

**CROSSABILITY, GENOME RELATIONSHIPS AND  
INHERITANCE STUDIES IN INTERGENERIC  
HYBRIDS OF PIGEONPEA**

**A THESIS SUBMITTED FOR THE DEGREE OF  
Doctor of Philosophy**

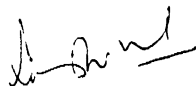
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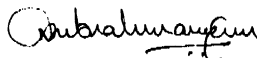
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C E R T I F I C A T E

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## ABSTRACT

The present investigation was initiated with the broad objectives of, assessment of genome relationships between the Cajanus cajan and its related wild species, screening the wide crosses for haploids through selective chromosome elimination, development of methods to improve crossability between species, studying the inheritance of discernible characters in the hybrids obtained and develop in vitro regeneration techniques in pigeonpea. The present study involved crossing C. cajan with twelve species of Atylosia and two species of Rhynchosia. A total of 24 intergeneric cross combinations including reciprocals were attempted.

Eight (A. albicans, A. cajanifolia, A. lanceolata, A. latisepala, A. lineata, A. scarabaeoides and A. sericea) out of the 12 Atylosia species hybridized with C. cajan when Cajanus was the female parent. From the reciprocal crosses a low rate of success was obtained in two combinations (A. albicans x C. cajan and A. sericea x C. cajan). When Cajanus was the female parent the degree of crossability varied not only with the Atylosia species in question, but also with the Cajanus genotype. One of the Cajanus cultivars, ICP-102 failed to cross with any of the Atylosia species. The rate of success was very high (1.37-19.5%) when A. lineata was the male parent followed by C. cajan x A. albicans crosses (1.09-9.6%). In the other six combinations the success was low ranging from 0.9-3.05%. C. cajan cultivars which had a common female parent in their pedigree did not vary much in their ability to cross with Atylosia species. Both the Rhynchosia species (R. rothii and R. minima) failed to cross with Cajanus.

Post-pollination hormone treatments were used to improve the crossability in successful Cajanus-Atylosia crosses and also to assess their influence in developing new hybrid combinations. Effects of gibberellic acid (GA<sub>3</sub>), kinetin and their mixture on Cajanus x Atylosia crossability were tested. GA<sub>3</sub> and kinetin were used independently at concentrations ranging from 10 ppm to 80 ppm or as a 1:1 mixture keeping the net hormone concentration in the same range. Following hormone treatments the rate of success was increased through increased pod setting and number of seeds/pod. GA<sub>3</sub> at a concentration of 50-60 ppm was found to be the most effective treatment. Higher hormone concentrations had a detrimental effect. In unsuccessful crosses hormone treatments delayed bud drop by 3-4 days, permitting prolonged ovule development. Results indicate that the hormone treatment helps in post-fertilization development leading to an increased rate of success. Results suggest that the Australian species of Atylosia are more diverged from Cajanus than the Asian Atylosias.

All the progeny were subjected to a detailed cytological analysis. There was no evidence of chromosome elimination in any of the Cajanus-Atylosia crosses.

Cytological studies in pigeonpea unequivocally established the presence of two nucleolar organizers per genome in *Cajanus* and *Atylosia* species. Detailed meiotic studies in the hybrids revealed a high degree of genome homology and recombination between the cultivated and the wild species. In the hybrids eleven bivalents were common at metaphase-I with occasional univalents. Univalents were more frequent in the hybrids with the Australian *Atylosias* (*A. grandifolia* and *A. latisejala*). Chiasma frequency was also low in these hybrids in comparison with the others. Anaphase separation was regular in most of the hybrids again with the exception of the hybrids with *A. grandifolia* and *A. latisejala*. In both these cases multipolar spindle abnormalities were common. Inversion loops at pachytene and anaphase I bridges were found in hybrids of *C. cajan* with *A. grandifolia* indicating inversion heterozygosity in the  $F_1$  hybrids.

Hybrids between *C. cajan* and *A. cajanifolia* were the most fertile while the hybrids with *A. grandifolia* and *A. latisejala* were highly sterile. Other hybrids exhibited a considerable amount of sterility in spite of regular bivalent formation at metaphase-I and normal disjunction. The genotype of the female parent also influenced the pollen fertility. Hybrids with the genotype, ICP-7035 were consistently less fertile in all combinations.

Nucleolar variation in number, size and distribution was recorded after division-I and division-II in three hybrids (*C. cajan* x *A. albicans*, *C. cajan* x *A. lineata* and *C. cajan* x *A. grandifolia*). In these three hybrids three to four bivalents were found attached to the diplotene/diakinesis nucleolus as against one to two bivalents in the parents. Variation in nucleolar number and distribution are interpreted to have originated from pairing and recombination between nucleolar organizer chromosome(s) of one parental species with the nonnucleolar organizer chromosome(s) of the other. Detailed analysis of these hybrids provided evidence for "cryptic structural hybridity" and for intergenomic (allosyndetic) recombination. Nucleolar behaviour was used as a marker to trace the products of recombination in these hybrids. The nucleolar variation in the hybrids suggests that the sterility in the hybrids in spite of regular meiosis is a consequence of duplications and/or deficiencies originating from allosyndetic recombination particularly between the structurally altered chromosomes in the hybrids.

Most of the *Atylosia* characters like seed strophiole, seed mottling, and pod hairiness were expressed in the hybrids indicating their dominant nature while leaflet shape and twining nature showed incomplete dominance. The  $F_2$  segregation for leaflet shape and pod hairiness revealed that these traits are governed by one locus while twining nature, seed strophiole and seed mottling were governed by two loci. Substantial variation for quantitative traits was found in the  $F_2$  generation which includes transgressive segregants for leaf length, leaf width, pod length, seed weight and protein content in the

Cajanus-Atylosia hybrids, underlining the importance of Atylosia species in the improvement of Cajanus. The F2 variation in these hybrids substantiated the cytological evidence for a close relationship between C. cajan and A. cajanifolia.

Techniques were standardized for regeneration of plants from immature embryos (embryos at least 11 days old) of C. cajan and from cotyledons of C. cajan, A. cajanifolia, A. albicans and A. sericea. Genotypic differences were evident with respect to frequency of regeneration in both embryo and cotyledon cultures. In cotyledon cultures the regeneration potential varied with the region of the cotyledonary explant with nodal halves being more effective while in embryo cultures an age dependent response was evident.

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**1. SCOPE OF THE INVESTIGATION**

Plants of greatest importance to agriculture belong to the families Graminae and Leguminosae. Leguminosae comprises a wide array of genera (600) and species (1300) (Delwiche, 1978) and occupies a unique place in the plant kingdom. Several legumes particularly from the subfamily Papilionoidae are of great economic importance next to cereals (Cobley and Steele, 1976). Grain legumes or pulses are the major source of dietary protein in developing countries. The high protein content and relatively low cost of these food legumes have earned them the title "Poor man's Meat" which effectively underlines their vital importance in developing nations. Legumes have also played a major role in patterning the agricultural systems in the tropics. Their nitrogen fixing ability enables them to sustain high yields in the face of minimum inputs, improve soil fertility along with their adaptability to diverse environments.

