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Karyotypic analysis of *Cajanus, Atylosia,* and *Rhynchosia* species

R. P. S. Pundir* and R. B. Singh**

Department of Genetics and Plant Breeding, Banaras Hindu University, Varanasi-221 005, India

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Summary. Somatic chromosomes of two cultivars of *Cajanus cajan,* eight species of *Atylosia* (*A. albicans, A. cajanifolia, A. lineata, A. platycarpa, A. scarabaeoides, A. serica, A. trinervia* and *A. volubilis*), and of *Rhynchosia rothii* were analysed. All species had 2n = 22. Eight of the 10 species studied had two pairs of sat-chromosomes while *A. scarabaeoides* and *A. sericea* had only one sat-chromosome pair. Based on relative chromosome length (L%), arm ratio (pa-value) and presence or absence of secondary constriction, a karyotype formula for each species was formulated. Based on these parameters the chromosome pairs could also be assigned to groups ranging from 8 to 10 in different species. Except for the asymmetrical karyotype of *A. albicans,* the other species had rather moderately symmetrical karyotypes.

Key words: Karyotype – *Cajanus* – *Atylosia* – *Rhynchosia* – Karyotype symmetry – Sat-chromosomes

Introduction

The tribe Cajaninae encompasses several hundred species, out of which only one, *Cajanus cajan* (L.) Millsp., is cultivated. *Cajanus cajan,* commonly called pigeonpea or red gram, is an important protein-rich food grain legume of the Indian subcontinent but its grain yield is low and unstable. Breeders have recognized the narrow genetic base of the cultivars and have wanted to enrich the genetic base through hybridization of *C. cajan* with its wild relatives in the genera *Atylosia* and *Rhynchosia.* Several of the allied species have been reported to be crossable with *C. cajan* with varying degrees of success. For an effective transfer of genes, an understanding of genomic relationships among the various forms is necessary.

A literature survey revealed that the information presently available is contradictory on karyotypic details of pigeonpea and its relatives (Deodikar and Thaker 1956; Kumar et al. 1958; Reddy 1973). Furthermore there are several related species whose karyotypic details or even the basic chromosome number are not yet known. The present report deals with the karyotypic details of two cultivars of *C. cajan,* seven species of *Atylosia,* and *Rhynchosia rothii.*

Materials and methods

Seeds of *C. cajan* (cvs. 'Pant A 2' and 'UPAS 120'), *A. albicans* (W. & A.) Benth. (JM 2356); *A. cajanifolia* Haines (JM 2739); *A. lineata* W. & A. (ICP 7469); *A. platycarpa* Benth.; *A. scarabaeoides* (L.) Benth. (ICP 7464); *A. sericea* Benth. ex Baker (ICP 7470), *A. trinervia* (D.C.) Gamble (JM 2668); *A. volubilis* (Blanco) Gamble (JM 1984) and *R. rothii* Benth. (JM 2296) were obtained from the Genetic Resources Unit. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT).

The root-tip squash technique used by early workers (Sikdar and De 1967; Shrivastava and Joshi 1972; and Reddy 1973) yielded unsatisfactory results. The squash technique developed by Pillai et al. (1981), however, with minor modifications, was found suitable for the present study.

Root-tips from 3 cm long roots were pretreated with 0.002 M 8-hydroxyquinoline for 3 h, washed in running water and fixed in a mixture of (1:1 v/v) methanol and glacial acetic acid for 24 h. The above fixative was replaced with modified Piennar's solution (isopropyl alcohol, propionic acid,

* Botanist, GRU, ICRISAT, Patancheru-502 324, India
** Regional Plant Production and Protection Officer, FAO, RAPA, Bangkok-2, Thailand

Table 1. Types of karyotype asymmetry as modified from Stebbins (1958)

