

# CYTOLOGICAL STUDIES OF AN INTERGENERIC HYBRID OF *CAJANUS CAJAN* (LINN.) MILLSP. AND *ATYLOSIA LINEATA*, W. & A.

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## I. INTRODUCTION

*Cajanus cajan* (Linn.) Millsp. commonly called as pigeon pea is cultivated in India as one of the important food crops. In pigeon pea there are annual and perennial forms and both of these forms are highly susceptible to wilt caused by *Fusarium udum* (Butler, 1910). From the work so far in progress in the Bombay State, it has not been possible to obtain a completely wilt-resistant type, either by direct selection or intervarietal hybridization of the cultivated forms.

*Atylosia lineata*, W. & A., commonly called the wild or *ran tur*, is a wild prototype of *C. cajan*. It is distinguished from *C. cajan* by the presence of arilate seeds bearing large, conspicuous, divided strophiole. When this was tested under heavy artificial infection, it was found to be highly resistant to fusarium wilt.

To take advantage of this factor, a cross between *C. cajan* (Linn.) Millsp. and *A. lineata*, W. & A. was made to combine the wilt-resistant character of the latter with all the other desirable characters of the former. In this paper is discussed the cytological behaviour of such an interspecific hybrid, the parents involved and of the further hybrid generations.

Basudeo Roy (1933) was the first to report the chromosome number of *C. cajan* as  $n = 11$ , while the detailed cytological observations of its meiosis were made by Rangaswamy and Krishnaswamy (1935). The cytological investigation of the two parents *A. lineata* and *C. cajan* and of the  $F_1$  hybrid were first begun by Deodikar and Thakar, who in 1956 have published the details of the cyto-taxonomy and chromosome morphology of the parents.

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## II. MATERIAL AND METHOD

The present investigations were started in 1954 in continuation of the work begun by Deodikar and Thakar on the plant material of *Cajanus cajan* (Linn.) Millsp., *Atylosia lineata*, W. & A. and the F<sub>1</sub> hybrid of a cross between them that were then growing in the experimental area of the Economic Botanist to Government of Bombay at the College of Agriculture, Poona. The strain of *C. cajan* used in the hybridization programme was the selection M-4-N made by Kumar and Gadre, the seed of which was obtained from the Superintendent, Agricultural School, Arbhavi, in May 1951. The seed of *A. lineata* was collected by Deodikar from Mahabaleshwar hills situated at 3,500' altitude.

The root tips of the parents and hybrid were fixed for 24 hours in Craff's A and B, washed in running water, dehydrated, cleared in ethyl-butyl alcohol series, infiltrated and embedded in paraffin. The sections were cut of 10 to 12  $\mu$  thickness and were stained in Flemming's modified triple stain for 24 hours (Johansen, 1940). For study of meiosis, the flower-buds were fixed in Craff's A and B solutions for six hours. Newton's iodine-gentian violet (Johansen, 1940) was found to give well-stained preparations.

Pollen grains were tested for their contents by using one per cent. aqueous iodine solution and for their functional behaviour, they were germinated in sugar-agar media (Johansen, 1940).

## III. CHROMOSOME NUMBER AND MORPHOLOGY

1. *C. cajan*.—The somatic number of 22 chromosomes determined agrees with the findings of previous workers. The chromosomes at prophase appeared long and well spread out and a pair of SAT-chromosomes was seen attached to the nucleolus. At metaphase the chromosomes were well stained and sufficiently long to observe the details regarding the length of their arms and the position of the constrictions (Fig. 1). The morphological details of the chromosomes are given in Table I.

In Table I, the *C. cajan* complement is grouped into three classes:

(i) The longest pair of chromosomes has a secondary constriction placed in the sub-terminal position.

