

## Pachytene Analyses in *Atylosia sericea* and *Cajanus cajan* × *A. sericea* hybrid<sup>1</sup>

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This is the second in a series of papers dealing with our investigation on inter-relationship of *Cajanus* and *Atylosia* species as revealed by hybridization and pachytene analysis. In the previous communication we (Reddy 1981) reported that 10 chromosomes are identical in *Cajanus* and *A. lineata* in so far as their pachytene morphology is concerned. However, on the basis of pachytene chromosome pairing in the hybrid only 9 chromosomes are common to them. *A. lineata* is an erect species and shows a high degree of affinity to *Cajanus*. *A. sericea* is another erect species which is also close to *Cajanus* in morphology and somatic karyology (Reddy and De, in press). The present work deals with the pachytene karyomorphology of *A. sericea* and *Cajanus* × *A. sericea* hybrid with a view to ascertain their affinity.

### Materials and methods

The materials used in this investigation are *A. sericea* and F<sub>1</sub> of *C. cajan* (Variety T-21) × *A. sericea*. The cytological techniques employed here have been described in an earlier report (Reddy 1981).

### Observations

#### Pachytene morphology of *Atylosia sericea*

The pachytene complement is constituted of 2 median, 8 submedian and 1 sub-terminal chromosomes. The individual bivalent length varies from 64.2 μ to 22.9 μ. The total chromatin length measures 429.5 μ of which 31.4 per cent is heterochromatic. Chromosomes I and II belong to the long group, chromosomes III to VIII to the medium group and chromosomes IX to XI to the short group.

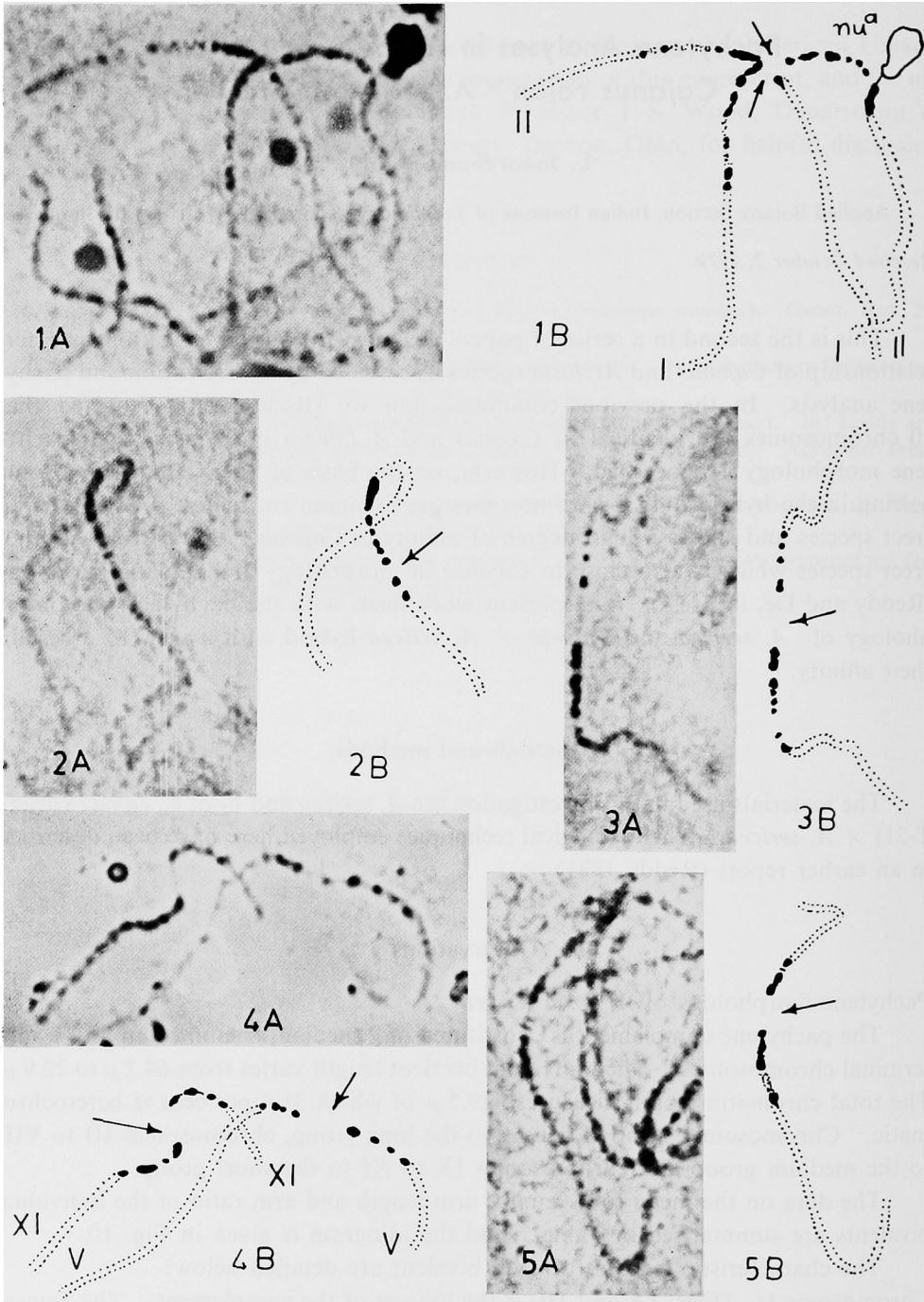
The data on the mean total length, arm length and arm ratio of the individual bivalents are summarised in Table 1 and the idiogram is given in Fig. 10.