Ratio largest/smallest	Proportion of chromosomes with arm ratio > 2:1					
	0	0.01–0.20	0.21–0.40	0.41–0.60	0.61–0.80	0.81–1.00
< 1.5:1	1a	2a	3a	4a	5a	6a
≧ 1.5:1–2:1	1b	2b	3b	4b	5b	6b
≧ 2:1–3:1	1c	2c	3c	4c	5c	6c
> 3:1	1d	2d	3d	4d	5d	6d

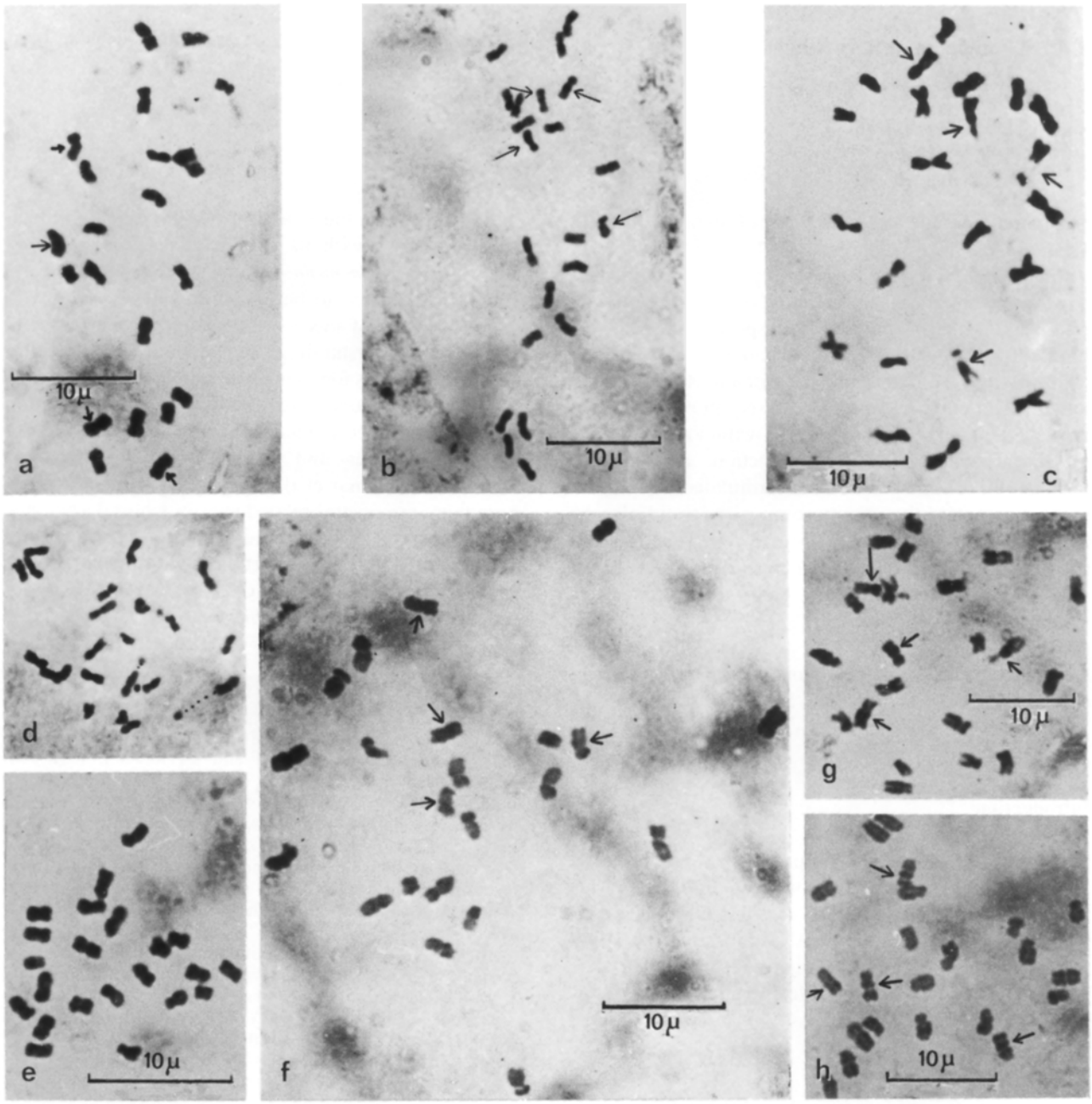


Fig. 1 a–h. Chromosomes at metaphase in root-tip cells of: **a** 'Pant A 2'; **b** *R. rothii;* **c** *A. cajanifolia;* **d** 'Pant A 2' (clear two pair sat-chromosomes); **e** *A. volubilis;* **f** UPAS 120; **g** *A. albicans;* **h** *A. lineata*

Table 2. Relative chromosome length (L%) of somatic chromosomes of *Cajanus, Atylosia,* and *Rhynchosia* species

Chromosome pairs	cv. 'Pant A 2'	cv. 'UPAS 120'	*A. albi.*	*A. cajif.*	*A. lin.*	*A. platy.*	*A. scar.*	*A. ser.*	*A. volub.*	*R. rothii*
1	5.18±0.10	5.30±0.16	7.11±0.33	5.56±0.19	5.07±0.09	5.45±0.14	4.65±0.03	4.75±0.14	5.54±0.04	4.54±0.19
2	4.54±0.07	4.68±0.07	5.69±0.21	4.29±0.09	4.60±0.19	4.34±0.13	5.77±0.13	5.58±0.16	5.11±0.05	3.88±0.24