Pigeonpea [*Cajanus cajan* (L.) Millsp.] the only cultivated species in the subtribe Cajaninae is an important pulse crop in the tropics and ranks fifth among the edible legumes. India has the largest area in the world under grain legume cultivation where pigeonpea occupies an area of 2.8 million ha. Pigeonpea is mostly consumed in the form of split pulse (dhal). Green pods and seeds are used as vegetable in some parts of the tropics. Seed protein content in pigeonpea averages about 23%. The average yield of pulses on a national basis compared to cereals has been very low and this gap continues to widen. The "green revolution" has not increased the pulse yields because of the emphasis on cereals. Apart from socio-economic considerations in

cultivating legumes on marginal lands without fertilizer and poor plant protection measures, genetic factors have also contributed to the slow pace of pigeonpea improvement. In pigeonpea as with many other legumes the inbreeding behaviour resulted in severe restriction on genetic variability and improvement has therefore been confined to selection and perpetuation of useful kinds of gene action. Plant breeding history shows that diverse gene pools are the foundations for effective crop improvement programmes. The primary objective in plant breeding is to widen the genetic base of a cultivated species. If the needed variation is limited as in the case of pigeonpea the options for the breeders are:

1. Incorporation of alien variation,
2. Induction of mutations or
3. Exploitation of somaclonal variation

The applicability of mutation breeding in crop improvement is limited since a vast majority of mutations are deleterious and it still remains a hit and trial method with limited scope for directed attempts.

Somaclonal variation is a recent technique which offers a lot of potential for the future but it has not been possible to exploit these variants on a field scale except in a few crops (Scowcroft and Larkin, 1982).

The other and possibly the most viable recourse of introducing variation into a species is through transfer of genetic material from one species to another by hybridization.

The genetic potential of wild relatives is widely demonstrated in plant breeding and in evolutionary studies. Wild relatives have helped to fill the voids in traditional breeding programmes and have helped to exploit the potential in several crops (Stalker, 1980).

The first recorded interspecific hybrid was made in 1717 between carnation and sweet William by Thomas Fairchild (see Allard, 1960). Since then there has been extensive literature on distant hybridization and the first man-made cereal, "Triticale" was an outcome of intergeneric hybridization. Extensive studies on distant hybridization have been made in crops like wheat (Sears, 1972; Sears, 1975;) barley (see Bothmer and Hagberg, 1983), Maize (see Mangelsdorf, 1974; Harlan and de Wet, 1977), Solanum (see Swaminathan and Magoon, 1961; Motskaitis and Vinituskus, 1975), cotton (Blank *et al.*, 1972; Meyer, 1973; Meyer, 1974), Nicotiana (see Smith, 1968; Mann *et al.*, 1963; Berbec, 1974), tomato (see Rick, 1982), and rice (see Nayar, 1973).

Distant hybridization studies have also been carried out in several important legumes such as, *Phaseolus*, *Vigna*, *Vicia*, *Pisum*, *Arachis* (Smartt, 1979). Species in the genus *Phaseolus* have been a subject of wide interest. The possibility of gene exchange between species has led to several studies on interspecific hybridization especially between *P. vulgaris* and *P. coccineus* (Mendel, 1866; Tschermak-Seysenegg, 1942; Lamprecht, 1948; Rudolf, 1953; Kedar and Bemis, 1960; Thomas, 1964; Al-yasiri and Coyne, 1966; Rutger and Beckman, 1970;

Smartt, 1970; Lemarchand, 1971; Marechal, 1971; Ibrahim and Coyne, 1975; Haq *et al.*, 1980; Savova, 1981; Shii *et al.*, 1982; Conti, 1983). Hybridization with other *Phaseolus* species including the wild forms has also received wide attention (Lorz, 1952; Honma, 1956; Coyne, 1964; Smartt, 1970; Braak and Koistra, 1975; Le Merchand *et al.*, 1976; Tan Boun Suy, 1979; Hwang, 1979). Interspecific hybridization in *Phaseolus* in recent years has been widely used in the improvement of *Phaseolus* sp. with respect to disease resistance, insect resistance, nitrogen fixation and several agronomic characters (Rgnalli *et al.*, 1981; Bannerot *et al.*, 1981; Zapata *et al.*, 1982; Hunter *et al.*, 1982; Alvarez, 1981; Lapinskas, 1980).

Wide hybridization has received a fair degree of attention in the genus *Glycine*. Studies on hybridization between *G. max* and *G. soja* have been extensive (Karasawa, 1936; Ting, 1946; Williams, 1948; Tang and Chen, 1959; Tang and Tai, 1962; Ahmed *et al.*, 1977 and 1979; Kiazuma *et al.*, 1980; Malik and Singh, 1982; Gai *et al.*, 1982). Ahmad *et al.* (1977 and 1979) made a detailed cytological analysis of these hybrids and found structural differences between the two genomes in the form of inversions. There have also been attempts at hybridization between the wild species (Palmer, 1965) and in 1968 Palmer and Hadley reported a successful cross between *G. tabacina* and *G. latifolia* (*G. tomentosa*). Later Broue *et al.* (1979) and Putievsky and Broue (1979) obtained two intraspecific and six interspecific hybrids. Recently Newell and Bymowitz (1983) reported a range of intra and interspecific hybrids in the

subgenus *Glycine* along with detailed cytological studies in the hybrids. Attempts at hybridization between cultivated soybean and the wild species were not successful with the conventional methods (Ladizinsky *et al*, 1979; Hood and Allen, 1980). Newell and Hymowitz (1982) were able to obtain a hybrid between *G. max* and *G. tomentella* with the aid of ovule culture. Apart from aiding in understanding the species relationships, interspecific hybridization in *Glycine* has also helped in widening the genetic base for quality characters of *G. max* particularly protein content (Ala *et al*, 1979; Erikson and Beversdorf, 1981; Kiazuma *et al*, 1980).

