CYTOLOGICAL STUDIES IN INDIAN PARASITIC PLANTS

I. The Cytology of *Striga*

BY L. S. S. KUMAR AND A. ABRAHAM

(*Botany Department, College of Agriculture, Poona*)

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VERY few of the Indian parasitic angiosperms have been investigated cytologically and even the chromosome numbers of most of them are not known. The present paper deals with the cytology of four species of *Striga*. Studies on other parasitic plants are in progress.

Material and Methods

Materials for the present study were collected locally. Three species, *S. lutea* Lour., *S. densiflora* Benth., and *S. euphrasioides* Benth., were collected from pot-cultures used for another investigation* and the host plant in all these cases was *Sorghum vulgare*. These three species of *Striga* are very similar to each other and bear a number of branches with numerous green leaves. They are hemi-parasites, while the fourth species, *S. orobanchoides* Benth., was collected locally from a marshy field. Preliminary examination in acetocarmine showed that the meiotic divisions take place when the buds are very small. The fourth bud above the flower which had opened in the morning of the day on which fixation was made, usually showed the meiotic divisions in at least one of the four anthers. Buds of this stage and slightly older as well as a little younger were taken and the sepals removed and after pretreatment with Carnoy's fluid for one minute were transferred to a chrome-acetic-formalin solution (2% chromic acid 5 c.c., 12% glacial acetic acid 5 c.c. and neutral formalin 5 c.c.) with a trace of saponin added. After the usual subsequent treatments, sections were cut at a thickness of 12 μ and stained in iodine gentian violet.

Observations

The somatic chromosome number was determined from metphase plates of ovary cells, in which the chromosomes were well spaced out. A certain degree of size variation in chromosomes was apparent even in

* Scheme of Research on the attack of *Striga* on Jowar.

adjoining metaphase plates in the same section. This appeared to be associated with size differences in the two cells, larger cells having chromosomes more evenly spaced out and more slender while smaller cells have chromosomes more crowded and more condensed. Chromosome counts at meiosis were made from polar views of I and II metaphases.

Mitosis :

S. densiflora.—Somatic chromosome number of this species is found to be $2n = 40$. Fig. 4 shows a metaphase plate in which the chromosomes are rather condensed. Two of the forty chromosomes are rather longer than the rest.

S. euphrasioides.—Fig. 5 shows a metaphase plate in which forty chromosomes are clearly counted. Here also two chromosomes are markedly longer than any of the other thirty-eight.

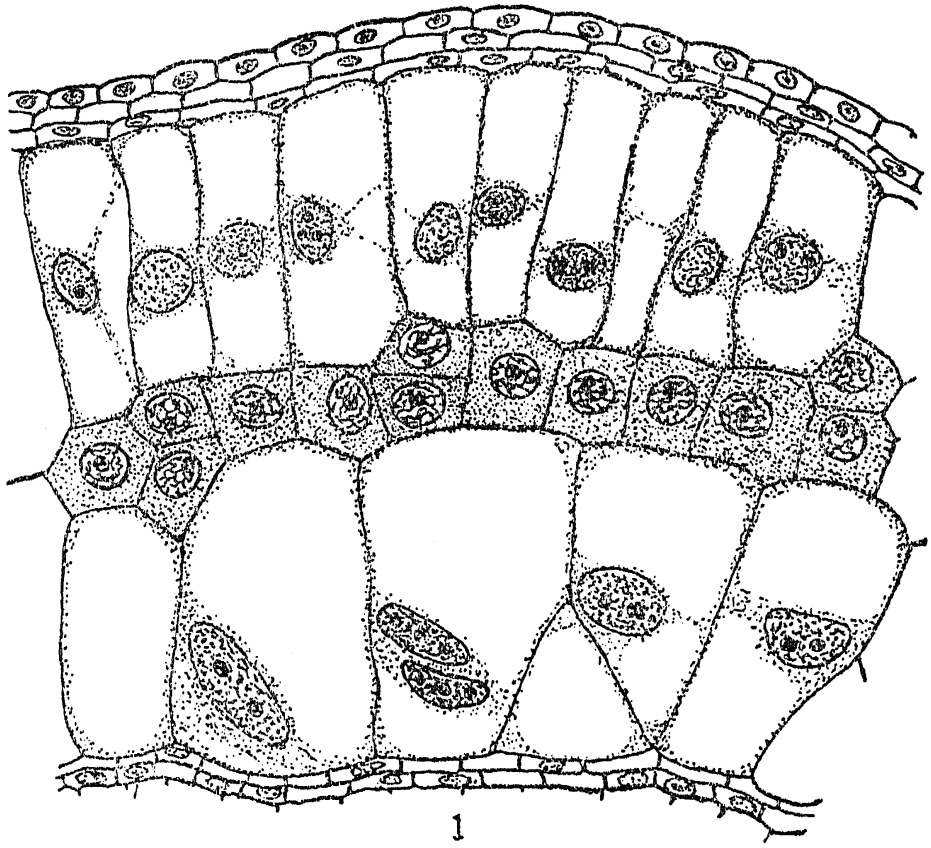
S. lutea.—Metaphase plates with chromosomes well spaced out were not observed, but the number as determined from a few plates in which chromosomes were rather crowded showed that the $2n$ number is forty in this species also.

