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(with 6 figures)

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# A CYTOLOGICAL INVESTIGATION OF THE DIFFERENT SPECIES OF *SANSEVIERIA* WITH THE AID OF THE IMPROVED TECHNIQUE

By

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(with 6 figures)

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## INTRODUCTION

The genus *Sansevieria* is represented in India by about ten species mainly growing in the tropical region. The plant is generally cultivated in the gardens for ornamentation in the leaves. Like most of the allied members it flowers twice in the year, once during the onset of monsoon and then during winter. The genus is interesting from taxonomic stand point. In the older classification of BENTHAM and HOOKER (1862-1883) its place was assigned under Haemodoraceae preceding Iridaceae in position. Later ENGLER and PRANTL (1929) included this under Liliaceae in association with *Dracaena*, *Yucca* and other genera. The genus has been placed in the latest classification of HUTCHINSON (1934) under Agavaceae.

In view of the debated systematic position of the genus as well as due to its ready availability, a cytological study was deemed highly desirable. In view of the existing knowledge of the chromosome complements of Liliaceous and Agavaceous members (SATO 1942), it was thought that a study of its karyotype might provide a clue to its affinity.

## MATERIALS AND METHODS

Three different types of the genus *Sansevieria* were utilised in the present investigation, all of which are cultivated for ornamental purposes. They mainly differ in the nature of the leaf, in its markings, texture etc. The characteristic features may be summarised as follows :

Type A — Plants with long leaves, ensiform above and boat shaped at the basal portion and thin in texture. The colour of the leaf is deep green with transverse markings at regular intervals.

Type B — Plants with comparatively long ensiform leaves, lamina throughout flat, characteristically thick, light green with white transverse markings.

Type C — Plants with very long ensiform leaves, longest in the series, lamina throughout flat, characteristically thick, light green with transverse deep green streak, margin being yellow in colour.