The genus *Vicia* exhibits dysploidy with  $x = 5, 6$  or  $7$ . Most of the interspecific crosses so far involve species with chromosome numbers  $x=6$  (subspecies *sativa*, *macrocarpa* and *nigra*). Hybrids between  $2n=10$  subspecies (*cordata*) have also been studied (Mettin and Hanelt, 1973). Very little information is available on crosses among subspecies with the chromosome number  $2n=14$ . Crosses between types with the same chromosome number result in fertile or semifertile hybrids and meiosis in the  $F_1$  hybrids indicated the presence of chromosomal structural hybridity as evidenced by heteromorphic bivalents, trivalents, quadrivalents etc (Mettin and Hanelt, 1973). Crosses involving forms with different chromosome numbers have also been attempted (Zohary and Plitmann, 1979) and hybrids were obtained in numerous combinations. Some of these hybrids exhibited hybrid vigour. Hybrid between *sativa* ( $x=6$ ) and *Cordata* ( $x=5$ ) showed pentavalents in the  $F_1$  generation and in  $F_2$ , segregants showed differences in

chromosome numbers ranging from 11-14 (Nerson, 1970). Watanabe and Yamada (1958) reported crosses between *sativa* and *angustifolia* in which the pollen fertility was less than 5% and the  $F_2$  segregated almost exclusively for parental types. Yamamoto (1971, 1974 a,b) studied crosses between *sativa*, *amphicarpa*, *pilosa* and *macrocarpa* and in the hybrids he found poor meiotic pairing and consequently low fertility. He reported a stable karyotype produced by the substitution of one chromosome of *sativa* in a *macrocarpa* karyotype derived from the interspecific hybrid. Later Yamamoto (1975) studying the amylose isozyme pattern in a *Pilosa* x *macrocarpa* hybrid showed the occurrence of recombination in the hybrid progeny. Cubero (1982) has summarized the interspecific hybridization studies in the genus *Vicia*. In general hybridization studies in this genus have shown that the various chromosome types are not fully reproductively isolated from one another, and hybridization studies have also shown that hybridization and recurrent recombination between distinct karyotypes contributed to the build up of chromosomal polymorphism. Species crosses in this genus helped in the generation of variability for traits like seed hardness (Donnelly, 1980).

In the genus *Vigna* the closest relative of green and black gram (*V. radiata* and *V. mungo*) is *V. sublobata*. Hybridization between these species is achieved with relative ease (AIRDC, 1977). Hybridization studies between *V. radiata*, *V. mungo*, *V. umbellulata* and *V. angularis* have been numerous (Dana, 1966a,b,c; Al-yasiri and Coyne, 1966; Biswas and Dana, 1975,



1976; Sawa, 1973; Chen *et al*, 1977; Chowdhry and Chowdhry, 1977; Ann and Hartmann, 1977, 1978a, 1978b; Machado *et al*, 1982; Chen *et al* 1983). Although Machado *et al* (1982) from their cytological studies could not conclude whether the pairing observed in the interspecific hybrid was autosyndetic or allosyndetic, Chen *et al*, (1983) from their F<sub>2</sub> studies concluded that recombination and gene exchange takes place between these species. In *Vigna* also the related species have been frequently used for the transfer of characters like disease resistance and protein content (Singh, 1981; Gill *et al*, 1983; and Lukoki and Otuole, 1981).

Several wild *Arachis* species have been successfully crossed with the cultivated species *A. hypogaea*. The cultivated species crosses easily with *A. correntina*. The first report of this cross came from Krapovickas and Rigoni (1952) with subsequent reports from Kumar *et al* (1957) and Raman (1959). Gregory *et al* (1973) have produced hybrids between *A. hypogaea* and wild diploids in the section *Arachis*. These hybrids have also been reported by Moss (1977). Raman (1976) has reported hybrids of *Arachis* with *A. villosa* and *A. duranensis*. Intersectional crosses such as *Arachis* x *Rhizomatosae*, *Erectoides* x *Rhizomatosae*, *Erectoides* x *Ambinervosae*, *Caulorhiza* x *Erectoides*, and *Arachis* x *Erectoids* are possible (Gregory *et al*, 1973). In addition Raman (1976) reported hybrids between *A. hypogaea* and *A. villosulicarpa*, *A. hagenbeckii* and *A. diogeni* and between *A. monticola* and *A. glabrata* though the authenticity of many of these has been questioned. In spite of ploidy differences the

wild species of *Arachis* have gained a lot of importance with respect to transfer of specific characters like resistance to insects, viruses, nematodes, fungi, etc. (ICRISAT, 1982).

Hybridization between species of *Pisum* (*sativum*, *elatum*, *humile*, *fulvum* and *abyssinicum*) has also received some attention (Zohary and Hopf, 1973; Gritton and Wiezbicka, 1975; Belova, 1979 and 1980). Success has also been reported from crosses involving *Pisum* and *Vicia* (Stegajlo, 1963; Sobolev et al, 1968; Sobolev and Burgil, 1970). The chromosome number of these hybrids was between 12 and 16 (2n). Sobolev et al (1968) reported nonhomologous chromosome pairing in the hybrids and the chromosomes formed distinct groups.

Cultivated pigeonpea is bestowed with a wealth of related wild species in the genus *Atylosia*. The genus *Rhynchosia* is also considered close to *Cajanus*. All the species of *Atylosia* and *Rhynchosia* have the same basic chromosome number as that of *Cajanus* (X=11). There are about 34 species of *Atylosia*, of which 18 are endemic to India, 15 are natives of Australia and one species is found in Africa (van der Maesen, pers. comm.). The taxonomic delimitation of *Cajanus* and *Atylosia* into distinct genera which has been done on the basis of the seed strophiole has been a point of contention (Mac Comb, 1975; van der Maesen, 1980). Many of the *Atylosia* species possess several desirable characters such as disease and pest resistance, high protein content, photoperiod insensitivity, drought tolerance (Remanandan, 1980). In spite of limited variability for certain important qualitative traits in *C. cajan* and the existence of a

wide wealth of *Atylosia* species, studies on intergeneric hybridization in pigeonpea are surprisingly few and far between.

Naithani (1941) was the first to give details about the meiotic division of pigeonpea although it was Roy (1933) who for the first time reported the chromosome number of cajanus as  $n=11$  which was confirmed by Akinola *et al* (1972). Krishnaswamy and Ayyangar (1935) provided information about meiotic pairing and chiasma frequencies in pigeonpea and this was followed by the studies of Kumar *et al* (1945, 1966), Bhattacharjee (1956) and Reddy and De (1983) all of which mentioned normal chromosome pairing and regular formation of eleven bivalents at meiosis.

The karyotype description of Naithani (1941) showed no variation for chromosome morphology between varieties. This attempt was followed by that of Deodikar and Thakur (1956) who gave the total complement length as 75.4  $\mu\text{m}$ .

These initial attempts were followed by the works of Shrivastava *et al* (1973) and Sinha and Kumar (1979) who reported significant varietal variation for arm ratios and chromosome lengths and by those of Sharma and Gupta (1982) and Pundir (1981).

There have been two publications on pachytene karyotype analysis (Reddy, 1981a and Dundas *et al* 1983). While Reddy identified the pachytene chromosomes by the length and centromere positions, Dundas *et al* (1983) used chromomere patterns for chromosome identification. Only recently the Cisma C banding technique was attempted for pigeonpea without much success

(Lavania and Lavania, 1982).