The characteristic features of each bivalent are detailed below:

Chromosome I: (Figs. 1A and 1B) is the longest of the complement. The heterochromatin (HC) of the long arm measures (12.3 μ) nearly half the length of euchromatin (EC) and appears as two chromatic blocks. The distal chromatic

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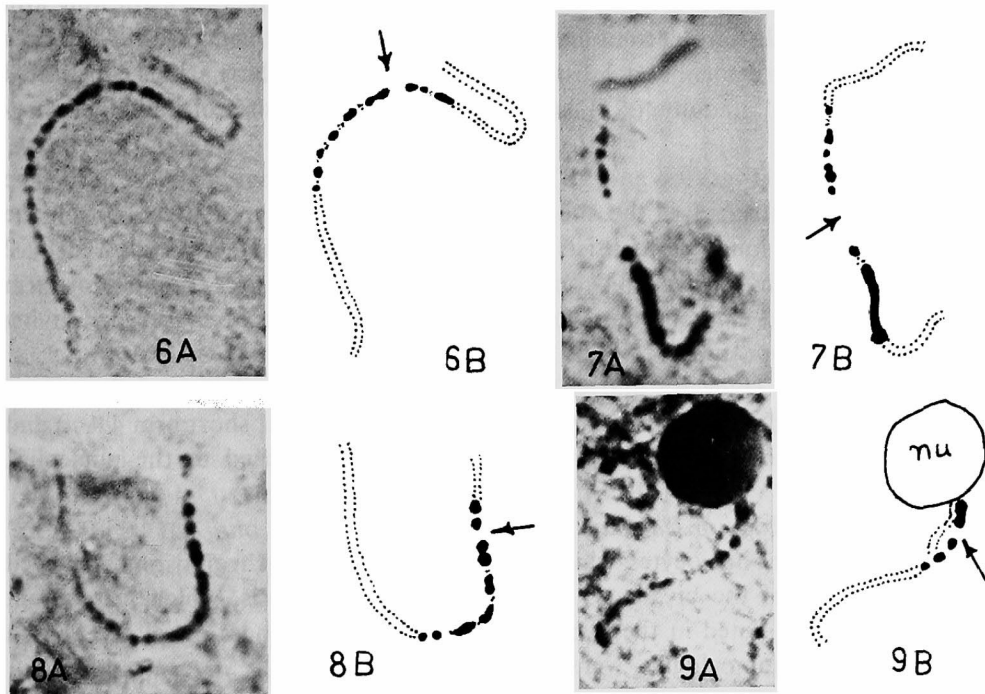


Figs. 1-5. *Atylosia sericea* pachytene chromosomes. In each pair of figures A is a photomicrograph ( $\times 2000$ ) and B is an interpretive drawing. Arrows indicate position of centromere. 1A and B, chromosomes I and II. Note an additional nucleolus ( $nu^a$ ) attached to the long arm of chromosome I. 2A and B, chromosome III. 3A and B, chromosome IV. 4A and B, chromosomes V and XI. 5A and B, chromosomes VI.

block is less deeply stained and in certain preparations an additional nucleolus is found to be associated with it. Slightly more than two-fifths of the short arm is constituted of HC ( $11.4 \mu$ ).

**Chromosome II:** (Figs. 1A and 1B) is submedian. The HC ( $1.0 \mu$ ) of the long arm possesses a single small chromomere. The HC ( $9.3 \mu$ ) of the short arm consists of two chromatic blocks. The proximal block consists of 4 closely appressed chromomeres followed by a block of small, faintly stained, inconspicuous chromomeres.