3	5.72±0.17	5.52±0.08	4.76±0.06	5.55±0.14	5.48±0.07	5.30±0.02	5.35±0.04	5.57±0.12	5.05±0.02	5.68±0.03
4	5.29±0.05	5.16±0.05	4.66±0.01	4.88±0.07	5.20±0.11	5.25±0.05	5.20±0.05	5.04±0.05	5.05±0.02	5.23±0.03
5	4.92±0.06	4.91±0.06	4.66±0.12	4.68±0.05	4.93±0.08	4.86±0.08	4.81±0.04	5.00±0.05	4.77±0.02	4.95±0.02
6	4.82±0.08	4.76±0.05	4.44±0.00	4.63±0.06	4.89±0.06	4.82±0.05	4.81±0.04	4.75±0.06	4.58±0.04	4.75±0.08
7	4.32±0.09	4.58±0.09	4.24±0.01	4.51±0.01	4.60±0.11	4.54±0.12	4.66±0.09	4.59±0.06	4.39±0.03	4.66±0.02
8	4.28±0.08	4.40±0.03	4.12±0.04	4.34±0.11	4.46±0.07	4.33±0.12	4.12±0.04	4.20±0.11	4.39±0.07	4.66±0.02
9	4.15±0.07	4.13±0.06	3.70±0.04	4.27±0.03	3.98±0.07	3.98±0.06	3.99±0.02	3.93±0.17	4.27±0.10	4.28±0.10
10	3.73±0.15	3.41±0.07	3.41±0.21	3.96±0.12	3.62±0.11	3.79±0.12	3.49±0.15	3.47±0.05	4.00±0.06	4.02±0.06
11	3.05±0.05	3.17±0.05	3.18±0.08	3.32±0.03	3.16±0.11	3.32±0.10	3.15±0.03	3.12±0.08	2.87±0.12	3.33±0.13

Table 3. Arm ratio (long/short arm) of somatic chromosomes of *Cajanus, Atylosia,* and *Rhynchosia* species