The satellite chromosome numbers in pigeonpea have been a subject of controversy and from the literature one can see that the number varies from 0 to 2. Sinha and Kumar (1979) and Shrivastava *et al* (1973) reported that some cultivars used in their studies had one Satellite (SAT) chromosome while others have none. The karyotypes presented by Sharma and Gupta (1982) do not show any SAT chromosome while those of Pundir (1981) showed two.

Among the species of *Atylosia*, *A. lineata* was used extensively by Kumar *et al* (1958), Sikder and De (1967) and Reddy and De (1983) in their studies on meiotic pairing. Kumar *et al* (1958), Deodikar and Thakur (1956), Sikder and De (1967) Shrivastava *et al* (1973) and Pundir (1981) studied the somatic karyotype of *A. lineata* and reported a close relationship between the karyotypes of *Cajanus* and *Atylosia lineata*. Sikder and De (1967), Reddy (1973) and Pundir (1981) studied the karyotypes of *A. lineata*, *A. sericea* and *A. scarabaeoides*. The karyotypes of these species were also presented by Reddy (1981 a,b,c) based on pachytene chromosome analysis. The other *Atylosia* species for which chromosome numbers were reported were *A. platycarpa* (Bir and Kumari, 1973, 1977; Pundir and Singh, 1981); *A. rugosa* (Sanjappa and Satyananda, 1979); *A. volubilis* (Rao, 1978 and Pundir and Singh, 1978); *A. trinervea* (Pundir and Singh, 1978), *Atylosia albicans* (Rao, 1978 and Pundir and Singh 1978) and *A. cajanifolia* (Pundir, 1981).

Attempts at Cajanus-Atylosia hybridization are presented in Table I. The first report on intergeneric hybridization in pigeonpea dates back to 1956 when Deodikar and Thakur crossed C. cajan with Atylosia lineata. The hybrid was fairly fertile. Kumar et al (1958) extended the earlier work on to hybrid cytology and found regular bivalent formation in the hybrid. A hybrid between C. cajan and A. scarabaeoides was obtained by Roy and De (1967) who expressed doubts about the generic status of Atylosia. Reddy (1973) analysed pachytene chromosome pairing in Cajanus cajan, Atylosia lineata, A. scarabaeoides and A. sericea and their hybrids. These pachytene studies in general revealed a high degree of chromosome homology between C. cajan and the three species of Atylosia. Studies on the inheritance of a few qualitative traits was also done in this study. Ariyanayagam and Spence (1978) reported hybrids between Cajanus and A. platycarpa while further attempts (Pundir, 1981; Reddy et al, 1980 and in the present study) to cross Cajanus with A. platycarpa failed. Further attempts at Cajanus - Atylosia hybridization by Pundir (1981) involved karyotype comparisons between the cultivated and the wild species and meiotic pairing in the  $F_1$  hybrids. These studies revealed a great degree of karyotypic similarities between species. All the studies on Cajanus-Atylosia hybridization revealed a close relationship between the species of the two genera and regular pairing in their hybrids which nevertheless exhibited a fair degree of sterility.

Table I. Studies on intergeneric hybridization in pigeonpea.

Year	<u>Atylosia</u> species used	Authors
1956	<u>A. lineata</u>	Deodkar and Thakur
1958	<u>A. lineata</u>	Kumar <i>et al</i>
1967	<u>A. scarabaeoides</u>	Roy and De
1973*	<u>A. lineata</u> , <u>A. scarabaeoides</u> and <u>A. sericea</u>	Reddy
1978	<u>A. platycarpa</u>	Ariyanayagam and Spence
1981	<u>A. albicans</u> , <u>A. sericea</u> , <u>A. lineata</u> , <u>A. scarabaeoides</u> , <u>A. trinervea</u> and <u>A. cajanifolia</u>	Pundir
1984	<u>A. reticulata</u> (sub-sp. <u>reticulata</u> ), <u>A. pluriflora</u> and <u>A. acutifolia</u>	Dundas

\*This work was published in 1981.

Except at ICRISAT there has been no report on the use of *Atylosia* species in pigeonpea breeding programmes. At ICRISAT their use is mostly confined to breeding high protein lines (Reddy et al, 1979) and to a limited extent for breeding for insect resistance, dwarfs and isolation of cytoplasmic male steriles (L.J. Reddy pers. comm.). Only a few *Atylosia* species have been used in this direction so far. While there has been a rapid accumulation of literature on distant hybridization in other crops, the limited interest in pigeonpea in this regard stems from the small chromosome size and the paucity of attempts in distant hybridizations in pigeonpea. Genome relationships between *Cajanus* and *Atylosia* species is still obscure. *A. cajanifolia*, which is morphologically very similar to *Cajanus* except for the seed strophile, was identified as early as 1920 (Van der Maesen, 1980) but an attempt to cross these two species was not reported until 1981 (Pundir).

A clear understanding of the cytogenetic relationships between the cultivated and wild species is a pre-requisite for the effective use of wild species in the crop's improvement. Distant hybridization is mostly aimed at introducing new genetic variability or to achieve a new genomic constitution in such a way that the characters of the parental species are recombined effectively. These possibilities are directly related to the degree of genetic relatedness between the parents. It has been found that, the closer the genome relationship between the cultivated and the wild species the greater the amount of genetic recombination and consequently variability.

Apart from being a source of novel genes and variability, wide hybridizations have also proved to be a potential means of haploid production. For instance Kasha and Kao (1970) reported a high frequency of haploids from crosses between *H. vulgare* and *H. bulbosum*. These *vulgare* haploids originate from fertilization followed by selective elimination of *bulbosum* chromosomes during early stages of embryo development (Subrahmanyam and Kasha, 1973). Selective chromosome elimination leading to haploid formation is widespread among *Hordeum* interspecific crosses (Subrahmanyam, 1977, 1979, 1980) and also from intergeneric crosses (Barclay, 1975; Shigenobu and Sakamoto, 1981; Bothmer *et al.*, 1984). Barclay (1975) reported wheat haploids (polyhaploids) from crosses between *T. aestivum* and *H. bulbosum*. Selective chromosome elimination is also known in hybrids between *N. tabacum* and *N. plumbaginifolia* (Gupta and Gupta, 1973). Haploid production via chromosome elimination has become a successful commercial venture in Barley breeding (Kasha and Reinbergs, 1980).

Pigeonpea being a long duration and photosensitive plant can normally be grown only once in a year. This places a severe constraint in pigeonpea breeding programmes. Development of haploids in pigeonpea would have an immense value in shortening the time required for breeding a cultivar. Unfortunately, the anther culture technique has not shown promise in any of the leguminous species. From the experience in barley it seemed worth while to screen *Cajanus - Atylosia* crosses for selective chromosome elimination as a source of haploids.