S. orobanchoides.—While the chromosome number in this species also is $2n = 40$, the chromosomes appear to be larger than in the other three species (Fig. 6). Two long chromosomes are seen in this complement also.

Meiosis:

Preparations showing all the different stages of meiosis were obtained in the case of *S. densiflora* and *S. euphrasioides*, while in the other two species a few of the stages were not observed. Details of the meiotic division are mentioned only very briefly as no very unusual aspects were observed.

In all the four species all the four anthers are fertile. The anthers are two-lobed in cross section. The primary archesporial layer appears very early in the ontogeny of the anther, and by periclinal division it gives rise to the primary wall layer and the primary sporogenous layer. The former gives rise to three layers of cells, the two near the epidermis being narrow and elongated, while the third surrounding the sporogenous layer constitutes the tapetum. The tapetum is very conspicuous on both sides of the sporogenous layer, by its very large size and prominent nuclei. In Fig. 1, a few of the tapetal cells show two nuclei in each cell, while the spore mother cell nuclei are still in the early stages of meiosis. In cross sections and in vertical sections through certain planes, the sporogenous layer is found to be composed of single row of cells while at certain regions they are two cells thick. This shows that the primary sporogenous cells undergo



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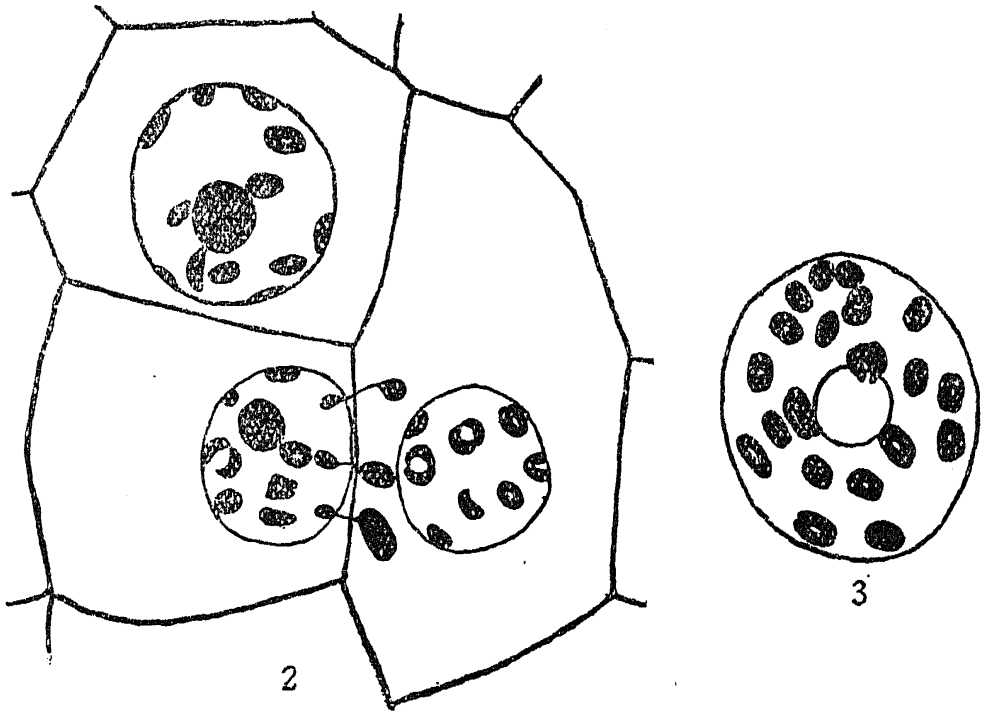