Chromosome pairs	cv. 'Pant A 2'	cv. 'UPAS 120'	*A. albi.*	*A. cajif.*	*A. lin.*	*A. platy.*	*A. scar.*	*A. ser.*	*A. volub.*	*R. rothii*
1	1.34±0.00	1.46±0.13	1.24±0.06	1.39±0.04	1.48±0.02	2.48±0.00	–	1.12±0.04	1.16±0.03	1.21±0.03
2	1.29±0.04	1.50±0.07	1.16±0.02	1.60±0.13	1.22±0.04	1.15±0.03	1.21±0.01	2.10±0.16	1.25±0.05	1.00±0.00
3	1.29±0.04	1.08±0.02	2.28±0.23	1.15±0.05	1.25±0.10	1.44±0.06	2.08±0.12	1.14±0.02	1.79±0.07	1.14±0.02
4	1.20±0.08	1.43±0.02	1.39±0.08	1.16±0.10	1.40±0.08	1.27±0.08	1.24±0.08	1.48±0.08	1.41±0.07	1.03±0.02
5	1.28±0.08	1.32±0.06	1.10±0.05	1.42±0.12	1.24±0.11	1.47±0.07	1.16±0.05	1.71±0.10	1.09±0.03	1.17±0.07
6	1.25±0.09	1.52±0.14	1.10±0.05	1.38±0.08	1.36±0.09	1.22±0.07	1.25±0.09	1.98±0.14	1.20±0.04	1.07±0.02
7	1.64±0.10	1.34±0.09	1.24±0.10	1.41±0.03	2.00±0.08	1.57±0.10	1.42±0.06	1.18±0.08	1.31±0.02	1.23±0.04
8	1.11±0.08	1.62±0.19	1.36±0.22	1.20±0.07	2.51±0.08	1.31±0.02	1.19±0.06	1.38±0.10	1.27±0.05	1.32±0.02
9	1.42±0.10	1.96±0.14	1.22±0.12	1.11±0.07	1.15±0.08	1.48±0.05	1.17±0.11	1.34±0.11	1.00±0.00	1.04±0.03
10	1.52±0.09	1.07±0.03	2.18±0.11	1.32±0.09	1.16±0.06	1.35±0.01	1.86±0.12	1.17±0.07	1.47±0.04	1.00±0.00
11	1.42±0.14	1.33±0.12	1.14±0.00	1.37±0.08	1.38±0.03	1.19±0.12	1.28±0.11	1.11±0.07	1.00±0.00	1.00±0.00

solvent ether and acetone, 6:3:1:1 v/v). These procedures were done at 10 °C. After 24 h the root-tips were washed in water and hydrolysed in 1N HCl for 10 min at 60 °C and stained with Feulgen. Root-tip squashes were prepared in a drop of 2% aceto-orcein. Microphotographs were taken using an oil immersion lens.

Mean chromosome lengths along with the standard deviation for each accession were obtained. To compensate for the differential contraction of chromosomes in various cells the length of each chromosome was expressed as percent (L%) of total length of the diploid complement while the centromere position was characterized by the estimate referred to as the Pa-value (long/short arm) (Blixt 1958, 1972). The chromosome complement was grouped into two classes: Class A consisted of satellite (sat) chromosomes, and class B consisted of the non-sat chromosomes. In each class the chromosomes were arranged in descending order of their length. Chromosomes with Pa-values 1.00 to 1.25 were arbitrarily designated as median (M), those with 1.26 to 2.00 as sub-median (SM) and the ones with greater than 2.00 as sub-terminal (ST). The statistical significant differences between L% and the centromere position were used in discerning these chromosomes with analogous morphologies.

Stebbins' method (1958) of karyotype asymmetry, which is based on relative chromosome lengths and centromere, was modified and used for characterizing individual accessions, as shown in Table 1.