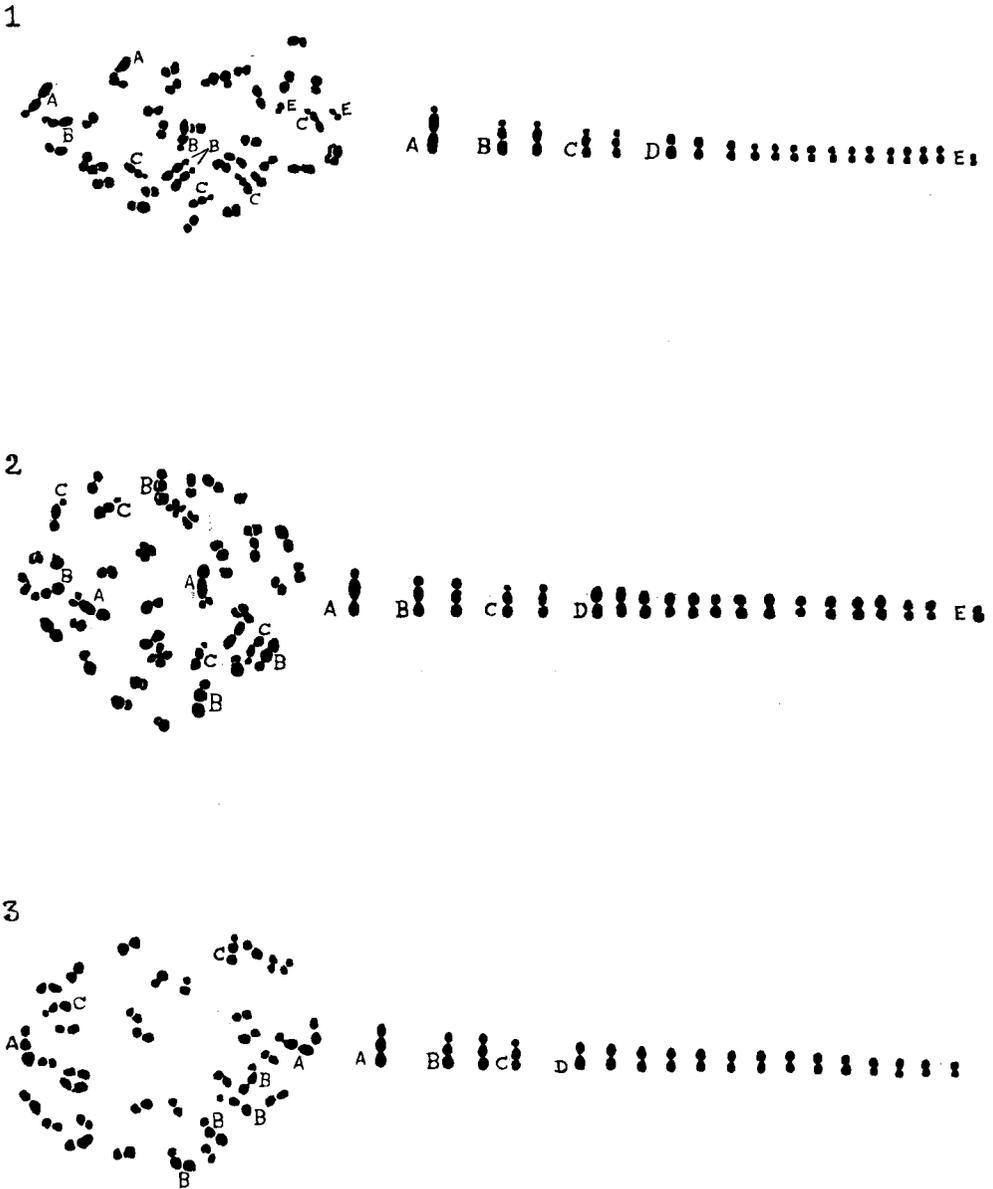
The first type has been identified as *S. cylindrica*. The latter two types were identified from the Herbarium, Indian Botanic Garden, Sibpur, as *S. nilotica*. But as the two types B and C show much difference in their morphological appearance and chromosome number they have been dealt with separately.

For the study of somatic and meiotic stages healthy root-tips and flower buds were collected from plants growing in pots in the college compound.

Smear preparation of flower buds were made after fixing the same in Nawaschin's fluid between 10 A. M. and 1 P. M. After smearing, the materials were kept in the fixing fluid for nearly 3 hrs. followed by overnight washing in running water and subsequent staining after Newton's crystal violet-iodine technique.

For the study of somatic chromosomes the use of both Coumarin (SHARMA and BAL 1953) and Paradichlorobenzene (SHARMA and MOOKERJEA 1955) were taken advantage of. Healthy root-tips, immediately after cutting were kept in a mixture containing saturated aqueous solution of Paradichlorobenzene and Coumarin in equal proportion for 3 hrs. at a temperature of 12° C-16° C. Subsequently the materials were heated for 5 to 6 seconds over a flame in Orcein/HCl mixture containing 2% aceto orcein and N.HCl in the proportion of 9 : 1. Too much heating was avoided as that has been found to cause fragmentation (SHARMA and ROY 1955). The procedure was followed by smearing in 1% aceto orcein on a dry slide and applying uniform and heavy pressure with the help of a filter put on the cover glass. After proper sealing, the preparation containing well spread metaphase plate could be kept as such for a number of days.

Figures were drawn with the help of a drawing prism using a compensating eye piece  $\times 20$ , an 1.3 apochromat objective and an aplanatic condenser 1.4 N. A. at a table magnification of  $\times 2300$  approximately.



Structure of chromosome of *Sansevieria* sp.

Figs. 1-3. — Somatic metaphase plates and the idiograms of *S. cylindrica*, *S. nilotica* and another variety of the latter showing  $2n = 40, 40$  and  $36$  chromosomes respectively.