2 3

TEXT-FIGS. 1—3

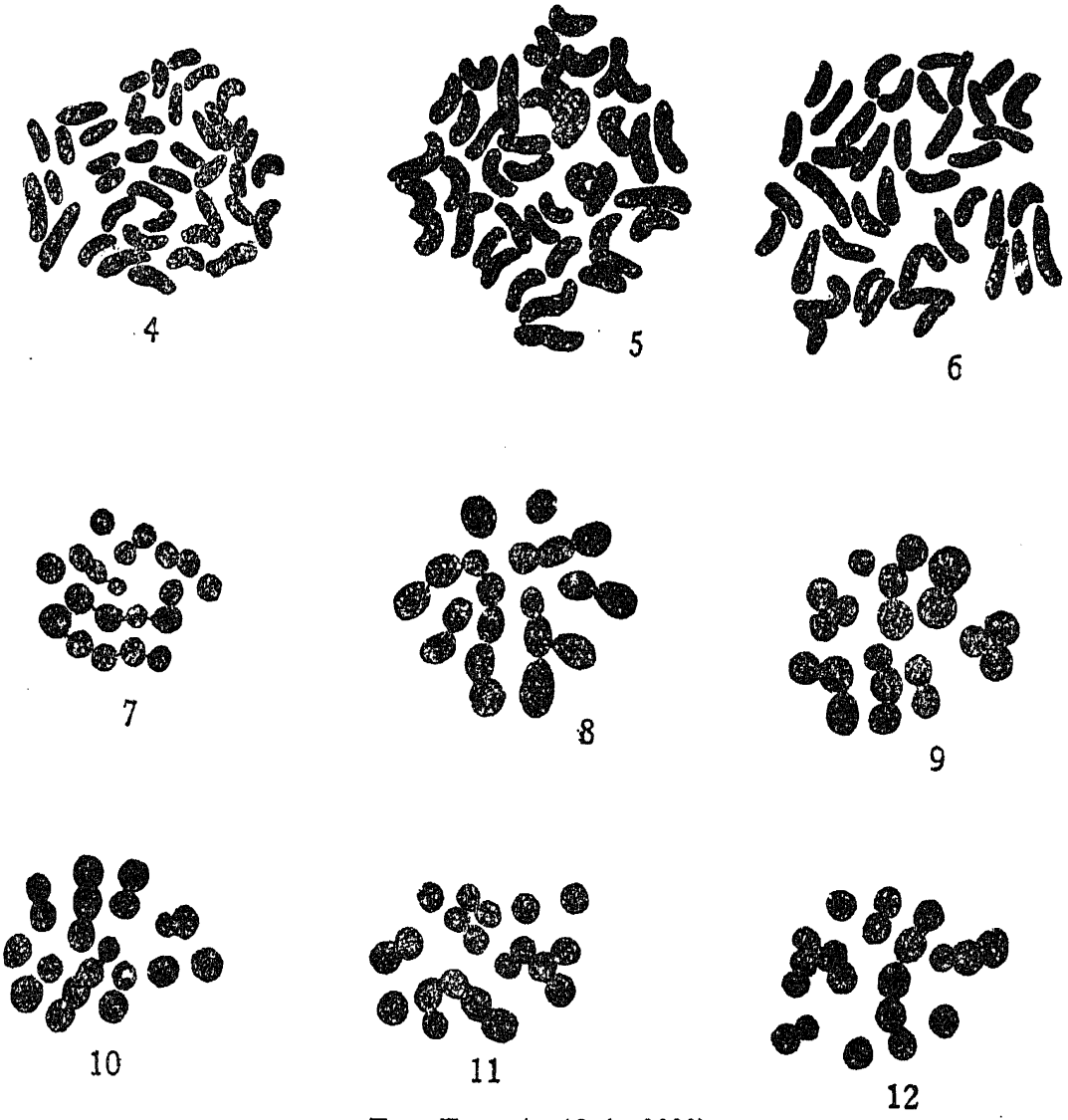
FIG. 1. *S. densiflora.*—Part of longitudinal section of an anther showing the large tapetal cells in some of which the nuclei have already divided. The pollen mother cell nuclei are in the early prophase of meiosis. ×540. FIG. 2. Chromatin extrusion in *S. euphrasioides.* ×2000. FIG. 3. *S. densiflora.*—A nucleus at diakinesis showing three bivalents in contact with nucleolus. One of the three bivalents is satellited and the satellites are lying on the nucleolus. ×3000.

mitosis only at certain regions, while at other regions they directly function as spore mother cells.