(ii) Of the three classes, Class C1 includes three pairs of chromosomes which are longer than 4.0 microns and have sub-median constrictions. Class C2 includes three pairs of chromosomes 3.6 microns in length. Out of these two pairs have sub-terminal and one sub-median primary constriction. The rest of the five pairs of chromosomes of Class C3 are between

TABLE I  
*Morphology of the somatic chromosome complement of Cajanus cajan (Linn.) Millsp.*

Class	Position of constriction		Length of arms in microns	Total length	Total number of chromo- somes
	Primary	Secondary			
C1	SM	ST	2.1+2.4+0.5*	5.0	2
	SM	..	2.1+2.4	4.5	2
	SM	..	2.2+1.8	4.0	2
C2	SM	..	2.0+1.6	3.6	2
	ST	..	2.7+0.9	3.6	2
	ST	..	2.5+1.0	3.5	2
C3	SM	..	1.6+1.3	2.9	2
	SM	..	1.3+1.1	2.4	4
	M	..	1.15+1.15	2.3	4

\* Length of satellite.

Total number of chromosomes = 22.

Total length of complement = 73.0 microns.

Average diameter = 0.45 micron.

M = Median; SM = Sub-median; ST = Sub-terminal.

2.3 and 2.9 microns in total length, with three pairs having sub-median and two pairs having median primary constrictions.

(iii) The total length of the whole complement is 73.0 microns with mean diameter of 0.45 microns.

The idiogram representing the diploid complement of *C. cajan* is given in Fig. 2.

2. *A. lineata*.—The diploid complement was found to be 22 which agrees with the number reported by previous workers.

The prophase chromosomes were well spread out in the nucleus and a pair of SAT-chromosomes was seen attached to the nucleolus. The morphological details of the chromosomes as observed at metaphase (Fig. 3) are given in Table II.

It will be seen from Table II that:

(i) As in the case of the *C. cajan* so in *A. lineata* there is a long pair of chromosomes having secondary constriction at sub-terminal position.

TABLE II  
Morphology of the somatic chromosome complement of  
*Atylosia lineata*

Class	Position of constriction		Length of arms in microns	Total length	Total number of chromo- somes
	Primary	Secondary			
A1	SM	ST	2.5+1.8+0.5*	4.8	2
	M	..	2.2+2.2	4.4	2
	SM	..	2.1+1.7	3.8	2
	SM	..	1.9+1.5	3.4	2
A2	M	..	1.7+1.7	3.4	2
	SM	..	1.7+1.5	3.2	2
A3	SM	..	1.6+1.0	2.6	4
	SM	..	1.3+1.1	2.4	4
	M	..	1.2+1.2	2.4	2

\* Length of satellite.

Total number of chromosomes = 22.

Total length of complement = 70.8 microns.

Average diameter = 0.45 micron.

M = Median; SM = Sub-median; ST = Sub-terminal.

(ii) The chromosome complement of *A. lineata* consists of three classes, the class A1 includes in all four pairs, all having length between 3.4 to 4.8 microns, three of which have sub-median and one median primary constriction. The class A2 includes two pairs of chromosomes between 3.2 to 3.4 microns, one with sub-median and the other with median primary constrictions. The class A3 includes in all five pairs, four of which have sub-median and one median primary constriction, with length between 2.4 to 2.6 microns.

(iii) The total length of the chromosome complement is 70.8 microns with average diameter of 0.45 micron.

In Fig. 4 is given the idiogram of the chromosome complement of *A. lineata*.

3.  $F_1$  (*C. cajan* × *A. lineata*).—The cytological studies of the hybrid of *C. cajan* × *A. lineata* have been made for the first time. The chromosome complement in the somatic tissue was  $2n = 22$  (Fig. 5). The haploid chromosome set of each parent is present in the  $F_1$ .

At mitotic prophase a long chromosome pair was attached to the nucleolus. The details of the chromosome morphology are given in Table III. The idiogram of the chromosome complement of  $F_1$  (*C. cajan* × *A. lineata*) is given in Fig. 6.