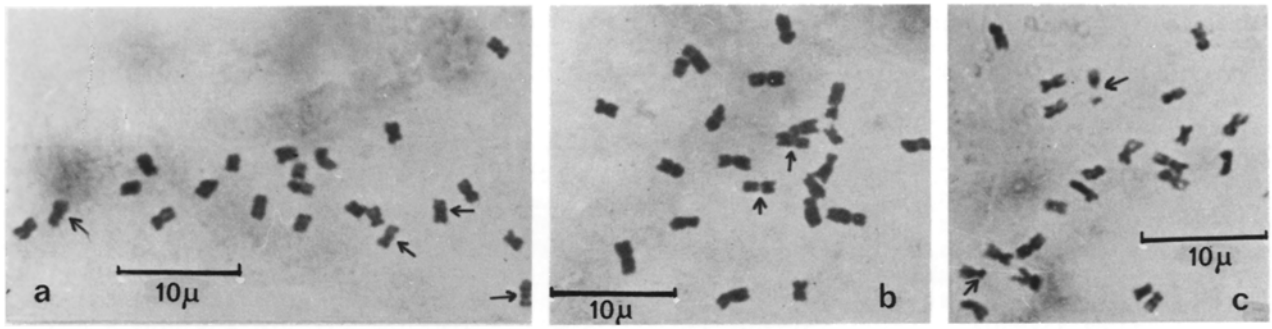
Results

The 11 taxa (including *A. trinervia*) studied each had a 22 somatic chromosome number. Nine of them had 2 pairs of sat-chromosomes while *A. scarabaeoides* and *A. sericea* each had only one pair. *Atylosia trinervia* could not be studied further due to a paucity of seeds. Relative chromosome length (L%) and arm ratio (long/short) appear in Tables 2 and 3, respectively. Mean chromatin length of each taxon and the grouping of the chromosomes based on satellite presence and significant length differences, and karyotype asymmetry derived are given in Table 4. Microphotographs of the somatic plates and karyograms of each taxon are depicted in Figs. 1 and 2.

Discussion

Chromosome number

The somatic chromosome number of *C. cajan* (= *C. indicus*) as n = 11 and 2n = 22 has been reported as early



