1948) may be due to the influence of fixatives which were not able to bring out clearly even the primary constrictions of the longer chromosomes.

The present observation shows the repeated occurrence of particular types of chromosome (Types H and L; Fig. 2) in the complement. This indicates that duplication of some of the members of the ancestral set has taken place and evidently the plant appears to be a secondarily balanced polyploid.

SUMMARY

1. The somatic chromosome number of *Kalanchoe pinnata* is 40 and the meiotic 20.

2. The morphology of the somatic chromosomes has been studied. They are of seventeen different types and fall into four categories, viz., long, medium, short and very short, according to size. The pair of satellited chromosomes belongs to the category of long chromosomes.

3. The meiotic chromosomes are all alike in form and structure and are globular in nature. Secondary association of the bivalents has been noted. Maximum association is found to be 1 (3) + 1 (2) + 15 (1), hence the basic number is 17.

4. The presence of extranuclear bodies in the cytoplasm at the metaphase of the second division is a characteristic feature. These bodies are Feulgen negative.

REFERENCES

1. Baldwin J. T., 1938. — *Kalanchoe: The genus and its chromosomes.* Amer. Jour. Bot., *25:* 572-579.
2. *Berger A., 0000. — *Crassulaceae.* Die Natürlichen Pflanzenfamilien. *18a:* 352-483, Leipzig. W. Engelmann.
3. Conagain C. H. T., 1951. — *Paradichlorobenzene for chromosome counts.* Stain. Tech., *26:* 274.
4. Gates R. R., 1950. — *Genetics and taxonomy of the cultivated Brassicas and their wild relatives.* Bull. Torr. Bot. Club, *77:* No. I, 19-28.
5. *Hamet R., 1907. — *Monographie du genre Kalanchoe.* Bull. Herb. Biossier, 7 *(Sér. 2):* 869-900.
6. *—, 1907. — *Monographie du genre Kalanchoe.* Bull. Her. Biossier, 8 *(Sér. 2):* 17-48.
7. *—, 1908. — *Monographie du genre Kalanchoe.* Bull. Her. Boissier, 8 *(Sér. 2):* 17-48.
8. *—, 1915. — *Sur Quelques Kalanchoe de la Flore Malgoche.* Ann. Mus. Coll. Marsille, *3 (Sér. 3):* 123-164.
9. *—, 1916. — *Sur Quelques Crassulacees Nonvelles.* Jour. Bot., *54:* Suppl. 1-33.
10. Longley A. E., 1941. — *Chromosome morphology in maize and its relatives.* Bot. Rev., *7:* 263-289.
11. Löve A., 1953. — *Subarctic Polyploidy.* Hereditus, *39:* 113-123.
12. Ludford R. J., 1942. — *Pathological aspects of cytology.* Chapter on Cytology and Cell physiology. Oxford Univ. Press, London, 226-260.

13. *Mauritzon J., 1933. — *Studien über die Embryologie der Familien Crassulaceae und Saxifragaceae.* Lund. Håken Ohlssons Buchdrucherie.

14. Meyer J. R., 1945. — *Prefixing with paradichlorobenzene to facilitate chromosome study.* Stain. Tech., *20:* 121-125.

15. *Persoon C. H., 1805. — *Synopsis Plantarum. I.* Parisus Lutetiorum, apud C. F. Camerum.

16. Pollister A. W. and G. I. Lavin, 1944. — *Possible conversion of chromonucleic acid during atypical spermiogenesis of Viviparus malleatus Reeve.* Anat. Rec., *89:* 557-558.

17. Sharma A. K. and (Miss) Chitra Ghosh, 1951. — *Oxyquinoline: its possibilities in other cytological procedures.* Sci. and Cult., *16:* 528-530.

18. Sharma A. K. and Mookerjea A., 1955. — *Paradichlorobenzene and other chemicals in chromosome work.* Stain. Tech., *30:* 1-7.

19. Sharma A. K., 1955. — *An investigation into the cytology of some of the members of Commelinaceae and its bearing on the manifestation of their interrelationship.* Genetica, *27:* 323-363.

20. Sheffield F. M. L., 1941. — *The cytoplasmic and nuclear inclusions associated with severe etch virus.* Jour. Roy. Microsc. Soc., London, Ser. III, *61:* 30-45.

21. Sparrow A. H. and Hammond M. R., 1947. — *Cytological evidence for the transfer of desoxyribose nucleic acid from nucleus to cytoplasm in certain plant cells.* Amer. Jour. Bot., *34:* 439-445.

22. Spiegelman S. and Kamen M. D., 1946. — *Genes and nucleoproliens in the synthesis of enzymes.* Science, *104:* 581-584.

23. Taylor W. R., 1924. — *Cytological studies on Gasteria. I: Chromosome shape and Individuality.* Amer. Jour. Bot., *11:* 51-59.

24. —, 1925*a*. — *The chromosome morphology of Velthemia, Allium, and Cyrtanthus.* Amer. Jour. Bot., *12:* 104-115.

25. —, 1925*b*. — *Cytological studies on Gasteria. II: Comparison of the chromosomes of Gasteria, Aloe and Howorthia.* Amer. Jour. Bot., *12:* 219-223.

26. —, 1925*c*. — *Chromosome constrictions as distinguishing characteristics in plants.* Amer. Jour. Bot., *12:* 239-243.

27. Taylor W. R., 1926. — *Chromosome morphology in Fritillaria Alstroemaria, Silphium and other genera.* Amer. Jour. Bot., *13:* 179-193.

28. *Toyohuku T., 1935. — *Chromosome numbers in Sedum.* Japanese Jour. Genetics, *11:* 316-317.

29. Uhl C. H., 1948. — *Cytotaxonomic studies in the subfamilies Crassuloideae, Kalanchoideae and Cotyledonoideae of the family Crassulaceae.* Amer. Jour. Bot., *35:* 695-706.

* Not seen in original.

RIASSUNTO

In *Kalanchoa pinnata* il numero cromosomico è 2n = 40, e quello meiotico è n = 20.

I cromosomi presentano 17 differenti tipi morfologici, che si raggruppano in quattro categorie distinte per le dimensioni, cioè lunghi, medi, corti e cortissimi. Un paio di cromosomi satelliferi appartiene alla categoria dei lunghi.

Alla meiosi è stata notata l'associazione secondaria dei gemini. La massima associazione trovata è 1 (3) + 1 (2) + 15 (1), che porta ad individuare un numero base x = 17.

Nel citoplasma durante la seconda divisione meiotica si osservano corpi estranucleari feulgen-negativi.