

CYTOMORPHOLOGICAL STUDIES IN *CAJANUS CAJAN* × *ATYLOSIA LINEATA***

L. J. REDDY* and D. N. DE

Applied Botany Section, Indian Institute of Technology, Kharagpur, West Bengal, India

ABSTRACT

Atylosia lineata was successfully hybridized with *Cajanus cajan* using the latter as female parent. Reciprocal crosses were not successful. The characters of *C. cajan*, viz., fugacious stipules, absence of pigmentation on the standard petal, deciduous petals, absence of hairs on the pods, non-shattering nature of pods reddish brown seed color without mottles and absence of strophiole on the seeds were recessive to those of *A. lineata*. The meiotic behaviour of the hybrid was regular except for minor abnormalities. Two bivalents frequently showed heteromorphism and there was a reduction in the chiasma frequency during diakinesis and metaphase-I. A few laggards and irregular distribution of chromosomes to the poles during anaphase-I was noticed in a few cells. On the basis of plant morphology, crossability, hybrid fertility and meiotic behaviour, *A. lineata* comes very close to *C. cajan*.

PIGEONPEA [*Cajanus cajan* (L.) Millsp.], a tropical grain legume is grown for its protein-rich seeds and is one of the oldest food crops. *Cajanus* is a monotypic genus consisting of a single species. The genus *Atylosia* is closely related to *Cajanus* and is the putative progenitor of the cultivated species. The affinity of these two genera, *Atylosia* and *Cajanus*, has been revealed by their successful hybridization (Deodikar and Thakar, 1956; Kumar and Thombre, 1958; Sikdar and De, 1967). In view of the very close affinity between these two genera, different species of *Atylosia* can be very well utilised in breeding superior strains of pigeonpea by incorporating some of the useful characters of *Atylosia* such as hardiness, resistance to pod-borers and diseases.

The extent of crossability which depends largely on the strength of genetic barriers, the similarity in the morphological and anatomical attributes and cytological behaviour as reflected from karyomorphology and pairing in meiosis are helpful in understanding the phylogenetic relationship of different species on one hand and the probable mechanisms involved in the evolution of cultivated species on the other. Such a knowledge is necessary not only to clarify phylogenetic pathways but also for planning more effective approach to recombining the wide spectrum of variability occurring within a genus and between related genera.

The present paper deals with the cytomorphological studies of *C. cajan*, *A. lineata*, an erect species of *Atylosia* and their hybrid.

**A part of the Ph.D. thesis submitted by the senior author to I.I.T., Kharagpur, India.

*Present address: ICRISAT, Patancheru P. O., Andhra Pradesh-502 324, India

MATERIAL AND METHODS

The materials used in this study are *Cajanus cajan* (Variety T-21), *Atylosia lineata* W. & A. and their hybrid. Pure seeds of T-21 were obtained from Pulses Geneticist, IARI, New Delhi, India and that of *Atylosia lineata* from Economic Botanist, Poona, India. The hybrid was produced by using *Cajanus cajan* as the female parent because the reciprocal crosses were not successful. For cytological studies the flower buds of the parents and their hybrid were fixed in propionic acid and ethanol mixture (1:3) with the acid component saturated with ferric acetate. The buds were kept overnight in the fixative and the anthers were stained in 1% propinocarmine.

OBSERVATIONS

Morphology: A comparative account of the morphological features of *C. cajan*, *A. lineata* along with their F₁ are given in Table 1. It can be seen from the table that the characters of *Cajanus*, viz, fugacious stipules, absence of pigmentation on the standard petal, deciduous petals, absence of visible hairs on the pods, non-shattering nature of mature pods, reddish brown seed color and absence of strophiole were recessive to those of *A. lineata*. Similarly, lanceolate nature of first pair of simple leaves of *Cajanus* was dominant. The shape, length and breadth of leaflets, length of petiole, size of the standard petal, beak of the pod, number of chambers per pod were intermediate in the hybrid. Pod length, and number of seeds per pod and seed weight were nearer to that of *C. cajan*. Pod color in *C. cajan* is green with purplish black streaks and that of *A. lineata* is uniformly green. However, in the hybrid the pods were uniformly reddish brown.

Pollen sterility: While pollen sterility in *C. cajan* and *A. lineata* was 2.9 and 3.2 per cent respectively, it was 44.9 per cent in the hybrid.

Pod-setting: Pod-setting in the hybrid was 38 per cent as against 76 and 71 per cent in *C. cajan* and *A. lineata* respectively.