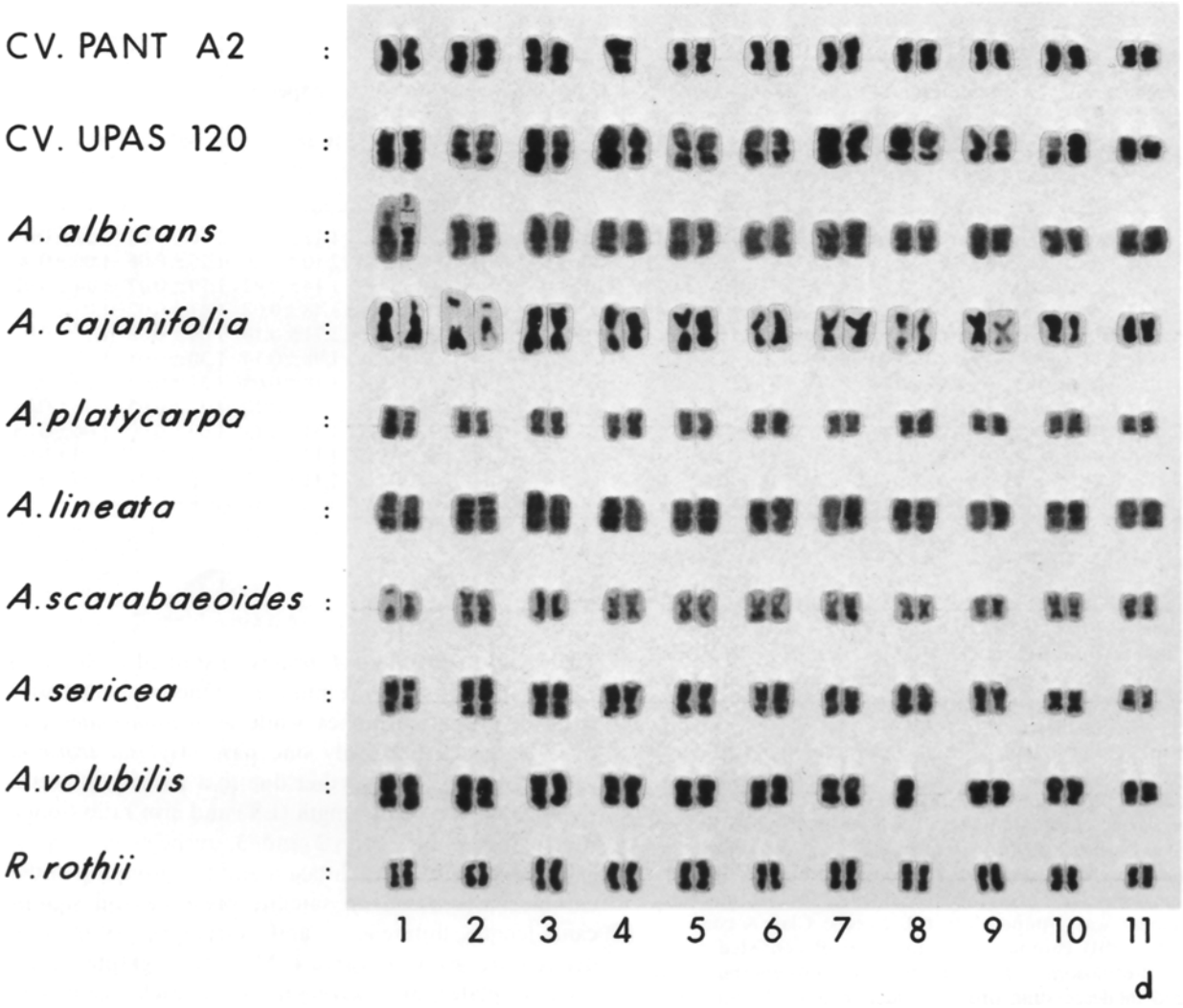


Fig. 2a–d. Chromosomes at metaphase in root-tip cells of: **a** *A. platycarpa;* **b** *A. sericea;* **c** *A. scarabaeoides;* **d** Karyograms

Table 4. Karyotype formulae and asymmetry score of *Cajanus, Atylosia,* and *Rhynchosia* species

Identity	Chromatin length (μ)	Karyotype formulae	Asymmetry score
C. cajan cv. 'Pant A 2'	43.77±3.28	$1A_1^{SM}, 1A_2^{SM}, 1B_1^{SM}, 1B_2^{M}, 1B_3^{SM}, 1B_4^{M}, 1B_5^{SM}, 1B_6^{M}, 2B_7^{SM}, 1B_8^{SM}$	1b
C. cajan cv. 'UPAS 120'	56.18±1.35	$1A_1^{SM}, 1A_2^{SM}, 1B_1^{M}, 1B_2^{SM}, 2B_3^{SM}, 2B_4^{SM}, 1B_5^{SM}, 1B_6^{M}, 1B_7^{SM}$	1b
A. albicans	56.14±2.58	$2A_1^{M}, 1B_1^{ST}, 1B_2^{SM}, 3B_3^{M}, 1B_4^{SM}, 1B_5^{M}, 1B_6^{ST}, 1B_7^{M}$	2c
A. cajanifolia	48.18±4.45	$1A_1^{SM}, 1A_2^{SM}, 1B_1^{M}, 1B_2^{M}, 2B_3^{SM}, 1B_4^{SM}, 2B_5^{M}, 1B_6^{SM}, 1B_7^{SM}$	1b
A. lineata	50.19±3.43	$1A_1^{SM}, 1A_2^{M}, 1B_1^{M}, 1B_2^{SM}, 1B_3^{M}, 2B_4^{SM}, 1B_5^{ST}, 2B_6^{M}, 1B_7^{SM}$	2b
A. platycarpa	41.82±1.74	$1A_1^{ST}, 1A_2^{M}, 2B_1^{SM}, 1B_2^{SM}, 1B_3^{M}, 2B_4^{SM}, 2B_5^{SM}, 1B_6^{M}$	2b
A. scarabaeoides	42.72±3.88	$1A_1, 1B_1^{M}, 1B_2^{ST}, 1B_3^{M}, 2B_4^{M}, 1B_5^{SM}, 2B_6^{M}, 2B_7^{SM}$	2b
A. sericea	46.73±2.40	$1A_1^{M}, 1B_1^{ST}, 1B_2^{M}, 2B_3^{SM}, 1B_4^{SM}, 1B_5^{M}, 2B_6^{SM}, 1B_7^{M}, 1B_8^{M}$	2b
A. volubilis	37.70±0.10	$1A_1^{M}, 1A_2^{M}, 2B_1^{SM}, 1B_2^{M}, 1B_3^{M}, 2B_4^{SM}, 1B_5^{M}, 1B_6^{SM}, 1B_7^{M}$	1b
R. rothii	37.80±3.84	$2A_1^{M}, 1B_1^{M}, 1B_2^{M}, 2B_3^{M}, 1B_4^{M}, 1B_5^{SM}, 2B_6^{M}, 1B_7^{M}$	1b