**Chromosome III:** (Figs. 2A and 2B) is submedian. The HC ( $7.9 \mu$ ) of the long arm appears as two chromatic blocks. The proximal block, adjacent to the



Figs. 6-9. *Atylosia sericea* pachytene chromosomes. In each pair of figures A is a photograph ( $\times 2000$ ) and B is an interpretive drawing. Arrows indicate position of centromere. 6A and B, chromosome VII. 7A and B, chromosome VIII. 8A and B, chromosome IX. 9A and B, chromosome X.

centromere consists of 3 to 4 chromomeres. The second block consists of 4 distinct chromomeres followed by the distal block of 2 small chromomeres. The HC ( $5.3 \mu$ ) of the short arm consists of 4 small, yet distinct, widely separated chromomeres.

**Chromosome V:** (Figs. 4A and 4B) is submedian. The long arm is approximately twice as long as the short arm. The HC ( $4.1 \mu$ ) of the long arm consists of 4 closely located chromomeres. The short arm consists of equal amounts of HC and EC. The HC ( $6.9 \mu$ ) of the short arm possesses 4 distinct widely separated chromomeres.

- Chromosome VI: (Figs. 5A and 5B) is submedian. The HC ( $8.0 \mu$ ) of the long arm is one and half times more than that of the short arm and consists of 6 chromomeres, 3 big and 3 small. The HC ( $3.4 \mu$ ) of the short arm consists of 2 distinct chromomeres followed by a big barrel shaped chromomere.
- Chromosome VII: (Figs. 6A and 6B) is submedian. The HC ( $9.0 \mu$ ) of the long arm is twice as long as that of the short arm ( $4.5 \mu$ ) and constitutes one-third of the length of the long arm.
- Chromosome VIII: (Figs. 7A and 7B) is median. The HC ( $6.9 \mu$ ) of the long arm consists of 6 distinct chromomeres. The short arm is made of equal amounts of HC ( $7.8 \mu$ ) and EC. Adjacent to the centromere, the short arm consists of a macrochromomere followed by a chromatic segment.
- Chromosome IX: (Figs. 8A and 8B) is subterminal. The HC ( $9.2 \mu$ ) of long arm consists of 2 distinct chromomeres proximal to the centromere followed by a big chromatin segment and 2 distal chromomeres. The short arm is constituted of nearly equal amounts of HC ( $2.0 \mu$ ) and EC and consists of 2 distinct chromomeres.
- Chromosome X: (Figs. 9A and 9B) is a nucleolar-organising chromosome. The nucleolus is attached to the short arm and the satellite measures  $4.5 \mu$ . The HC ( $3.2 \mu$ ) of long arm consists of 2 big distinct chromomeres followed by 2 to 3 faintly stained, inconspicuous chromomeres. The HC ( $2.0 \mu$ ) of short arm also possesses 2 macrochromomeres which are closely appressed at times, giving a knob like appearance.
- Chromosome XI: (Figs. 4A and 4B) is submedian. Both the long and short arms are constituted of equal amounts of HC (long arm  $6.0 \mu$ ; short arm  $4.4 \mu$ ) and EC. In certain cells, this chromosome was seen attached to the nucleolus.

#### Pachytene morphology of *Cajanus* $\times$ *A. sericea* hybrid

Majority of the meiocytes which exhibited well-spread chromosomes showed complete pairing of the bivalents. In others, partial synapsis, which was absent in the parents was noted in the hybrid. Most of the differences in relative length and arm ratio of homoeologues were somehow adjusted.

In eight out of the eleven bivalents the homoeologues participating in synapsis were identified. The synapsis is complete in these bivalents except for certain interstitial and terminal loose pairing regions in a few microsporocytes. Out of these eight bivalents, two exhibited heteromorphism.

Two translocations were noticed in less than 10 per cent of analysable cells. A detailed account of the pairing behaviour of specific chromosomes of the two parents is given below. A comparative statement of the cytological values of the parents and the hybrid is given in Table 1.

Chromosome I of *Cajanus* pairs with the chromosome I of *A. sericea* which has slightly longer arms. In most of the microsporocytes of the hybrid, 1 to 2 duplication loops were seen in the long arm (Figs. 11A and 11B).

Chromosome II of *Cajanus* pairs with the chromosome II of *A. sericea* which has higher arm ratio. In the hybrid, this bivalent is easily identified by the presence of a single chromomere in its long arm and was found to be completely paired (Figs.

12A and 12B).

Chromosome IV of *Cajanus* pairs with the chromosome IV of *A. sericea*. Both are median chromosomes but differ in the distribution of HC. *Cajanus* chromosome possesses two chromatic blocks in each of the arms in contrast to *A. sericea* chromosome which possesses three and one chromatic blocks in the long and short arms respectively. In the hybrid, non-homologous association of centromeres was observed. The spatial differences between the chromomeres in the short arm of the parents is clearly brought out in the hybrid pachytene during which these regions normally remain unpaired (Figs. 13A and 13B).

Chromosome V of *Cajanus* pairs with the chromosome VII of *A. sericea* the long arm of which is relatively shorter. In the hybrid, the bivalent resembles *A. sericea* chromosome in its length and arm ratio (Figs. 14A and 14B).