Pundir (1981) reported that some of the *Alyosia* species used in his study failed to cross in any direction. Natural barriers to interspecific hybridization may be of pre- or post-fertilization nature. The advent of the embryo culture technique has helped in overcoming such problems especially the post-fertilization barriers. Rescue of interspecific progeny through embryo culture was done for the first time in a *Linum perenne* x *L. austriacum* cross by Liabach (1929). Raghavan (1977) lists 75 species (updated by Stewart, 1981) in which the embryo culture techniques have been standardized. New hybrid combinations through the use of embryo culture include several legumes (Honma, 1955; Bajaj and Bopp, 1971; Braak and Kooistra, 1975; Alvarez et al, 1981; Mok et al, 1978; Ann and Hartman, 1978; Newell and Hymowitz, 1982; Sastri and Moss, 1982; Gosal and Bajaj, 1983; Chen et al, 1983).

In several instances it has been found that the embryo culture technique helps in rescuing the hybrid progeny only in combination with hormone treatments (Islam, 1964; Kruse, 1974; Bajaj et al, 1980; Alonso and Kimber, 1980; Sastri et al, 1981; Sastri and Moss, 1982; Kuwada and Mabuchi, 1976). Hormone treatments prolong the embryo development on the female parent so that the embryo at excision is more amenable to culture. Further, hormone treatments are also known to improve the rate of success in compatible crosses. In the light of the literature it seemed worth while to standardize embryo culture technique for pigeonpea and to study the effect of hormone treatments in intergeneric crosses involving pigeonpea.

Obtaining species hybrids and assessment of genome relationships is a first step in the exploitation of wild species in the improvement of any cultivated species. The next logical step is the utilization of such hybrids in the breeding programme before which it would be essential to study the inheritance pattern and also assess the quantum of variability generated. Studies on inheritance provide information on the possible number of genes governing a character and their interaction. Evaluation of the variation in the  $F_2$  generation helps in understanding the extent of recombination and variability. Genetic studies provide a clear direction to the handling of segregating generations. There have been several studies on the genetics of qualitative and quantitative traits in pigeonpea (Deshpande and Jeswani, 1956; D'cruz and Deokar, 1970; Munoz and Abrams, 1971; Pandey, 1972; Sharma et al, 1972; Joshi, 1973; Chaudhary and Thombre, 1977; Dahiya and Brar, 1977; Dahiya et al, 1977; Kapur, 1977; Malhotra and Sodhi, 1977; Reddy et al, 1979; Sidhu and Sandhu 1980; Saxena and Sharma, 1981 to name a few). Only two studies have so far been conducted on the genetics of Cajanus x Atylosia crosses (Reddy et al, 1980 and Pundir, 1981).

Tissue culture technique are emerging as a major supplementary aid to traditional plant breeding procedures in crop improvement programmes (Vasil et al, 1982) due to their increasing importance in clonal propagation (Mura-shige, 1974), production of haploids (Chih-Ching Chu, 1982), production of disease free plants (Gengenbach et al, 1977) and induction of genetic variants (Scowcroft and Larkin, 1982).

A basic knowledge of differentiation is essential for the effective use of this technique. Tissue culture techniques have been developed for several grain legumes (Kantha et al, 1981) but such studies on pigeonpea are limited (Rao and Swamy, 1975; Mehta and Ram, 1981).

In the light of the above considerations and constraints the present investigation was undertaken to understand the relationship between Cajanus and Atylosia and Rhynchosia in order to allow easier transfer of useful traits from the wild to the cultivated species with the following broad objectives:

1. Assessment of crossability relationships between Cajanus, and certain Atylosia and Rhynchosia species.
2. Development of methods to improve species crossability.
3. Assessment of genome relationships between C. cajan and its wild relatives.
4. Screening for haploids produced by selective chromosome elimination in Cajanus - Atylosia crosses.
5. Study of the inheritance and variation for qualitative and quantitative traits in the intergeneric hybrids and
6. Development of in vitro regeneration techniques in pigeonpea.

## 2. MATERIALS AND METHODS

**SEED SOURCE:** The Genetic Resources Unit, ICRISAT was the source of seed for all the *Atylosia* and *Rhynchosia* species while the seed of the cultivars of *C. cajan* was obtained from the pigeonpea breeding subprogram at ICRISAT. The salient morphological features and economic importance of the species used in this study are presented in Tables VIII, IX and X, and Table II respectively.

**PLANT CULTURE:** The field investigations in the present study were carried out at the ICRISAT research farm which is situated 18°N, 78 E at an altitude of 536 m. Prior to planting the land was ploughed, harrowed and ridged. The field was divided into 4m plots and the seed was sown manually at a spacing of 30-60 cm between plants depending upon the plant habit and 75 cm between rows. Support was provided wherever necessary for the climbing types. In cases where plants were grown in pots the potting mixture consisted of three parts of soil and one part of farm yard manure.

Seed of the wild species and also the  $F_1$ 's possess a hard seed coat, hence their seed was sacrificed with a scalpel before sowing to facilitate easy germination. Irrigation was provided whenever necessary. In field plantings hand weeding was done twice to keep the crop clean. Endosulphan (0.2%) was sprayed at regular intervals to check insect damage.

**HYBRIDIZATION:** In *C. cajan* flower opening begins in the morning at about 7 A.M. with the anthesis continuing until late in the

Table II. Origin and salient economic features of the Atylosia species used in the study.

Species	Acc. No.	Origin	Important Characteristics
<u>A. albicans</u>	NKR-177	Asia	Sterility mosaic resistant and high seed protein content
<u>A. cajanifolia</u>	PR-4876	Asia	High seed protein content
<u>A. grandifolia</u>	PR-4221	Australia	High seed protein content
<u>A. lanceolata</u>	CO-1619	Australia	Frost and drought tolerant
<u>A. latisepala</u>	CO-1618	Australia	Frost and drought tolerant
<u>A. lineata</u>	JM-3366	Asia	Sterility mosaic resistant and high seed protein content
<u>A. mollis</u>	JM-4331	Asia	
<u>A. platycarpa</u>	PR-4557	Asia	Blight resistant, annual, high seed protein content
<u>A. rugosa</u>	KM-4180	Asia	Antibiosis to <u>Heliothis armigera</u> and high seed protein content
<u>A. scarabaeoides</u>	JM-2367	Asia	Antibiosis to <u>Heliothis armigera</u> and high seed protein content
<u>A. sericea</u>	JM-1961	Asia	Blight and sterility mosaic resistant and high seed protein content
<u>A. volubilis</u>	JM-4208	Asia	Sterility mosaic resistant and high seed protein content

afternoon. The flowers remain open for about 20-24 h and the anthers dehisce before flower opening. The species of *Atylosia* have a similar floral biology except that they have delayed flower opening in some cases. Anther dehiscence in all the *Atylosia* species occurs before flower opening.