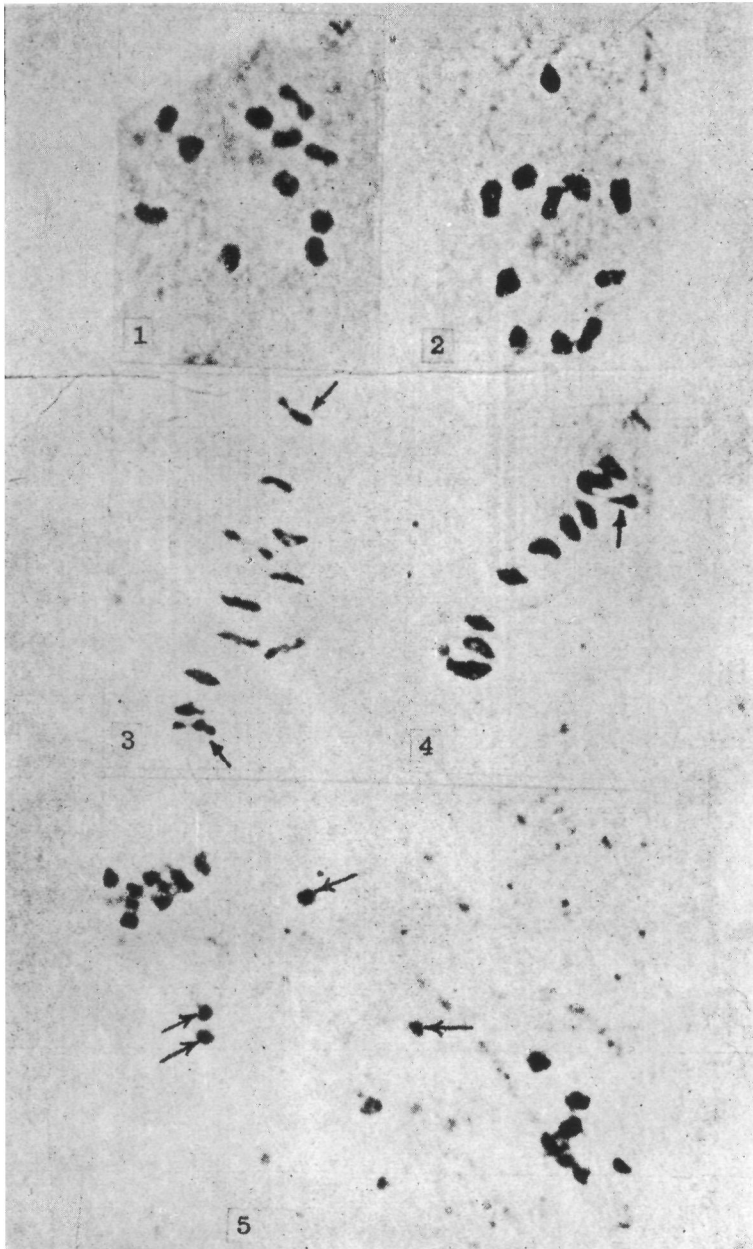
Seed-setting: The hybrid had three categories of pods, one-, two-, and three-chambered pods of which two-chambered pods were predominant (71 per cent). *C. cajan* had one- to four-chambered pods of which three-chambered ones were predominant (76.5 per cent). In *A. lineata*, pods were one- to two-chambered, of which two chambered pods were predominant (85.5 per cent). A detailed account of seed-setting in the hybrid and the parents is given in Table 2. It is evident from the table that although in the hybrid the filling percentage was very good (73.6 per cent), most of the seeds (68.5 per cent) were found to be shrunken.

Cytology: In the hybrids different degrees of synapsis were observed during pachytene while pairing was perfect in the parents. Even the microsporocytes of the same anther showed varying degrees of synapsis. The nuclei exhibiting partial synapsis contained partially paired chromosomes with terminal and/or interstitial unpaired segments, and rarely completely unpaired chromosomes together with completely paired chromosomes. Such partial synapsis was seen only in the hybrids but not in the parents. In some microsporocytes almost complete synapsis of all the chromosomes was noticed indicating thereby that the differences in relative lengths and arm ratios of the chromosomes of the two parents are somewhat accommodated during pairing in the hybrid. A