Chromosome VII of *Cajanus* pairs with the chromosome VIII of *A. sericea* which is characterized by an additional HC segment in its short arm. In most of the microsporocytes this bivalent is intimately paired in the HC regions of both the arms indicating thereby a non-homologous association of the HC of *A. sericea* chromosome with the EC of *Cajanus* chromosome in its short arm. Occasionally non-pairing in the EC region of long arm was observed (Figs. 15A and 15B). In the hybrid, the bivalent possesses a slightly lower arm ratio than both the parental chromosomes.

Chromosome IX of *Cajanus* pairs with the chromosome XI of *A. sericea*. These two chromosomes differ in their length and arm ratio. In the hybrid, this bivalent is heteromorphic in its long arm (Figs. 16A and 16B). In spite of the length differences, the short arm is completely paired in the hybrid.

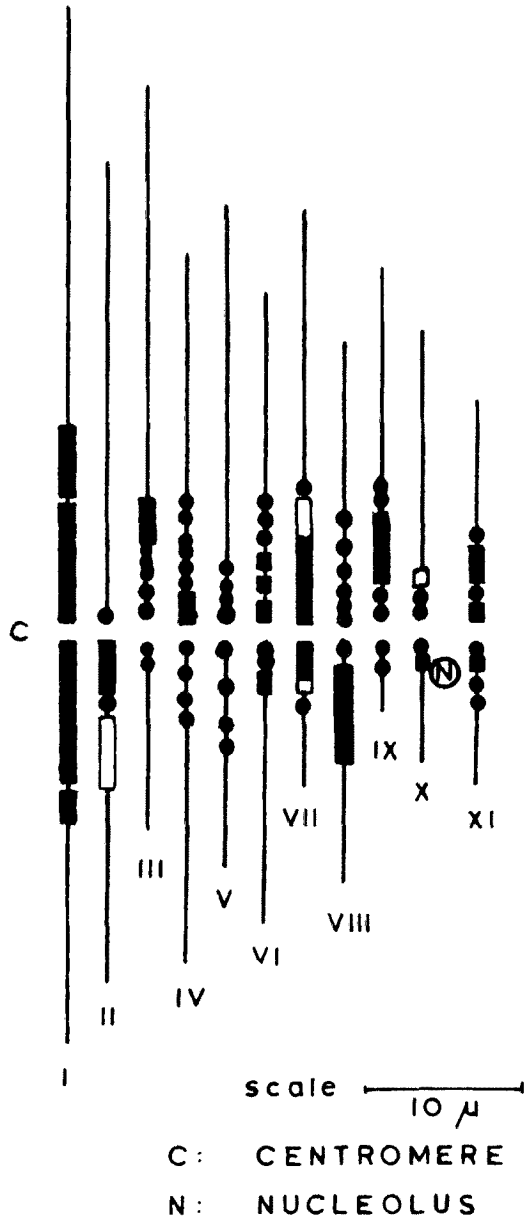


Fig. 10. Idiogram of the pachytene karyotype of *A. sericea*.

Table 1. Cytological values for the pachytene chromosomes of *Cajanus cajan*, *Atylosia sericea* and their hybrid