For hybridization, buds of the appropriate size were opened with the help of a forceps and anthers were removed without injuring the stigma. The forceps was dipped in spirit after each emasculation. Initially two methods of pollination were attempted.

1. Emasculation and immediate pollination and
2. Emasculation and pollination on the next morning.

The first method proved to be marginally better and it was followed in all the later studies.

**HORMONE TREATMENTS:** Cultivar Pant A2 was used as the *Cajanus* parent for hormone treatment studies. Gibberellic acid (GA3) and kinetin were used independantly at concentrations ranging from 10 to 80 ppm or as a 1:1 mixture keeping the net hormone concentration in the same range (10 to 80 ppm). A No.22 hypodermic needle was used to place the hormone solution so as to fill the bud cavity surrounding the pistil. The treatments were given twice, 24 and 48 h after pollination. Care was taken to avoid physical injury to the buds during hormone application. One hundred pollinations were made for each treatment. Bud drop, pod set, pod length, and seeds per pod were recorded for each cross and treatment.

**CYTOLOGICAL INVESTIGATIONS:** For cytological studies buds of the appropriate size were fixed between 9 to 12 h in Carnoy's fluid (6 parts ethyl alcohol: 3 parts chloroform: 1 part glacial acetic acid). After 48 hours in the fixative the buds were transferred to 70% alcohol and stored in a cool place. For chromosomal studies the anthers were squashed in 1% acetocarmine. For nucleolar scoring the anthers were first squashed in 1% acetocarmine followed by destaining with 45% acetic acid and restaining with 2% acetocarmine. Detailed observations were made on metaphase configurations, anaphase disjunctions and nucleolar size and distribution in the parents and hybrids. Chiasma frequencies were recorded in both parents and hybrids. Observations were also made on pachytene chromosome pairing.

**POLLEN FERTILITY:** Pollen stainability in 1% acetocarmine was taken as an index of pollen fertility. All shrivelled, unstained and poorly stained grains were counted as steriles, Pollen fertility was recorded for all the parents and hybrids.

**PHOTOGRAPHY:** Desirable cells were photographed using a photomicroscope with oil immersion lens and a green filter. Kodak high contrast copy film (ASA 64) was used for micro photography.

**ELECTRON MICROSCOPY:** Leaf bits were made to adhere over a thin film of dolite silver paint on specimen stubs, dried and were sputter coated with a thin film of gold. Photographs were taken on a JOEL-35 scanning electron microscope.



**SEED PROTEIN ESTIMATION:** Seed protein percentage was determined with the aid of Technicon Auto Analyzer. Fifty grams of seed sample was needed for the estimation of protein content.

**GENETIC STUDIES:** The  $F_2$  population was raised from the crosses *C. cajan* x *A. albicans*, *C. cajan* x *A. cajanifolia*, *C. cajan* x *A. sericea*, *C. cajan* x *A. scarabaeoides* and *C. cajan* x *A. lineata*. The germinability of  $F_2$  seed is presented in Table III. In all the above five hybrids the *Cajanus* parent used was Pant A2. The  $F_2$  populations were scored for seed strophiole (presence, absence), seed mottles (presence, absence), pod hairiness (short hairs, long hairs), leaflet shape (lanceolate, intermediate, *Atylosia* type) and twining nature (erect, intermediate, twining). The plants were classified into distinct groups for the above characters and the goodness of fit of the observed segregation was calculated by the standard chi-square test using the following formula

$$\chi^2 = \frac{\sum \frac{(O-E)^2}{E}}$$

Where  $\Sigma$  = Summation

O= Observed frequency and

E= Expected frequency

The variation in the  $F_2$  population was computed for the characters, mid-leaf length, mid-leaf width, pod-length, seeds per pod and 100-seed weight. The leaf measurements were made on

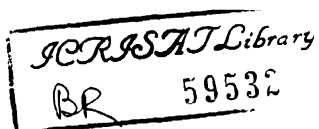


Table III. Seed germinability in F<sub>2</sub> population of hybrids between *C. cajan* and *Atylosia* species.

Population source	No. sown	No. germinated	Germination percentage
<i>C. cajan</i> × <i>A. albicans</i>	224	182	81.25
<i>C. cajan</i> × <i>A. cajanifolia</i>	329	207	62.91
<i>C. cajan</i> × <i>A. sericea</i>	206	163	79.12
<i>C. cajan</i> × <i>A. scarabaeoides</i>	281	211	75.08
<i>C. cajan</i> × <i>A. lineata</i>	330	291	88.18

the terminal leaflet and the measurements were on the average of measurements based on five samples. Observations on pod and seed characters were from the mature pods.

IN VITRO REGENERATION STUDIES: MS medium (Murashige and Skoog, 1962), B5 medium (Gamborg *et al.*, 1968) and White's medium (White, 1939) were used as the basal media. The composition of these media is presented in Table IV. Apart from these media, potato starch extract medium (Anonymous, 1976) was used in anther culture experiments. Stock solutions were prepared for the different hormones used in the study and used to supplement the basal media depending upon the requirement. Sucrose at 3% concentration was used as the carbon source. Solidification of the medium was achieved by Difco grade agar (8%). The pH of the media was adjusted to 5.6-5.8 by means of 0.1N HCl or 0.1N NaOH. The media were sterilized by autoclaving.

In *vitro* regeneration response was evaluated from the cotyledonary explants of the *Cajanus* cultivars ICP 4726, ICP 7035, Pant A2 and GS 4 and from *A. cajanifolia*, *A. albicans* and *A. sericea*. The regeneration potential of distal and nodal halves of cotyledons was evaluated. The *Cajanus* cultivars, Pant A2, ICP 7035, Prabhat and C 11 were used to standardize the culture conditions for regeneration from immature embryos. The *in vitro* response of the anthers of *C. cajan* (Pant A2), *A. albicans*, *A. grandifolia* and *A. volubilis* was tested.

CULTURE CONDITIONS: Seeds (for cotyledon and embryo cultures)

Table IV. Constituents of different basal media used *in vitro* regeneration studies.