Observations : *S. cylindrica*.

The diploid chromosome number of the species as could be seen from metaphase plate is 40, corroborating the previous report of PATEL and NARAYANA (1937). The complement is characterised by short chromosomes, size difference between most of them being not so marked. On the basis of the size, as well as the position of primary and secondary constriction the chromosomes can be classified under the following categories :

Type A — One pair of comparatively long chromosomes with nearly submedian primary constriction and a secondary constriction, median to the long arm.

Type B — Two pairs of chromosomes similar to type A in morphology, but shorter in size.

Type C — Two pairs of short chromosomes with nearly median primary constriction and a secondary constriction at the end of the comparatively long arm.

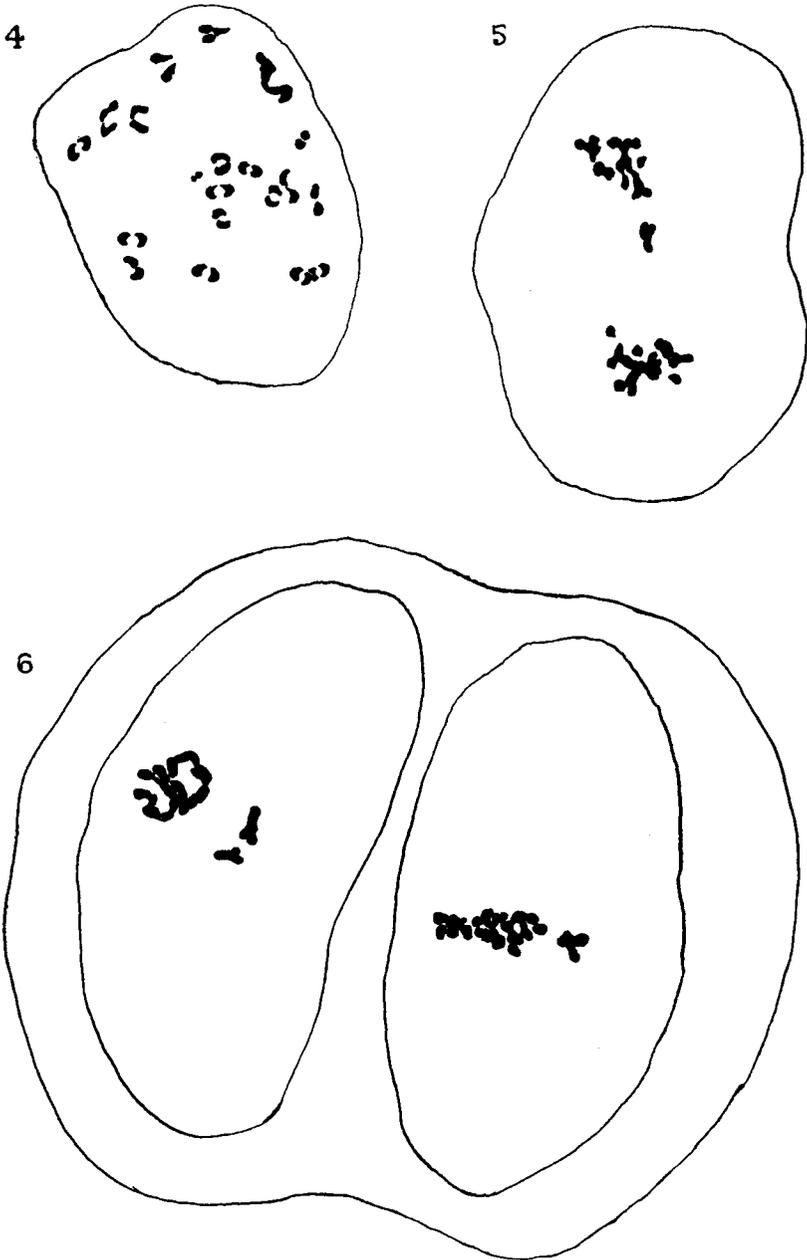
Type D — Fourteen pairs of short chromosomes with nearly median to submedian primary constrictions.