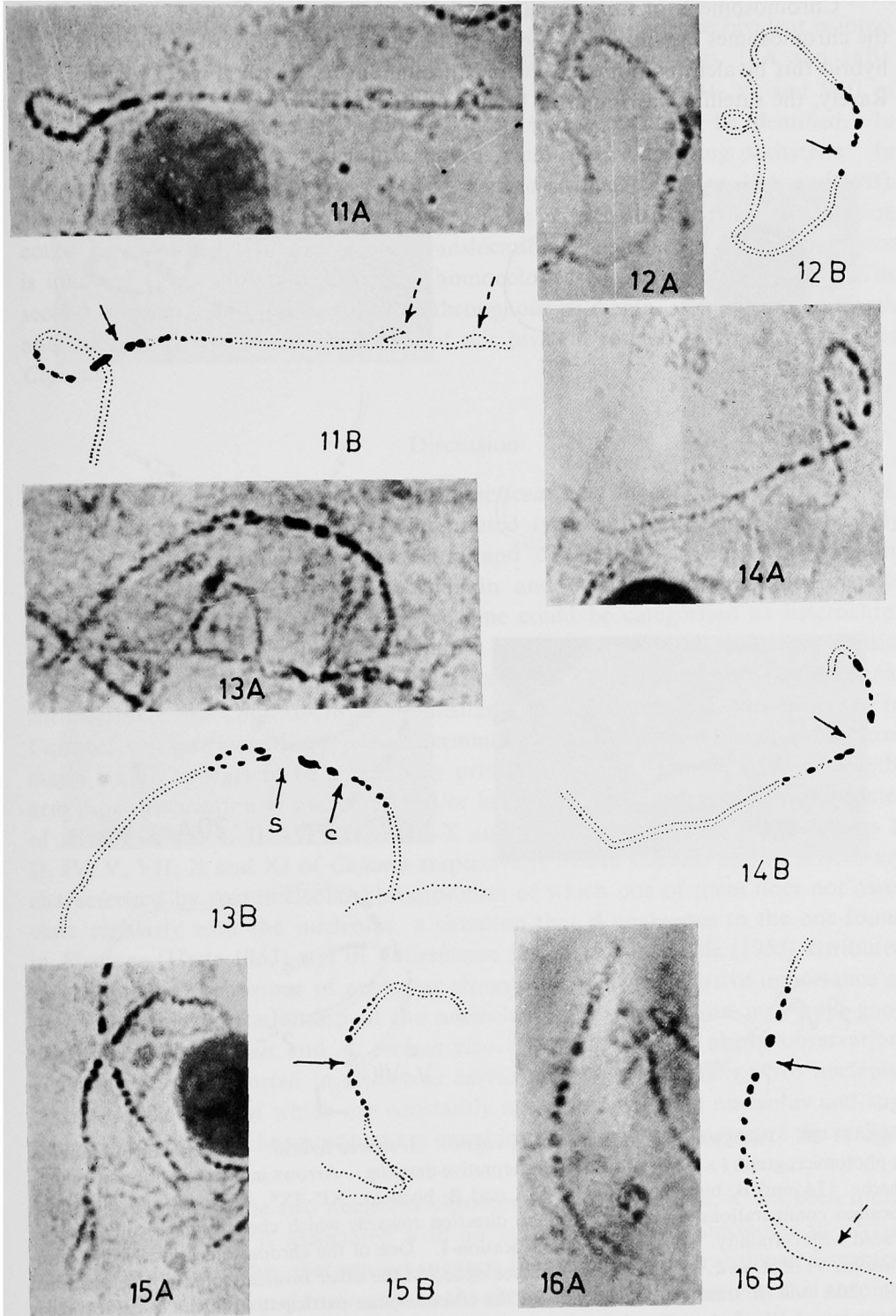
Sl. no.	Chromosome	Mean arm length in $\mu$		Mean total length ( $\mu$ ) $\pm$ S. E. (a + b)	Mean arm ratio (a/b)	⊙ Position of centromere
		Short arm (a)	long arm (b)			
1	I <sup>C</sup> †	23.9	37.1	61.0 $\pm$ 6.2	0.64	SM
	I <sup>S</sup>	25.4	38.8	64.2 $\pm$ 8.7	0.65	SM
	F <sub>1</sub>	24.5	37.5	62.0 $\pm$ 10.1	0.65	SM
2	II <sup>C</sup>	18.6	32.9	51.5 $\pm$ 7.8	0.56	SM
	II <sup>S</sup>	21.3	28.8	50.1 $\pm$ 7.3	0.73	SM
	F <sub>1</sub>	20.2	30.2	50.4 $\pm$ 6.9	0.65	SM
3	IV <sup>C</sup>	21.2	22.6	43.8 $\pm$ 5.7	0.93	M
	F <sub>1</sub> as IV <sup>C</sup>	21.5	23.2	44.7 $\pm$ 4.8	0.92	M
	as IV <sup>S</sup>	19.6	22.0	41.6 $\pm$ 4.5	0.89	M
	IV <sup>S</sup>	20.1	23.5	43.6 $\pm$ 6.9	0.89	M
4	V <sup>C</sup>	10.1	33.0	43.1 $\pm$ 4.3	0.30	ST
	VII <sup>S</sup>	9.7	26.6	36.3 $\pm$ 4.2	0.36	SM
	F <sub>1</sub>	10.2	27.1	37.3 $\pm$ 6.1	0.37	SM
5	VII <sup>C</sup>	14.4	17.2	31.6 $\pm$ 3.4	0.83	SM
	VIII <sup>S</sup>	15.3	17.9	33.2 $\pm$ 3.1	0.85	M
	F <sub>1</sub>	14.1	18.4	32.5 $\pm$ 4.6	0.76	SM
6	IX <sup>C</sup>	6.4	19.7	26.1 $\pm$ 2.9	0.32	ST
	as IX <sup>C</sup>	8.6	20.7	29.3 $\pm$ 3.5	0.41	SM
	F <sub>1</sub> as XI <sup>S</sup>	8.6	14.2	22.8 $\pm$ 3.4	0.60	SM
	XI <sup>S</sup>	9.0	13.9	22.9 $\pm$ 2.5	0.64	SM
7	X <sup>C*</sup>	6.3	17.5	23.8 $\pm$ 2.1	0.36	SM
	X <sup>S*</sup>	7.8	18.4	26.2 $\pm$ 2.4	0.42	SM
	F <sub>1</sub> *	7.7	18.7	26.4 $\pm$ 2.9	0.41	SM
8	XI <sup>C</sup>	5.3	16.9	22.2 $\pm$ 2.0	0.31	ST
	IX <sup>S</sup>	4.9	22.4	27.3 $\pm$ 5.3	0.22	ST
	F <sub>1</sub>	5.1	19.3	24.4 $\pm$ 4.7	0.26	ST
Chromosomes whose homoeologues could not be identified in the hybrid						
9	III <sup>C</sup>	19.8	27.8	47.6 $\pm$ 5.5	0.71	SM
10	VI <sup>C</sup>	11.3	20.6	31.9 $\pm$ 2.6	0.54	SM
11	VIII <sup>C</sup>	15.2	15.9	31.1 $\pm$ 2.5	0.95	M
12	III <sup>S</sup>	12.2	33.8	46.0 $\pm$ 6.1	0.36	SM
13	V <sup>S</sup>	14.2	26.6	40.8 $\pm$ 5.8	0.53	SM
14	VI <sup>S</sup>	17.8	21.1	38.9 $\pm$ 5.6	0.84	SM