as 1933 by Roy. Later these numbers were confirmed in *C. cajan,* and in *A. lineata, A. sericea* and *A. scarabaeoides* (Krishnaswami and Krishnaswami Aiyanger 1935; Deodikar and Thakar 1956; Kumar et al. 1958; Shrivastava et al. 1973 and Reddy 1973). As far as chromosome number is concerned, the findings of the present study on root tips as well as PMC chromosome counts on *C. cajan, A. lineata, A. sericea* and *A. scarabaeoides* (Pundir 1981) agree with those of the earlier reports. The somatic chromosomes of *A. albicans, A. cajanifolia, A. platycarpa, A. volubilis, A. trinervia* and *R. rothii* are examined here for the first time, and they are similar to other species. Thus, all the species belonging to the three genera, *Cajanus, Atylosia* and *Rhynchosia,* have 2n=22. The 2n=22 being the only known chromosome number in the three genera and the absence of secondary associations in the meiotic cycle of these taxa (Pundir 1981) suggest that the basic, haploid and monoploid chromosome number is same for these taxa, i.e. x=n=11 and thus, the species are diploid.

Karyotype

Chromatin length as reported for individual species by different workers varies widely. The present studies show that in addition to interspecific differences, the intraspecific differences, such as those between 'Pant A 2' and 'UPAS 120' of *C. cajan,* could be significant. These differences could be attributed to differential contractions of the chromosomes, the stages at which they were studied, and genotypic differences.

Cajanus cajan cv. 'Pant A 2' and 'UPAS 120'

Two pairs of sat-chromosomes were distinct as against one pair reported by earlier workers on *C. cajan.* The average chromatin length in the present case was 43.77±3.28 μm, which is far less (about two-fifths to three-fifths) than the values reported by Deodikar and Thakar (1956) and Kumar et al. (1958). The present measurements, however, are in close agreement to those reported by Shrivastava et al. (1973). Further, in the case of 'UPAS 120', 9 chromosome pairs possessed SM centromeres and 2 pairs M centromeres. The entire complement could thus be put into 9 statistically different groups whereas the corresponding figures for 'Pant A 2' were 8, 3 and 10. Deodikar and Thakar (1956) observed 6 chromosome pairs to have SM centromeres and 5 pairs to possess M centromere positions, which were classified into 10 groups. Although Kumar et al. (1958) placed the chromosomes into 9 groups, their estimates concerning centromere position is in disagreement with the present as well as with earlier findings.