Constituents (mg/l)	Murashige and Skoog (1962) MS	White (1919)	Gamborg <i>et al</i> (1968)B5
NH <sub>4</sub> NO <sub>3</sub>	1650	-	-
KNO <sub>3</sub>	1900	80	3000
Ca(NO <sub>3</sub> ) <sub>2</sub> H <sub>2</sub> O	-	288	-
KH <sub>2</sub> PO <sub>4</sub>	170	-	-
NaH <sub>2</sub> PO <sub>4</sub> 4H <sub>2</sub> O	-	19	-
KCl	-	65	-
MgSO <sub>4</sub> 7H <sub>2</sub> O	370	737	500
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	-	-	134
CaCl <sub>2</sub> 2H <sub>2</sub> O	440	-	150
FeSO <sub>4</sub> 7H <sub>2</sub> O	27.8	-	-
Na <sub>2</sub> SO <sub>4</sub>	-	200	-
NaEDTA	37.3	-	-
Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	-	2.5	-
Na-Fe-EDTA	-	-	28
H <sub>3</sub> BO <sub>3</sub>	6.2	1.5	3.0
MnSO <sub>4</sub> 4H <sub>2</sub> O	22.3	6.7	10
ZnSO <sub>4</sub> 7H <sub>2</sub> O	10.6	2.2	2
KI	0.83	0.75	-
Na <sub>2</sub> MoO <sub>4</sub> 2H <sub>2</sub> O	0.25	-	0.25
CuSO <sub>4</sub> 5H <sub>2</sub> O	0.025	-	0.025
CoCl <sub>2</sub> 6H <sub>2</sub> O	0.025	-	0.025
Thiamine HCl	0.1	0.1	10
Pyridoxine HCl	0.5	0.1	-
Nicotinic acid	0.5	0.5	1.0
Myo-inositol	100	-	100
Glycine	2.0	3.0	-

and flower buds (for anther culture) were surface sterilized for 15 minutes in 10% chlorox (CHLOROX, USA), washed thrice in sterile water and retained for about half an hour in sterile water and dried before inoculation. For cotyledon cultures, the seed coat was removed and the seed was split vertically to get two distal and two nodal halves. All the operations were conducted under aseptic conditions in a laminar flow hood. The cultures were maintained at a temperature of  $25 \pm 2^{\circ}\text{C}$  under cool fluorescent light ( $6.8 \text{ watts m}^{-2}$ ).

### 3. SPECIES CROSSABILITY AND HYBRID MORPHOLOGY

### 3.1 RESULTS

#### 3.1.1. CROSSABILITY

Eight species of *Atylosia* (*A. albicans*, *A. cajanifolia*, *A. lineata*, *A. sericea*, *A. scarabaeoides*, *A. grandifolia*, *A. lanceolata* and *A. latisepala*) hybridized successfully with *C. cajan* while the other four *Atylosia* species (*A. mollis*, *A. volubilis*, *A. platycarpa* and *A. rugosa*) and the two *Rhynchosia* species (*R. rothii* and *R. minima*) used in this study failed to cross with the cultivated pigeonpea. The *Cajanus* - *Atylosia* crossability was influenced by the genotype of the *Cajanus* parent (♀) and the cross combination and the hybridization attempts in all resulted in 24 successful combinations (Table V).

Among the 8 successful *Cajanus* - *Atylosia* crosses, the crossability was highest when *A. lineata* was the male parent with 19.5%, 12.30%, 1.37% and 15.20% of the pollination resulting in hybrids in crosses with Pant A2, Baigani, ICP 7035 and C 11 respectively. This was followed by crosses involving *A. albicans* as the male parent with the successful pollinations averaging 9.16% with Pant A2, 7.23% with Baigani, 1.09% with ICP 7035 and 5.12% in crosses with C 11.

The degree of success was low in crosses involving *A. sericea*, *A. scarabaeoides* and *A. cajanifolia* as male parents. *A. sericea* as a pollen parent yielded hybrids in 2.36%, 3.03%, 0.75% and 1.7% of pollinations with Pant A2, Baigani, ICP 7035 and C 11 respectively and when the pollen source was

Table 7. Crossability of *Atylosia* and *Rhynchosia* species with five genotypes of *C. cajan* showing number of pollinations attempted (a), pod setting percentage (b) and seeds/pod - [average(range)] (c)

<i>C. cajan</i> (genotype)	<i>A. albicans</i>		<i>A. saricosa</i>			<i>A. scarabaeoides</i>			<i>A. calanifolia</i>			<i>A. grandifolia</i>			<i>A. latiseppala</i>			
	a	b	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	
Pant A2	731 <sup>1</sup>	9.16	1.79	762	2.36	1.44	542	4.61	1.92	443	2.70	2.33	581	1.72	2.0	222	0.90	2
	904	0.22	[1-3]	1016	0.19	[1-3]	612	0.0	(1-3)	697	0.0	(2-3)	154	0.0	(1-3)	NA	NA	(2)
			1.5		(1)													
Baiganf	442	7.23	2.06	329	3.03	2.0	509	2.35	1.83	491	3.05	1.9	NA	-	NA	-	-	-
	754	0.0	(1-4)	NA	(1-3)	NA	(1-3)	NA	(1-3)	NA	(1-3)	NA	(1-3)	-	-	-	-	-
ICP 7085	632	1.09	2.37	531	0.75	2.5	419	0.47	3	517	0.58	3.0	NA	-	168	0.0	-	-
	NA	NA	(1-4)	NA	(2-3)	NA	(3)	NA	(2-4)	NA	(2-4)	NA	(2-4)	-	NA	-	-	-
C 11	683	5.12	1.91	411	1.70	1.57	683	1.46	2	381	2.09	1.87	682	3.07	2.28	102	1.96	1.5
	NA	NA	(1-3)	382	0.0	(1-2)	411	0.0	(1-3)	NA	(1-2)	63	0.0	(1-3)	NA	(1-3)	NA	(1-2)
ICP 102	642	0.0	-	607	0.0	-	406	0.0	-	270	0.0	-	NA	-	NA	-	-	-
	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

	<i>A. lanceolata</i>			<i>A. linxata</i>			<i>A. mollis</i>			<i>A. platycarpa</i>			<i>A. volubilis</i>			<i>A. rugosa</i>			<i>R. rothlii</i>			<i>R. minima</i>		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
Pant A2	118	1.69	1	415	19.51	2.29	647	0	-	1021	0	-	804	0	-	292	0	-	383	0	-	89	0	-
	NA	NA	(1)	821	0.0	(1-4)	118	0	271	0	211	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Baiganf	NA	-	-	382	12.30	2.27	NA	-	-	NA	-	-	NA	-	-	NA	-	-	NA	-	-	NA	-	-
	NA	NA	(1-5)	NA	(1-5)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
ICP 7085	58	0	-	364	1.37	3.00	NA	-	-	NA	-	-	NA	-	-	NA	-	-	NA	-	-	NA	-	-
	NA	NA	(2-4)	NA	(2-4)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
C 11	107	0.93	Single	407	15.20	2.30	NA	-	-	NA	-	-	NA	-	-	NA	-	-	NA	-	-	NA	-	-
	NA	NA	pod with a single seed	244	0.0	(1-4)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
ICP 102	NA	-	-	512	0.0	-	NA	-	-	NA	-	-	NA	-	-	NA	-	-	NA	-	-	NA	-	-
	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

0 - Value given above the line represent crosses involving *C. cajan* as female parent while those below the line represent



A. scaraboides the crossability with the respective Cajanus parents was 4.61%, 2.35%, 0.47% and 1.46%, while in crosses involving A. cajanifolia 2.7%, 3.05%, 0.58% and 2.09% of the pollination with Pant A2, Baigani, ICP 7035 and C 11 respectively resulted in pod set. Crosses involving the genotype ICP 102 with the above mentioned species of Atylosia failed. In crosses involving the three Australian Atylosias the crossability was low with a pod set of 1.72% and 3.07% in Pant A2 x A. grandifolia and C 11 x A. grandifolia crosses respectively, 0.9% and 1.96% in the crosses Pant A2 x A. latisepala and C 11 x A. latisepala and 1.69% and 0.93% in Pant A2 x A. lanceolata and C 11 x A. lanceolata combinations.