Prophase pairing is normal and at diplotene we find bivalents equidistant from one another. They condense further and at diakinesis the bivalents appear to be held together by two terminal chiasmata, giving rise to a ring-like appearance. In Fig. 3 showing a nucleus at diakinesis in *S. densiflora* three bivalents appear to be in contact with the nucleolus, but only one is satellited and intimately connected to it. Chromatin extrusion was observed in some pollen mother cells of one anther of *S. euphrasioides* (Fig. 2).

The equidistant orientation of bivalents in the periphery of the nucleus at diakinesis must be due either to a repulsion between bivalents as suggested by Lawrence (1931) or due to a repulsion force acting from the centre of the nucleus. However, this arrangement disappears with the loss of the nuclear membrane at pro-metaphase and the bivalents move towards the equator of the cell where they arrange into a plate. During this movement from the periphery to the centre some bivalents naturally come closer to each other. Whether these bivalents maintain this close association to give the appearance described as " secondary association " or whether there is a re-orientation of bivalents before metapahse plate is formed could not be determined. But the question arises as to how far secondary associations represent attraction due to residual homology and how far chance proximity of bivalents during pro-metaphase movement contributes to this appearance. It was found in some cases that, probably due to the action of fixatives or due to other undetermined causes, metaphase bivalents come together much more closely than is normal at that stage. This did not suggest a general collapse of the bivalents, as only a few bivalents are so closely associated while others are well spaced.

In *S. densiflora* and *S. euphrasioides* the haploid number is found to be twenty (Figs. 8–12). The haploid number in *S. lutea* was determined from a polar view of I anaphase and in *S. orobanchoides* from diakinesis nuclei. In both these species also the haploid number was found to be twenty. Figs. 8 and 9 show I metaphase plates from *S. euphrasioides* and Figs. 10–12 show the same stage from *S. densiflora.* In both cases very clear secondary association of the bivalents into eight groups is seen.



TEXT-FIGS. 4—12 (×3000).

FIG. 4. *S. densiflora.*—Somatic metaphase. $2n = 40$. FIG. 5. *S. euphrasioides.*—Somatic metaphase, $2n = 40$. FIG. 6. *S. orobanchoides.*—Somatic metaphase, $2n = 40$. FIG. 7. *S. lutea.*—Polar view of I anaphase showing 20 chromosomes. FIGS. 8 and 9. *S. euphrasioides.*—I metaphase showing 20 bivalents. Secondary association into 7 and 8 groups respectively. FIGS. 10, 11 and 12. *S. densiflora.*—I metaphase showing 20 bivalents in each. Fig. 10 shows secondary association into 10 groups, while in Figs. 11 and 12 the typical arrangement into 8 groups is shown.

TABLE I

Analysis of I metaphase secondary association in S. densiflora

Number of bivalent groups	Number of I metaphase plates	Frequency percentage
6	1	3·3
7	2	6·6
8	16	52·8
9	5	16·5
10	2	6·6
11	1	3·3
12	3	10·0
Total	30	

This was the most frequent type of grouping found in a count of thirty metaphase plates in *S. densiflora* and twelve metaphase plates in *S. euphrasioides*. A few cases were observed where the twenty bivalents formed into groups of 10, 11 or 12 (*cf.* Fig. 10), while very rarely groups of 6 and 7 were also noticed. The secondary association is maintained at anaphase I and metaphase II as well, as observed by Raghavan and Srinivasan (1940) in *Angelonia*, another genus of the Scrophulariaceæ.

Anaphasic separation is very regular and the chromosomes move as one plate so much so the metaphase association could be followed in some polar views of anaphase. The second division rapidly follows the formation of daughter nuclei in the first division. Cell wall formation takes place only after completion of the second nuclear division.