TABLE III  
Morphology of the somatic chromosome complement of  
 $F_1$  (*C. cajan* × *A. lineata*)

Pair No.	Position of constriction			Length of arms in microns	Total length
	Primary	Secondary			
1	C	SM	ST	2.4+2.0+0.5*	4.9
	A	SM	ST	2.2+2.1+0.5*	4.8
2	C	SM	..	2.3+2.0	4.3
	A	M	..	2.2+2.1	4.3
3	C	SM	...	2.2+1.8	4.0
	A	SM	..	2.1+1.7	3.8
4	C	SM	..	2.1+1.5	3.6
	A	SM	..	1.9+1.5	3.4
5	C	ST	..	2.6+1.0	3.6
	A	SM	..	1.8+1.7	3.5
6	C	ST	..	2.5+1.1	3.6
	A	SM	..	1.8+1.6	3.4
7	C	SM	..	1.6+1.3	2.9
	A	SM	..	1.6+1.0	2.6
8	C	SM	..	1.3+1.1	2.4
	A	SM	..	1.3+1.1	2.4
9	C	SM	..	1.3+1.1	2.4
	A	SM	..	1.3+1.1	2.4
10	C	SM	..	1.3+1.1	2.4
	A	M	..	1.3+1.1	2.4
11	C	M	..	1.2+1.2	2.4
	A	M	..	1.1+1.1	2.2

\* Length of satellite.

Total number of chromosomes = 22.

Total length of chromosomes = 71.7 microns.

Average diameter = 0.45 micron.

M = Median; SM = Sub-median; ST = Sub-terminal; C = Cajan; A = Atylosia.

Unlike the parents *C. cajan* and *A. lineata* the pairs of chromosomes in the hybrid were heteromorphic as will be seen from columns 4 and 5 of Table III. The first pair has secondary constrictions but the individual lengths of the two chromosomes differ, nevertheless, the position of the

centromere is sub-median and that of the secondary constriction is sub-terminal.

Chromosome pairs 3, 4 and 7 differ in length but the position of the primary constriction is sub-median. The chromosomes of pairs 8, 9 and 11 have equal length and are identical both for the total lengths and the positions of primary constrictions. The pairs 2, 5, 6 and 10 do not appear to show much difference in the total length, but the position of the centromere differs within each pair as shown in column 2 of Table III.

The total length of the chromosome complement of the  $F_1$  is 71.7 microns which is different from that of the two parents and lies midway between the two complements as it should.

#### IV. MICROSPOROGENESIS

1. *C. cajan*.—In this, the stages after diakinesis were studied. At diakinesis the chromosomes had formed bivalents and the chiasma had terminalised. There were eleven bivalents spread out and were lying nearer to the nuclear membrane. A nucleolus was seen with two chromosomes attached to it (Fig. 7). These correspond to the pair of SAT-chromosomes seen in the somatic cells.

Metaphase-I showed 11 pairs of chromosomes (Figs. 8 and 9). 100 p.m.c.'s showing metaphase in polar and side-view were studied. There were no major irregularities observed except the occurrence of precocious chromosomes. In six per cent. of the cases showing side-view of metaphase-I, the precocious chromosomes were observed (Fig. 10). The other stages in meiosis were normal. The gametic number of chromosomes observed was eleven.

2. *A. lineata*.—At diakinesis, the chromosomes were forming bivalents and were held together by the terminalising chiasmata. A pair of SAT-chromosomes was attached to the nucleolus (Fig. 11).

At metaphase-I, polar as well as side-views showed the presence of 11 bivalents (Fig. 12). The chromosome separation at anaphase and further stages in meiosis was normal. It was found that the haploid complement was 11.

3.  $F_1$  (*C. cajan*  $\times$  *A. lineata*).—At diakinesis the chromosome though in bivalent condition were very much shortened with the terminalised chiasma. 30 nuclei showing diakinesis were studied and except for one, the others showed that chromosomes were formed into eleven pairs (Fig. 13). In one there were 9 bivalents and a multivalent with four chromosomes (Fig. 14)