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

In all, therefore, of forty chromosomes in the complement, ten have been found to be provided with secondary constrictions. Even in the absence of any data on the chromosome-nucleolus relationship in this genus, in view of the enormous evidences gathered from widely different groups of plants (JACOB 1940; GATES 1942; BHADURI and SHARMA 1946; BHADURI and BOSE 1947; SHARMA 1947; BHADURI and KAR 1948; CHAKRAVORTI 1948), it may be suggested that all these constrictions are nucleolar.

Meiotic chromosomes so far studied show regular behaviour. Forty chromosomes forming clear twenty bivalents could be observed in the first meiotic division. The bivalents are manifested in various, configurations due to different degrees of terminalisation of the chiasmata.

In addition to normal bivalent formation and subsequent segregation, cases of abnormalities in chromosome division could be noted in a few percent of cases. Pollen mother cells were observed with a bivalent lagging at the equator during anaphase. Some of the second meiotic nuclei reveal certain interesting peculiarities. In these cases, a single chromosome was found to lie outside the metaphase plate in both the nuclei of the pollen mother cells.



Behaviour of chromosomes of *S. cylindrica*.

Figs. 4 and 5. — Diakinesis stage showing 20 bivalents and anaphase I having a lagging bivalent respectively.

Fig. 6. — Second meiotic metaphase in polar and side views showing the staying apart of two chromosomes.

The diploid chromosome number of the type B is 36. In chromosome morphology, it is similar to those of the other two types, though one pair of chromosomes with secondary constriction and the « E » pair are missing.

The diploid number of chromosome in case of type « C » is 40, and the chromosome complements in their morphology exactly correspond to the type « A ».

#### DISCUSSION

It needs no emphasis that the chromosomes of most of the Lilies, the Amaryllids and the Agaves are well known due to the sustained researches of a number of workers in different centres (FERNANDEZ 1931; SATO 1942; MATSUURA 1949; SHARMA and BAL 1954; SHARMA and BHATTACHARYYA 1954; SHARMA and GHOSH 1954). Most of the members of Liliaceae are characterized by having long chromosomes showing not much of size differences within their complements. The types are well exemplified in *Lilium*, *Trillium* and *Allium* etc. Considerable foreshortening of the chromosome arms is a feature characteristic of such chromosomes. Amaryllids on the other hand show considerable shorter chromosomes in general, much thinner in width having constriction regions in various positions, the types being best represented in *Crinum*, *Pancratium*, *Hymenocallis* etc. Certain genera included within this family also show to some extent size difference in the complement, the best example being the genus *Haemanthus*. But in this case most of the chromosomes are acrocentric.

The family Agavaceae on the other hand present a remarkable admixture of extremely long and very short chromosomes in the complement. Their arrangements at the equator in most cases are characteristic, long chromosomes being located at the periphery and the shorter ones towards the centre.

The chromosomes of *Sansevieria* reported in the present paper do not evidently fall under any of these categories. The complement is characterised by high number of short chromosomes, size difference within the complements being not marked. The two species with three forms investigated here may be regarded as forming a distinct homogeneous assemblage representing a single evolutionary line. The 36 chromosomed type is also related to the other two in its morphology. The type finds

parallel to a little extent with the chromosomes of the Irids investigated so far. In the preceding part of the discussion it has been pointed out that none of the allied families show such complement among their members. Even if one takes into account the possibility of the presence of diverse types of complements within a particular family, such wide differences cannot at all be ignored. Moreover, the fact that the Lillies, the Amaryllids and the Agaves etc. are quite homogeneous assemblages as far as their karyotypes are concerned, goes against the inclusion of *Sansevieria* in association with the Lillies or the members of Agavaceae. In full consideration of these relevant facts in taxonomy, the inclusion of *Sansevieria* in a separate family co-ordinate in rank with Liliaceae and Agavaceae seems highly reasonable. The justification of its being regarded as a member of the family Haemodoreceae would await further researches on other Haemodoreceous genera.