Discussion

Basic number of chromosomes in Striga.—The previous work on the cytology of the Scrophulariaceæ has been reviewed by Raghavan and Srinivasan (1940) who have also recorded the chromosome numbers in six genera and eight species belonging to this family. They have also discussed the significance of secondary association and the probable basic number of chromosomes in the family.

Secondary association of bivalents at I metaphase is now being used as a reliable criterion for determining secondary polyploidy in a genus and also to infer the basic chromosome number of the genus or family. Though it is generally accepted that secondary association is a manifestation of residual homology between the bivalents, it is not unlikely that chance position occupied by a bivalent during pro-metaphase movement may also lead to a closer orientation of two or more bivalents. This possibility together with the fact that the action of chemicals may also accentuate this appearance, necessitates great caution in the use of this evidence.

The meiotic prophase chromosomes are slender, long and coiled and still we normally find homologous chromosomes closely pairing. This shows that when there is an attraction of a sufficient magnitude between any two chromosomes, they could pair at the meiotic prophase in spite of possible mechanical difficulties due to presence of intervening chromosome strands or the very elongated and coiled condition of these chromosomes. Such being the case with respect to primary association, it is difficult to understand why, if secondary association is really due to a residual attraction (which must also be assumed to be of a specific nature if we are to draw any inference regarding ancestral homology from it) all bivalents capable of

secondary pairing do not do so frequently. Even if this secondary attraction is not of such a magnitude as that determining the prophase pairing, it should be expected, at least in cases where the chromosomes are few and small in size and the pollen mother cell of sufficient diameter—thus eliminating any mechanical barrier to bivalents coming together—that all bivalents capable of secondarily associating would do so. If this view is admissible, then the most frequent type of secondary association observed should be taken as representing the real maximum association to be expected on the basis of secondary homology, while those deviating from this may only be the result of other causes which apparently increase or decrease the number of bivalent groups. In this connection it is interesting to note the observations of Catcheside (1937) on *Brassica*. From counts of a very large number of I metaphase plates, he observed 80 cases of $3_{(2)} + 3_{(1)}$ association and 122 cases of $2_{(2)} + 5_{(1)}$ association. Though the latter showed the maximum frequency he has taken the former as indicating the basic number ($3+3=6$). But unlike some recent authors who have relied solely on the maximum association irrespective of the observed frequency, Catcheside considers higher association showing comparatively very low frequency as merely aberrant types of associations.

The most frequent type of secondary association found in a random selection of thirty metaphase plates in *S. densiflora* is into eight groups. A smaller sample of twelve metaphase plates examined in *S. euphrasioides* also showed nearly the same frequency percentage of association of bivalents into eight groups as in *S. densiflora*. Based on this finding and taking into account the fact that a large number of genera of the Scrophulariaceæ have eight as their basic chromosome number (Srinath, 1940), we are inclined to take eight as the basic number in *Striga* also, though it is strictly not the maximum association observed. On the basis of maximum association the number should be six; but this need be considered as an aberrant association only in view of the fact that it is unrelated to basic numbers in other allied genera and is seen only once in thirty plates examined. Raghavan and Srinivasan (1940) also found the most frequent type of secondary association in *Angelonia grandiflora* to be eight, though they found in two metaphase plates out of a total of thirty-one examined, a maximum association into five groups and from this took five as the basic number of the genus and even went so far as to suggest that this may be the primary basic number in the family. But a large number of genera of the Scrophulariaceæ have eight as their basic number and the present study shows yet another genus with the same basic number. If the most frequent association and not the maximum association is taken into account, then *Angelonia* too should be considered as having eight as the basic number.

For verifying the applicability of our suggestion, based mainly on theoretical considerations, that in plants showing secondary associations the most frequent type of such associations should be taken as indicating the basic number, it would be necessary to statistically analyse the chromosome arrangement in a number of closely related genera exhibiting secondary association and which in possessing only a small number of small-sized chromosomes are otherwise also favourable materials for such studies. An investigation on these lines is in progress in our laboratory.

Summary

The cytology of four species of *Striga*, *viz.*, *S. densiflora*, *S. euphrasioides*, *S. lutea* and *S. orobanchoides* is described. The chromosome numbers have been found to be $n = 20$ and $2n = 40$ in all the four species.

The basic number of chromosomes of the genus is shown to be eight. Based on theoretical considerations it is suggested that in using evidence from secondary associations the most frequent type of association should be taken as indicating the basic number.

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