The chromosomes were well spread out along the nuclear wall with one pair attached to the nucleolus. It was, however, interesting to note that at diakinesis six to seven peculiar rod-like configurations of bivalents were observed in each nucleus which were not observed in either parent. The common type of configuration observed in both the parents, in the case of bivalents at diakinesis, was in the form of dark circles thus indicating that the total chiasma in each parental nucleus was more than that in  $F_1$ . The chiasma were formed in both the arms. The rod-like configurations indicated that the bivalent chromosomes were held by the chiasma formed in only one arm and as no chiasma was formed in the other arm, which would therefore be free. In the case of the bivalents that formed circles they would be held together by chiasma at both the ends so that the arms would not be free. At metaphase-I, the paired bivalents were observed in the polar and side-views. In the polar view as well as in the side-view the difference in the size of the bivalents could be seen (Figs. 15 and 16).

In all 70 cells showing metaphase-I were studied in the  $F_1$ . Out of these 67 showed 11 bivalents. The remaining 3 showed 9 bivalents and a multivalent consisting of four chromosomes (Fig. 17).

50 cells at anaphase-I were studied, out of which 48 showed normal separation of bivalents and the remaining two showed the normal separation of 10 bivalents with the 11th bivalent forming a bridge with a fragment (Fig. 18).

The stages of meiosis-II did not present any abnormalities.

#### V. POLLEN GERMINATION AND SEED SETTING

The percentage of seed setting from the flowers produced by the parents and the  $F_1$  were studied. The parents *C. cajan*, *A. lineata* and their  $F_1$  showed 81, 60 and 30 as the percentages of seed set. Similarly the pollens of all these plants were germinated on sugar-agar media (Johansen, 1940) and the parents *C. cajan*, *A. lineata* and the  $F_1$  gave 79, 66 and 51 per cent. pollen germination respectively.

From the information given above it will be seen that the  $F_1$  has a very low percentage of normal seed setting and also of germination pollen.

#### VI. DISCUSSION

1. *Chromosome number and morphology.*—Basudeo Roy (1933), Rangaswamy and Krishnaswamy (1935) and Deodikar and Thakar (1956) have reported the chromosome number of *C. cajan* as  $2n = 22$ . This number has been confirmed in these investigations.

Deodikar and Thakar were the first to report the somatic number of *A. lineata* as  $2n = 22$ . In the investigations presented here this number has also been confirmed.

The cytological studies in the  $F_1$  hybrid of *C. cajan*  $\times$  *A. lineata* are reported for the first time and the chromosome complement in the somatic tissue represents the haploid complement of each of the two parents and therefore  $2n = 22$ .

Deodikar and Thakar have given the details of chromosome morphology of the *C. cajan* and *A. lineata*. Their observations on chromosome length and position of primary and secondary constrictions differs from the results obtained during this study. The total chromosome length observed by Deodikar and Thakar and the authors in *C. cajan* does not show a great difference, which is 2.4 microns whereas this difference in *A. lineata* is much greater being 11.6 microns. The position of the primary constrictions of the chromosomes of *C. cajan* and *A. lineata*, as reported by Deodikar and Thakar, are different to those observed by the present investigators.

Both the differences in the length of the chromosome complement and the position of the centromere may possibly be due to the varietal characteristics, differences in the technique and the stages at which observations were made. The other significant point to be noted is that Deodikar and Thakar have not recorded even a single pair of chromosome of *A. lineata* having secondary constrictions whereas in the investigations presented here, a pair of chromosomes having sub-terminal secondary constrictions has been observed. It is likely that Deodikar and Thakar were not able to report the presence of the secondary constrictions in the chromosome complement of *A. lineata* because they did not examine the correct stage of meiosis and mitosis.

The somatic chromosome complement of the  $F_1$  consists of two haploid sets one derived from the parent *C. cajan* and the other from *A. lineata*. The morphological characters of these two haploid sets of the  $F_1$  are not different from those of the two parents from which they are derived.

The morphology of the chromosome complement of *C. cajan* is different from that of *A. lineata*. These differences observed between the complements of the two parents have been maintained in the  $F_1$  as evident from the heteromorphic character of each pair.