The high chromosome number, viz., forty in the somatic complement apparently suggests its polyploid constitution. But the meiotic data gathered in the present investigation do not provide evidences supporting such a speculation. Neither multivalent formation, a clear evidence of autopolyploidy, nor secondary association of bivalents indicating their allopolyploid or precisely, amphidiploid constitution have been obtained.

The karyotype reveals on the other hand duplication of chromosome in the set. It is well known that multivalent formation in polyploidy is dependent to a large extent on the frequency of chiasma formation in bivalents of diploids. Chiasma and as such their frequency further, is directly correlated with the length of the chromosomes, the longer the chromosome the higher being the frequency. The polyploid constitution of the species investigated here might be obscured by the failure to form multivalents due possibly to low frequency of chiasma, an inherent characteristic of short chromosomes. In view of these facts, therefore, even in the absence of multivalents the possibility of its being a polyploid cannot at all be excluded.

Cases of a few percentage of irregular P. M. C. s need consideration. It may be noted that a single bivalent is always involved in such abnormal behaviour. The lagging of the bivalent at the equator at the first meiotic division and the staying apart of one chromosome in each of the nuclei of second meiotic metaphase seem to be the manifestation of a single bivalent member. It may be surmised that in cases where the two members instead of lagging move to two different poles fall out

of step from the other chromosomes during second meiotic division. The behaviour suggests that the species concerned might not be a true simple diploid but rather one in which the intermixture of different complements to certain extent has been caused during evolution.

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#### SUMMARY

1. The cytology of different species of *Sansevieria* belonging to Haemodoraceae of Bentham and Hooker has been worked out.

2. The somatic chromosome number has been worked out to be forty in *S. cylindrica* and in one variety of *S. nilotica*. The other variety of *S. nilotica* shows thirty-six chromosomes in the diploid set. Karyotypes show five types of chromosomes, short in size, distinguishable on the basis of primary and secondary constrictions. The two species with forty chromosomes correspond exactly in chromosome morphology, the variety with thirty-six being devoid of one satellited and one non-satellited pair.

3. Meiotic studies reveal regular behaviour excepting abnormality in division of a single bivalent in a few percent of mother cells.

4. From a comparison of its karyotypes with that of members of Amaryllidaceae, Agavaceae etc., the families to which the genus is often assigned, its position in a separate family has been justified.

5. Though evidences of polyploidy have not been obtained from meiotic data of the species studied it has been suggested that the probability of its being a polyploid cannot at all be overruled, because of the short chromosomes having low chiasma frequency and as such less chance of forming multivalents.

6. The irregular behaviour of a single bivalent has been considered indicative of the species being not a true simple diploid.

## RIASSUNTO

Nel genere *Sansevieria* (Haemodoraceae), il numero cromosomico somatico di *S. cylindrica* e di una varietà di *S. nilotica* è  $2n = 40$ ; quello di un'altra varietà di *S. nilotica* è  $2n = 36$ . Il cariotipo presenta cinque tipi di cromosomi di breve lunghezza distinguibili in base alle costrizioni primarie e secondarie. Le due specie con 40 cromosomi si corrispondono perfettamente per la morfologia cromosomica; la varietà con 36 cromosomi manca di un paio satellifero e di uno non satellifero. La meiosi ha un decorso normale, ad eccezione di qualche anomalia nella separazione di un singolo bivalente in una piccola percentuale di cellule madri, fenomeno considerato indicativo di una specie che non è un semplice diploide ma molto probabilmente un poliploide. La posizione del genere in una famiglia separata dalle famiglie affini (Amaryllidaceae, Agavaceae, ecc.) è giustificata dal punto di vista carilogico.

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