TABLE 1

Morphological comparison of C. cajan, A. lineata and their hybrid

Character	<i>C. cajan</i>	<i>A. lineata</i>	<i>C. cajan</i> × <i>A. lineata</i>
Shape of first pair of simple leaves	Lanceolate	Ovate	Lanceolate
Branching	Racemose with wide angled branches	Racemose with nearly right angled branches	Racemose with intermediate angled branches
Leaflets:			
(a) Shape	Lanceolate	Obovate acute or subobtuse	Intermediate
(b) Length	6.89 cm	3.23 cm	4.78 cm
(c) Breadth	2.66 cm	1.40 cm	1.92 cm
(d) Venation	Palmately reticulate	Palmately reticulate	Palmately reticulate
Length of petiole	5.27 cm	1.83 cm	3.54 cm
Nature of stipules	Fugacious	Persistent	Persistent
Days from sowing to the first flush of flowers	105 days	164 days	129 days
Size of the standard petal (L×B)	2.08 × 1.39 cm	1.32 × 0.74 cm	1.82 × 0.93 cm
Color of the standard petal	Yellow	Yellow with purple pigmentation	Yellow with purple pigmentation
Nature of petals	Deciduous	Persistent	Persistent
Pod-length	8.6 cm	1.9 cm	3.7 cm
Pod color	Green with purplish black streaks	Green	Uniformly reddish brown
Hairs on mature pods	Absent	Present	Present
Beak of the pod	Prominant	Minute	Intermediate
Nature of mature pods	Non-shattering	Shattering	Shattering
No. of chambers per pod	2.95	1.85	2.18
No. of seeds per pod	2.89	1.81	1.60
Seed color	Reddish brown to whitish brown	Brown with black speckles	Brown with black speckles
Strophiole	Absent	Present	Present
100-seed weight	7.60 g	3.74 g	4.95 g



Figs. 1 & 2 Meiocytes of parents, *C. cajan* and *A. ineata* respectively at diakinesis.

Figs. 3 to 5. Meiocytes of *C. cajan* × *A. ineata* hybrid showing 2 heteromeric bivalents (Fig. 3, arrows) and a quadrivalent (Fig. 4, arrow) at metaphase-I and laggards at anaphase-I (Fig. 5, arrows). All photographs × 1900.

TABLE 2

Seed-setting in C. cajan, A. lineata and their hybrid. No. of pods analysed in each case: 200

Pedigree	Total No. of chambers	No. of filled chambers	Filling percentage	No. of shiunken seeds	Percentage of shiunken seeds
<i>C. cajan</i>	590	579	98.1	63	10.8
<i>A. lineata</i>	371	362	97.5	42	11.6
<i>C. cajan</i> × <i>A. lineata</i>	436	321	73.6	220	68.5

detailed account of the pairing behaviour of specific chromosomes of the two parent. in the hybrid is reported elsewhere (Reddy, 1981).

In both the parents 11 bivalents were always observed during diakinesis and metaphase-I (Figs. 1 and 2). The ring bivalents were predominant with a very few rod bivalents. At anaphase-I regular 11-11 separation of chromosomes to the poles was observed. In the hybrid, during diakinesis and metaphase-I, heteromorphism was quite frequently observed in two bivalents (Fig. 3). A quadrivalent (Fig. 4) and a range of 0-4 univalents were found in the hybrid. The quadrivalent and univalents were observed in 16.0 and 55.7 per cent of the meiocytes respectively during diakinesis and metaphase-I. The various quadrivalent, bivalent and univalent configurations and their frequency are given in Table 3. The range and the mean of ring and rod bivalents observed in the

TABLE 3

Frequency of chromosome configurations at diakinesis and metaphase-I in C. cajan × A. lineata hybrid. No. of cells scored: 106

Chromosome configuration	No. of cells per each type	Percentage
11 II	35	33.01
10 II + 2 I	18	16.98
9 II + 4 I	36	33.96
1 IV + 9 II	12	11.32
1 IV + 8 II + 2 I	5	4.71

hybrid and the parents are given in Table 4. It is evident from the table that there was an increase in the rod bivalents and decrease in the ring bivalents in the hybrid. At anaphase-I irregular separation of the chromosomes to the poles with laggards (Fig. 5) and unequal distribution to the poles were observed. The frequency of various anaphase-I configurations is given in Table 5. No abnormalities were encountered in the second division except for micronuclei at telophase II in 4.7 per cent of the cells.

TABLE 4

Frequency of ring and rod bivalents at metaphase-I of C. cajan, A. lineata and their hybrid

Pedigree	No. of cells scored	Ring bivalents		Rod bivalents		*Others	
		Range	Mean	Range	Mean	Range	Mean
<i>C. cajan</i>	32	7-11	9.07	0-2	0.77	0-3	1.15
<i>A. lineata</i>	41	8-11	9.10	0-2	0.70	0-3	1.20
<i>C. cajan</i> × <i>A. lineata</i>	97	4-10	6.97	1-6	2.92	1-3	1.10

*Could not be classified.