#### VII. RELATIONSHIP OF THE TWO SPECIES: *C. cajan* AND *A. lineata*

Stebbins (1945) has reviewed the investigations on the interspecific and intergeneric hybrids with slight irregularities of meiosis and has also



discussed the possible causes of sterility in such hybrids. He seems to conclude that in such cases structural differences in the chromosomes are responsible for sterility in the hybrids. Muntzing (1938) studied certain crosses in *Galeopsis* and observed partial sterility in their species hybrids. He also explained the partial sterility on the basis of the structural hybridity involving small chromosomal segments. The  $F_1$  of the cross *C. cajan*  $\times$  *A. lineata* reported in this paper has shown partial abortion of the seed and rather low pollen germination. This partial sterility in this hybrid may be due to the structural differences in the chromosome complements of the two parents, as suggested by Stebbins and Muntzing. Certain amount of evidence in favour of this structural differences in the two chromosome sets in the hybrid is supported by the presence of quadrivalents and bridges with fragments observed during the reduction division.

The mitotic and the meiotic studies of the two parents *C. cajan*, *A. lineata* and their hybrid indicate that the two species are closely related and as suggested by Deodikar and Thakar their taxonomic classification could be revised on the basis of the cytological information presented in this paper.

#### VIII. SUMMARY

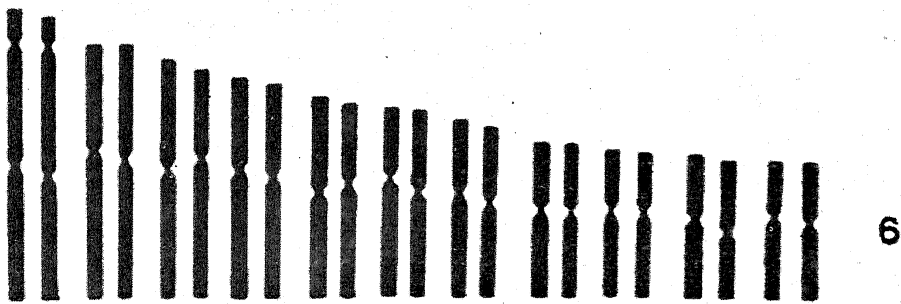
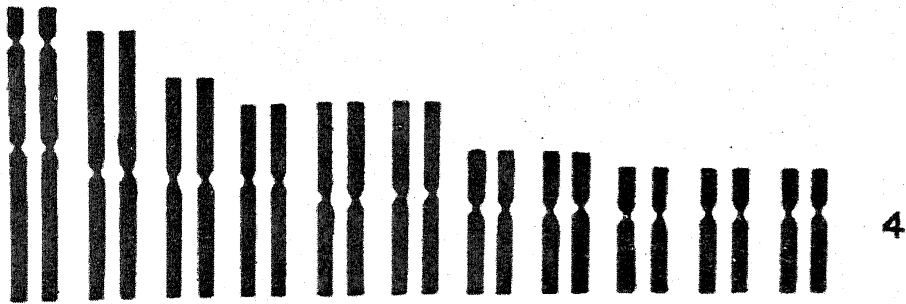
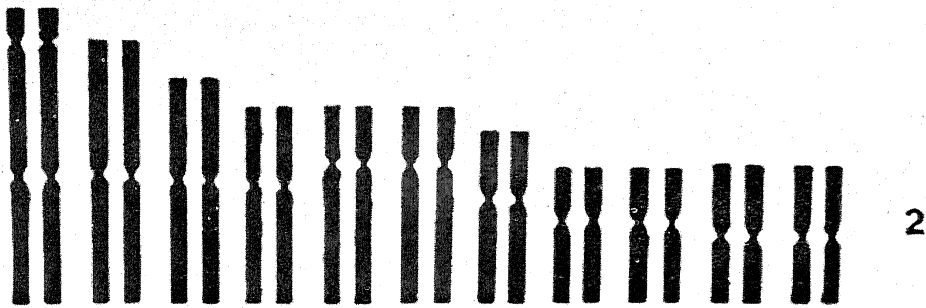
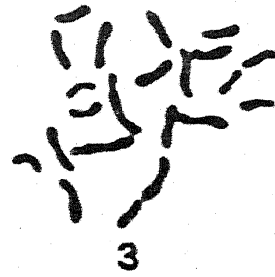
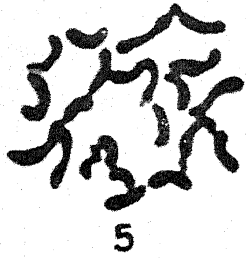
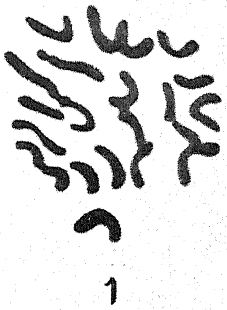
An intergeneric hybrid between *C. cajan* (Linn.) Millsp. ( $2n = 22$ )  $\times$  *A. lineata*, W. & A. ( $2n = 22$ ) was obtained. The morphology of the somatic chromosomes of the parents and the  $F_1$  was studied. It was observed that there was difference in the chromosome morphology of the two species. Some meiotic irregularities were observed in the  $F_1$  which showed only partial fertility. It is suggested that the sterility of  $F_1$  is due to the structural differences in the chromosome of the two parents. The homology between the chromosome complements of the two parents suggests close relationship between them.