A. lineata

There were two pairs of sat-chromosomes. Earlier workers had reported none or one pair of sat-chromosomes. As regards the position of the centromere, the present study revealed 5 chromosome pairs with M centromere positions, 5 pairs with SM, and 1 pair with ST: the 11 chromosome pairs could be put into 9 groups. Deodikar and Thakar (1956) reported 3 M, 5 SM and 3 ST chromosome pairs, classified into 7 groups. Kumar et al. (1958) observed no ST centromere and identified 3 M and 8 SM chromosomes assigned to 9 groups, while Sikdar and De (1967) confirmed the centromere position reported by Kumar et al. (1958). They assigned the chromosomes to 10 groups. The groupings done by Shrivastava et al. (1973) disagreed considerably from the present study and from other reports. They recorded 7 M, 2 SM and 2 ST chromosome pairs.

Atylosia sericea

Unlike previous cases, this species had only one pair of sat-chromosomes. The observation is in agreement with that of Deodikar and Thakar (1956) but differs from those of Reddy (1973) and De (1974), who reported two pairs of sat-chromosomes. Five M, five SM and one ST pairs of chromosomes were seen in the present study, and placed into 10 statistically different groups, whereas Deodikar and Thakar (1956) observed 4 M, 5 SM and 2 ST chromosome pairs and had put them into 7 different groups.

Atylosia scarabaeoides

This species, like *A. sericea,* had only one pair of sat-chromosomes; the same observation made by earlier workers. The centromere position in the sat-chromosome could not be resolved clearly, but the remaining 10 pairs were placed into 8 groups. Although the number of groups (10) identified by Sikdar and De (1967) were not very different from those in the present study, their identification of the centromere position was quite different from the one in the present case as they found 2 pairs with M and 9 pairs with SM centromeres.

The other species. A. albicans, A. cajanifolia, A. platycarpa, A. volubilis, A. trinervia and *R. rothii* were investigated for the first time. Since the observations are based on an efficient technique of karyotype analysis and careful measurements, the reports for these species along with the others studied on number of sat-chromosomes, chromosome length and centromere positions should be of general applicability for this group of plants.

Regarding the differences in centromere positions of the group of chromosomes, and also the location of the satellites, it may be suggested that intra-chromosomal rearrangements might have taken place, resulting in the shift of the centromere position. However, no evidence of inversion heterozygosity or any other structural heterozygosity was observed either in the parents or in the interspecific hybrids. Even if these supposed rearrangements had taken place, it is quite possible that these involved rather small segments (individual chromosome average length itself is low) and thus could not be detected under the meiotic analysis made (Pundir 1981). Further, the studies involving analysis of banding patterns of the chromosomes should be undertaken to help resolve this issue. However, the preliminary investigation has not been of much consequence in the identification of individual chromosomes (Lavania and Lavania 1982).

Karyotype asymmetry

Structural changes in chromosomes can be identified to some extent by comparing the karyotype of the species with the same chromosome number as well as those with different ones. This is best expressed in terms of symmetry-asymmetry (Levitsky 1931). More primitive species tend to have karyotypes with chromosomes possessing the M centromere. Based on the revised diagram of karyotype asymmetry (Table 1), increasing numbers and alphabet sequences represent increasing asymmetry of the karyotype. On the other hand, in general, those species having symmetrical karyotypes are expected to have high recombination indexes and to be in active states of proliferation with restricted genetic differentiation and specific adaptation. The species *A. albicans* which showed the highest karyotype asymmetry (Table 4) is rather restricted in its distribution and has a specific adaptation strategy. On the other hand, many of the *Atylosia* species of the present study, those occurring in the Western or the Eastern Ghats of India, or even the most widely occurring ones, such as *A. scarabaeoides* and *A. volubilis* (L.J.G. van der Maesen – pers commun.), are under no special selection pressures and thus are not required to assimilate specific genomic configurations to lock-up specific genetic variability. They consequently continue to have relatively more symmetrical karyotypes than the *A. albicans.*

Similarly, the domesticated species, *C. cajan,* and its supposedly closest relative, *A. cajanifolia,* have faced no intense directional selection for conserving a specific gene sequence and thus possess more "generalized" or symmetrical karyotypes.

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