⊙ M=Median; SM=submedian; ST=subterminal,

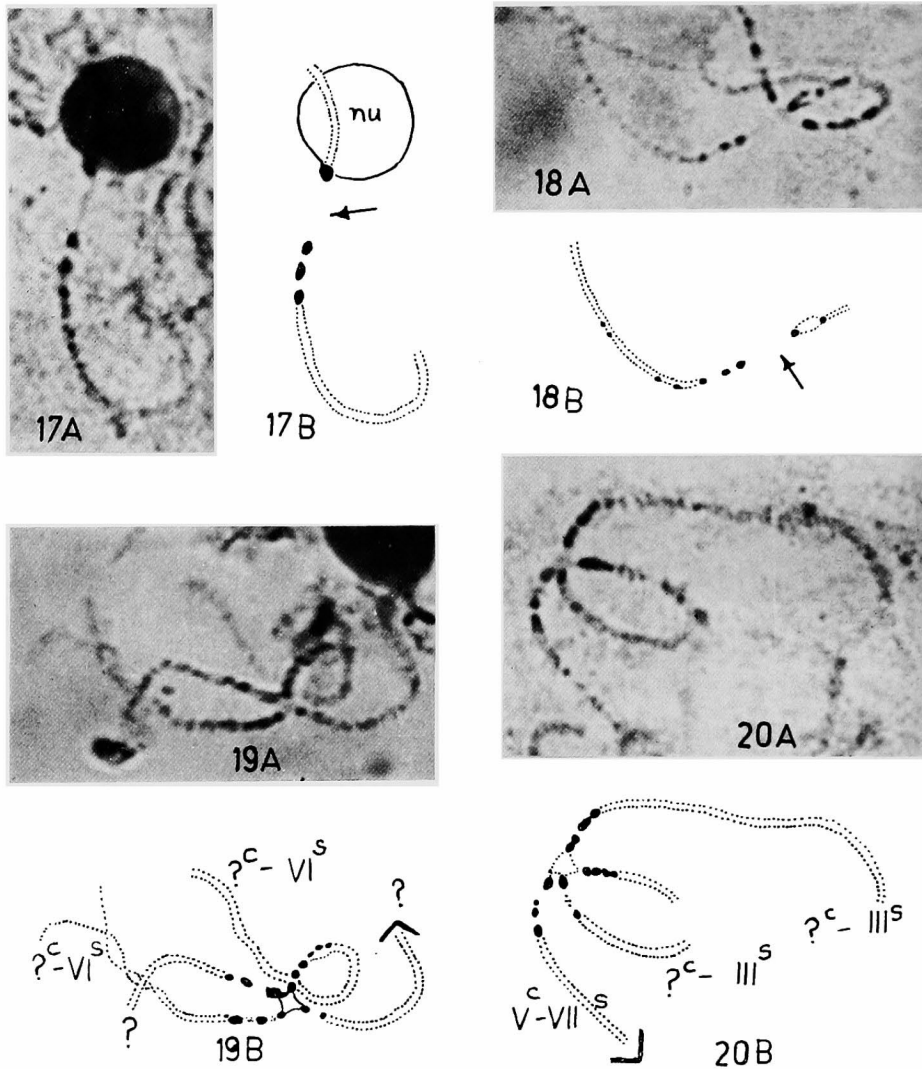
† C=*C. cajan* chromosome; S=*A. sericea* chromosome,

\* denotes nucleolar chromosome.