#### IX. ACKNOWLEDGEMENTS

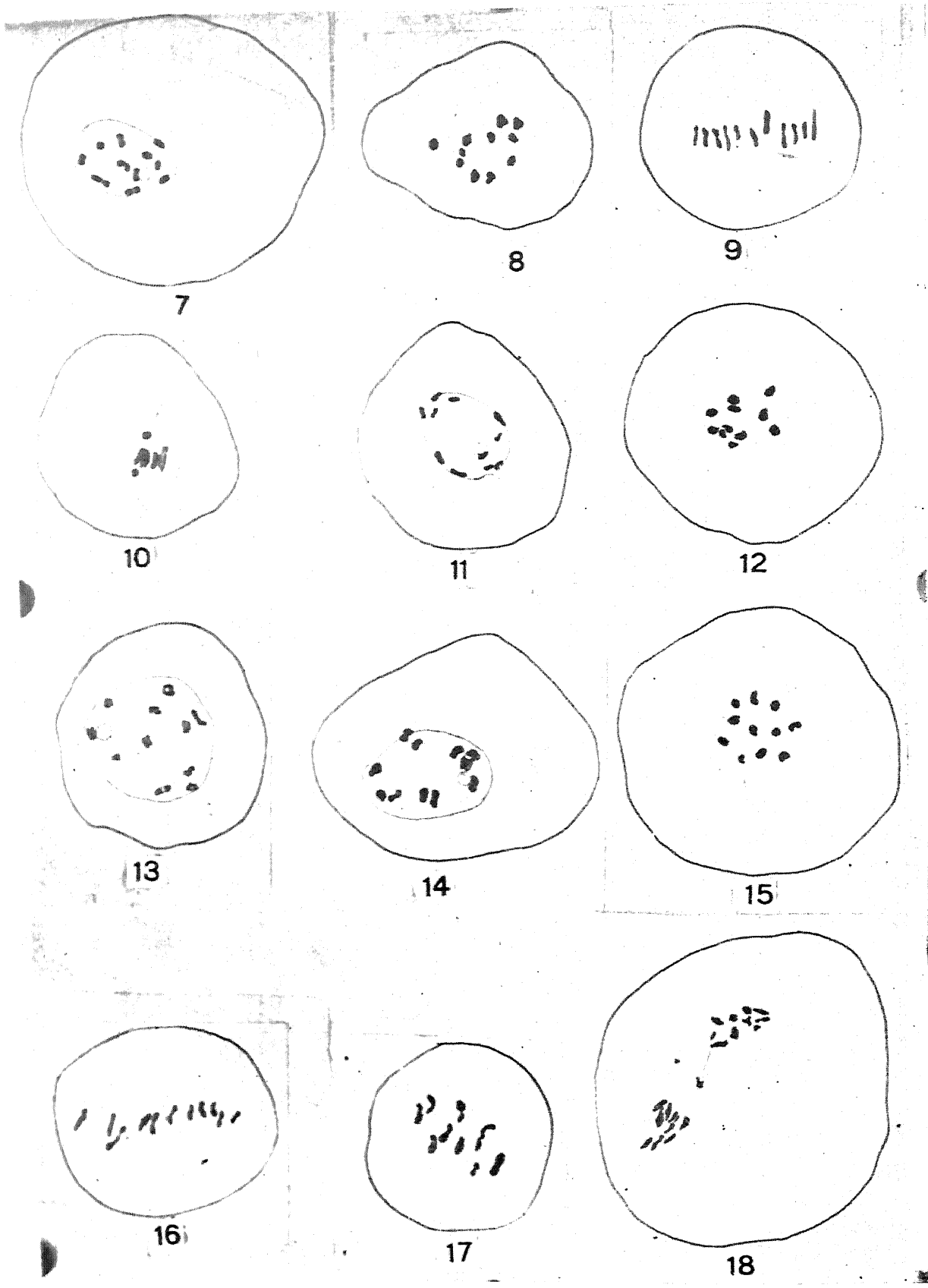
We are thankful to Dr. G. B. Deodikar for the collection of the seed of *A. lineata*. Our thanks are also due to Shri B. N. Nagras, Field Assistant of the Botany Section, for the help rendered in the care of plant material.