TABLE 5

Anaphase-I configurations in C. cajan × A. lineata hybrid. No. of cells scored: 95

Sl. No.	Distribution	Nc. of cells per each type	Percentage
1.	11-11	82	86.3
2.	10-12	4	4.2
3.	Irregular distribution with laggards	9	9.4

DISCUSSION

Cajanus and *Atylosia* belong to the tribe, Phaseoleae, sub-tribe Cajaneae and sub-order Papilionaceae of the order Leguminosae. *Cajanus* is a monotypic genus and is separated from its allied genus, *Atylosia* on the basis of presence of arillate seeds in the latter (Hooker, 1879). *A. lineata* is an erect species of *Atylosia* and could be hybridized with *C. cajan* (Cultivar T-21) only when latter is used as the female parent indicating thereby a one way cross-compatibility. Similar

unidirectional success has been reported in interspecific crosses of *Phaseolus* (Strand, 1943; Lorz, 1952; Honma, 1955, Buishand, 1956; Honma and Heecht, 1958; Sen and Ghosh, 1960; Dana, 1964, 1965). *Nicotiana* (Kostoff, 1943; Swaminathan and Murthy, 1957) and *Arachis* (Muhammad, 1970). In view of the absence of pre- and post-fertilization barriers and also internal barriers between *Cajanus* and erect species of *Atylosia*, especially *A. lineata*, the geographical barriers must have been mainly responsible for the absence of natural hybrids between *C. cajan* and *A. lineata*. This is borne out by the fact that these two species are allopatric, *Cajanus* being confined to plains and the erect species of *Atylosia* to hilly regions. In that case, one might expect to observe natural hybrids between these two species when they are brought together. In fact, such a natural hybrid between *C. cajan* and *A. lineata* has been observed by Deodikar and Thakar (1956).

In the hybrid, most of the characters of *A. lineata*, viz., persistent stipules, presence of pigmentation on the standard petal, persistent petals, hairiness of pods, shattering of mature pods, dark brown seeds with black mottles and presence of strophiole on the seeds were dominant over those of the cultivated species, *C. cajan*.

The meiotic behaviour of hybrid was found to be normal without any major abnormalities both in the first and second division of meiosis. This indicates a very close homology between the chromosomes of the two species. However, the frequent heteromorphism exhibited by two bivalents indicate that the two species have attained some degree of non-homology in their chromosomes. This is further reflected in the reduced chiasma frequency in the hybrid as evidenced in the decline of ring bivalents and increase in rod bivalents.

ACKNOWLEDGEMENTS

The senior author is highly grateful to Atomic Energy Department, Trombay, India for the financial support given through the award of senior research fellowship during the course of this work.

REFERENCES

- Buaishad, T. J. (1956). The crossing of beans (*Phaseolus* spp.). *Euphytica*, **5**: 41-60.
 Dana, S. (1964). Interspecific cross between tetraploid *Phaseolus* species and *P. ricciardianus* Ten. *Nucleus*, **7**: 1-10.
 Dana, S. (1965). *Phaseolus aureus* Roxb × Tetraploid *Phaseolus* species cross. *Revista de Biologia*, **5**(1-2): 109-114.
 Deodikar, G. B. and C. V. Thakar. (1956). Cytotaxonomic evidence for the affinity between *C. indicus* Spreng and certain erect species of *Atylosia* W. and *A. Proc. Indian Acad. Sci.* **43**(B): 37-45.
 Honma, S. (1955). A technique for artificial culturing of bean embryos. *Proc. Amer. Soc. Hor. Sci.*, **65**: 405-408.
 Honma, S. and O. Heecht. (1958). Bean interspecific hybrid involving *Phaseolus coccineus* X *P. lunatus*. *Proc. Amer. Soc. Hor. Sci.*, **75**: 360-365.
 Hooker, L. (1879). Flora of British India. Reeve and Co., Ltd., **11**: 212-217.
 Kostoff, D. (1943). *Cytogenetics of the genus Nicotiana*. States print House, Sofia, P. 1073.
 Kumar, L. S. S. and M. V. Thombre. (1958). An intergeneric hybrid of *Cajanus cajan* (L.) Millsp. × *Atylosia lineata* W. and *A. J. Univ. Poona*, **12**: 13-16.
 Lorz, A. P. (1952). An interspecific cross involving the lima bean, *Phaseolus lunatus* L. *Science*, **115**: 702-703.

- Muhammad, V. S. (1970). Studies on interspecific hybrids in the genus *Arachis* L. Unpublished Ph D. thesis submitted to the Madras University, India.
- Reddy, L. J. (1981). Pachytene analyses in *Cajanus cajan*, *Atylosia lineata* and their hybrid. *Cytologia*, **46**: 397-412.
- Sen, N. K. and A. K. Ghosh. (1960). Interspecific hybridization between *Phaseolus aureus* Roxb (Green gram) and *Ph. mungo* L (Black gram). *Bull. Bot. Soc., Bengal*, **14**: 1-4.
- Sikdar, A. K. and D. N. De. (1967). Cytological studies of two species of *Atylosia*. *Bull. bot. Soc., Bengal*, **21**(1): 25-26.
- Stenzel, A. B. (1943). Species crosses in the genus *Phaseolus*. *Proc. Am. er. Soc. hort. Soc.*, **42**: 409-572.
- Swaminathan, M. S. and B. R. Murty. (1957). One way incompatibility in some species crosses in genus *Nicotiana*. *Indian J. Genet.* **17**: 23-26.