Figs. 11–16. Pachytene chromosomes of *C. cajan*  $\times$  *A. sericea* hybrid. In each pair of figures A is photomicrograph ( $\times 2000$ ) and B is an interpretive drawing. Arrows indicate position of centromere. 11A and B, bivalent, I<sup>C</sup>–I<sup>S</sup>. Note two duplication buckles in the long arm (Broken arrows). 12A and B, bivalent II<sup>C</sup>–II<sup>S</sup>. 13A and B, bivalent, IV<sup>C</sup>–IV<sup>S</sup>. See non-homologous association of centromeres (S and C are centromeres of *A. sericea* and *C. cajan* respectively). Also note loose pairing in the HC and heteromorphic nature of the bivalent. 14A and B, bivalent, V<sup>C</sup>–VII<sup>S</sup>. 15A and B, bivalent VII<sup>C</sup>–VIII<sup>S</sup>. Note failure of pairing in the EC of long arm. 16A and B, bivalent, IX<sup>C</sup>–XI<sup>S</sup>. See the heteromorphic nature and loose pairing in the long arm (Broken arrow).



Chromosome X of *Cajanus* pairs with the chromosome X of *A. sericea*. Both the chromosomes organize a nucleolus in the short arm. In most of the cells in the hybrid this bivalent is intimately paired throughout its length (Figs. 17A and 17B). Rarely, the satellite was found to be unpaired.



Figs. 17-20. Pachytene chromosomes of *C. cajan* × *A. sericea* hybrid. In each pair of figures A is photomicrograph (×2000) and B is an interpretive drawing. Arrows indicate position of centromere. 17A and B, bivalent, X<sup>c</sup>-X<sup>s</sup>. 18A and B, bivalent, XI<sup>c</sup>-IX<sup>s</sup>. Figs. 19 and 20, translocation configurations. Arrows point the direction towards which chromosomes could not be traced with certainty. 19A and B, translocation-1. One of the chromosomes involved is chromosome VI of *A. sericea*. Its *Cajanus* homoeologue and the other bivalent could not be identified (?). 20A and B, translocation-2. One of the chromosomes participating in the translocation is chromosome III of *A. sericea*. Its *Cajanus* homoeologue could not be identified (?). The other bivalent although could not be followed throughout its length, on the basis of HC length and pattern of short arm and HC pattern of long arm is identified as V<sup>c</sup>-VII<sup>s</sup> bivalent.



Chromosomes XI of *Cajanus* pairs with chromosome IX of *A. sericea* which has slightly more length and higher arm ratio. In the hybrid, the bivalent is intermediate to parental chromosomes in length and arm ratio (Figs. 18A and 18B).

In the hybrid, the bivalents formed by the chromosomes III, VI and VIII of *Cajanus* and chromosomes III, V and VI of *A. sericea* could not be identified. In a few cells of the hybrid, two translocations were observed during pachytene. In one translocation, chromosome VI of *A. sericea* is involved (Figs. 19A and 19B). Neither its homoeologue nor the other bivalent participating in the translocation could be identified. In the second translocation, chromosome III of *A. sericea* is involved (Figs. 20A and 20B). Its homoeologue could not be identified. The second bivalent could not be followed throughout its length. However, the length and the HC pattern of the short arm of this bivalent resembles chromosome V of *Cajanus*.

### Discussion

The pachytene chromosomes of *A. sericea* like those of *Cajanus* and *A. lineata* (Reddy 1981) are of the differentiated type with centromeres flanked by proximal dark staining heterochromatin and distal light staining euchromatin. Moreover, in this species heterochromatin and euchromatin are quite distinct and a particular section of the chromosome could be categorised as heterochromatic or euchromatic without difficulty. Both *Cajanus* and *A. sericea* consist of 2 median chromosomes, but *A. sericea* contains 8 submedian and 1 subterminal chromosomes, in contrast to 6 submedian and 3 subterminal chromosomes in *Cajanus*. *A. sericea* with a fewer subterminal chromosomes and higher total chromatin length appears to be a relatively primitive species. On the basis of length, arm ratio, association of nucleolus and/or heterochromatic pattern, 7 chromosomes of *A. sericea* viz. I, II, IV, VII, VIII, X and IX correspond with chromosomes I, II, IV, V, VII, X and XI of *Cajanus* respectively. Both *Cajanus* and *A. sericea* are characterised by two nucleolar chromosomes of which one of them does not associate regularly with the nucleolus, a situation that is analogous to the one found in *Plantago* (Hyde 1953) and in *Antirrhinum* (Ernst 1939). Hyde (1953) attributed this differential behaviour of nucleolar chromosomes to the relative importance of their biochemical relationship to the nucleolus. The explanation may hold good in the case of *Cajanus* and *A. sericea* also. Contrary to the above observation, Krishnan (1966) reported in *Phaseolus aureus* and *Phaseolus mungo* two nucleolar chromosomes both of which are constantly associated with the nucleolus and suggested that both the chromosomes are equal in their capacity to organize the nucleolus.