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FIGS. 1-6



FIGS. 7-18

*Intergeneric Hybrid of C. cajan (Linn.) Millsp. and A. lineata, W. & A. 261*

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EXPLANATION OF PLATES

PLATE XXI

Somatic complement and idiogrammatic representation of chromosomes in *C. cajan*, *A. lineata* and their  $F_1$ .

- FIG. 1. Somatic chromosome complement of *C. cajan*,  $\times 3,000$ .
- FIG. 2. Idiogram of the somatic complement of *C. cajan*.
- FIG. 3. Somatic chromosome complement of *A. lineata*,  $\times 3,000$ .
- FIG. 4. Idiogram of somatic complement of *A. lineata*.
- FIG. 5. Somatic chromosome complement of  $F_1$  of *C. cajan*  $\times$  *A. lineata*,  $\times 3,000$ .
- FIG. 6. Idiogram of somatic complement of  $F_1$  of *C. cajan* and *A. lineata*.

PLATE XXII

Microsporogenesis in *C. cajan*, *A. lineata* and their  $F_1$ . (Drawing reduced to  $\frac{1}{4}$  original.)

*C. cajan*

- FIG. 7. Diakinesis showing 11 bivalents with one pair attached to nucleolus,  $\times 3,000$ .
- FIG. 8. Polar view of Metaphase-I,  $\times 3,000$ .
- FIG. 9. Side-view of Metaphase-I,  $\times 3,000$ .
- FIG. 10. Side-view of Metaphase-I showing precocious separation of a pair of chromosomes to each pole,  $\times 3,000$ .

*A. lineata*

- FIG. 11. Diakinesis showing 11 bivalents with one pair attached to nucleolus,  $\times 3,000$ .
- FIG. 12. Polar view of Metaphase-I,  $\times 3,000$ .

$F_1$  of *C. cajan*  $\times$  *A. lineata*

- FIG. 13. Diakinesis showing 11 bivalents with one pair attached to nucleolus,  $\times 3,000$ .

- FIG. 14. Diakinesis showing bivalents and a quadrivalent,  $\times 3,000$ .  
FIG. 15. Polar view of Metaphase-I,  $\times 3,000$ .  
FIG. 16. Side-view of Metaphase-I,  $\times 3,000$ .  
FIG. 17. Side-view of Metaphase-I showing bivalents and 1 quadrivalent,  $\times 3,000$ .  
FIG. 18. Anaphase-I showing bridge and fragment,  $\times 3,000$ .