In addition to the two nucleolar chromosomes in *A. sericea*, it has been found that chromosome I is associated with an additional smaller nucleolus in certain microsporocytes. This observation suggests the presence of a translocated segment of the regular nucleolar chromosome in the chromosome I; alternatively this chromosome may have had the nucleolus organising function in the past which has been suppressed during evolution. The phenomenon of suppression of nucleolar organ-

ising function as reported by Krishnan and De (1968) in tetraploid *Phaseolus* and by Magoon and Shambulingappa (1961) in *Sorghum* lends credence to the occurrence of the latter process in *A. sericea*.

The chromosomes of these two genera appear to have attained certain degree of diversity as is evidenced by a few structural differences observed. For example, the morphology and distribution of chromomeres in chromosome IV of *Cajanus* and that of *A. sericea* suggests an occurrence of a pericentric inversion during the evolution of these species resulting in the shifting of one of the chromatic blocks of the short arm to the long arm in *A. sericea* or shifting of one chromatic block of long arm to the short arm in *Cajanus*.

The following discussion is limited to the information available from the pachytene analysis of *Cajanus* × *A. sericea* hybrid. Since complete synapsis was found in most of the microsporocytes of the hybrid it is concluded that there exists a high degree of homology between *Cajanus* and *A. sericea*. Wherever there exists a difference in the total length of the chromatin of the homologues, the hybrid shows an intermediate length as in the case of *Cajanus* × *A. lineata* hybrid. Although most of the differences in the length of HC of the parent chromosomes are adjusted in the hybrid, non-homologous association between excess of HC of chromosome VIII of *A. sericea* and the corresponding EC of chromosome VII of *Cajanus* was also observed.

In the meiocytes of the hybrid, during pachytene, two translocations and a duplication loop were observed. In one translocation, chromosome VI of *A. sericea* is involved. Neither its *Cajanus* homoeologue nor the other bivalent participating in the translocation could be identified. In the second translocation, chromosome III of *A. sericea* is involved. Its *Cajanus* homoeologue could not be identified. The second bivalent involved is V<sup>c</sup>-VII<sup>s</sup>. The chromosome which exhibits duplication loop could not be identified.

On the basis of the direct comparison of pachytene chromosome morphology, chromosome IV of *Cajanus* and that of *A. sericea* which reveal an occurrence of pericentric inversion do not show reverse-loop pairing in the hybrid. Instead, these chromosomes show non-homologous association in the inverted regions. The very first cytological study on chromosome pairing in an inversion heterozygote (McClintock 1931, 1933) showed quite unambiguously that such reverse-loop pairing need not characterise all inversions. Thus, in a maize type heterozygous for a pericentric inversion in chromosome 4 reverse-loop synapsis was seen by McClintock (loc. cit) only in four out of four hundred sporocytes examined. The remainder showed non-homologous association in the inverted regions. Similarly, White and Morley (1955) have consistently failed to find evidence for reverse-loop pairing in pericentric inversions involving grasshopper chromosomes and are of the opinion that they pair straight as indeed Coleman (1948) has demonstrated in *Trimerotropis gracilis*. Where pairing is by non-homology, chiasmata cannot form and there appears no further complications to meiosis (John and Lewis 1965). This is borne out by the failure to notice any major abnormalities during later stages of meiosis of the hybrid in the present study.

On the basis of somatic and pachytene karyomorphology of parents and hybrids

of *Cajanus* × *A. lineata* (Reddy loc. cit.) and *Cajanus* × *A. sericea*, it is concluded that *A. sericea* has a lesser degree of homology to *Cajanus* than *A. lineata*. This is also borne out by unpublished observations of the author on crossability, germinability, survival, pollen fertility, pod-setting and seed-setting of the hybrids.

### Summary

On the basis of relative length, arm ratio, chromomere pattern and nucleolar association all the eleven pachytene chromosomes of *A. sericea* were identified. The pachytene complement consists of 2 median, 8 submedian and 1 subterminal chromosomes. On the basis of comparison of parental pachytene chromosomes, 7 are common to *A. sericea* and *Cajanus*. Complete synapsis in majority of the microsporocytes of *Cajanus* × *A. sericea* hybrid indicates a high degree of homology. In the hybrid, out of eleven bivalents, eight bivalents, including two exhibiting heteromorphism were identified. Occasionally two translocations and a duplication loop were observed in a few cells. On the basis of various evidences, it is concluded that *A. sericea* has a lesser degree of affinity to *Cajanus* than *A. lineata*.

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