Reciprocal success of a very low frequency was obtained in two cross combinations viz. A. albicans x C. cajan (.22%) and A. sericea x C. cajan (.19%) (Table V). In the crosses A. mollis x C. cajan and A. volubilis x C. cajan pod development in the crossed buds was normal but the seeds from such pods were extremely shrivelled and inviable.

Seed set per pod also varied with the female genotype, with ICP 7035 consistently averaging a higher number of seeds (about 3 per pod) (Table V).

In the study in which A. albicans and A. sericea were used as the male parents in crosses with ICPL 32, ICPL 47, ICPL 59 and ICPL 95 all of which have a common female background in their parentage variation in the crossability was narrow ranging from 2.5% to 5.5% in crosses with A. albicans and 1.5 to 4% in those

Table VI. Crossability of *Atylosia* species with *Cajanus cajan* having a common female background\*.

Cultivar	Parentage	<i>A. albicans</i>			<i>A. sericea</i>		
		a	b	c	a	b	c
ICPL 32	T 21 x Brazil 1465	200	5.5	31.6	200	3.5	32.9
ICPL 47	T 21 x Ja 2772	200	5.0	36.2	200	1.5	34.7
ICPL 59	T 21 x EC 100467	200	4.5	34.9	200	4.0	29.8
ICPL 95	T 21 x NP(WR)15	200	2.5	33.2	200	2.5	31.4

\*a: Number of pollinations attempted

b: Percentage of successful pollinations

c: Pollen sterility in the F<sub>1</sub> hybrids

involving *A. sericea* (Table VI).

### 3.1.2. EFFECT OF HORMONE TREATMENTS

*C. cajan* cv Pant A2 was the female parent in the hormone studies. The successful combinations exhibited a uniform response to hormone treatments. GA3 was found to be superior irrespective of the cross combination. For example when *A. grandifolia* was the pollen parent, pod-set increased from 2% in the control to 14% in treatments with 50 ppm of GA3. The optimum concentration of GA3 was found to be 40-50 ppm. Concentrations above 50 ppm were detrimental with complete failure of pod-set at 80 ppm or above. Kinetin treatments showed no consistent response at the concentrations tested. Treatments with GA3 + Kinetin mixture also did not improve pod-set but were detrimental at higher concentrations. Similar trends were apparent in crosses of *C. cajan* with *A. albicans*, *A. cajanifolia*, or *A. sericea* as male parents (Table VII).

In the four successful crosses studied for hormone response, GA3 alone or in combination with kinetin increased pod length and seeds per pod (Fig.1). In these crosses the average pod length at physiological maturity increased from 4.5 to 5.5 cm in the control to 7 cm. Furthermore, the seeds per pod increased from the range of 1.6-2.2 in controls to 3.5-4 when GA3 or GA3 + kinetin treatments were given. However, hormone treatments did not influence seed size.

Table VII. Percent pod-set in intergeneric crosses of *Cajanus cajan* and *Alysicarpus* species followed by hormone treatments.

Crosses Hormones (ppm)	<i>C. cajan</i> x <i>A. albicans</i>		<i>C. cajan</i> x <i>A. sajanifolia</i>		<i>C. cajan</i> x <i>A. grandifolia</i>		<i>C. cajan</i> x <i>A. saricosa</i>					
	GA3	Kin	GA3+Kin	GA3	Kin	GA3+Kin	GA3	Kin	GA3+Kin			
Control	7	7	7	2	2	2	2	2	1	1	1	1
10	8	8	6	3	2	1	1	3	1	2	0	1
20	8	5	8	2	3	2	2	2	2	3	2	1
30	10	7	7	4	2	1	4	2	1	3	2	2
40	13	6	8	11	3	1	5	1	0	7	3	1
50	17	8	3	7	3	0	14	2	0	5	2	1
60	4	5	1	3	2	0	3	3	0	10	1	0
70	1	4	0	0	2	0	0	2	0	0	2	0
80	0	5	0	0	1	0	0	2	0	0	1	0

Fig. 1. Effect of gibberellic acid and/or kinetin on percent pod and seed set in four *Cajanus-Atylosia* crosses.

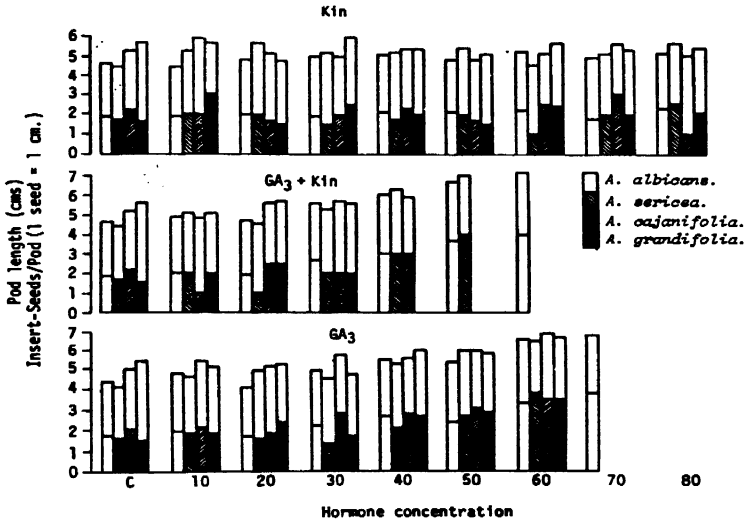


Figure 1.

Among the unsuccessful crosses bud drop commenced within two days after pollination (Fig.2). GA3 treatments prolonged ovary development and delayed bud drop by varying periods depending upon the cross combination and the hormone concentration (Fig.2). For example, when *A. platycarpa* and *A. volubilis* were the pollen parents, bud drop commenced two days after pollination in the control. GA3 prolonged this period up to five days at 60 ppm concentration in *A. volubilis* and at 50 ppm concentration in *A. platycarpa*. When *A. mollis* was the male parent bud drop was delayed by 3-4 days. Increase in ovule size was evident in cross combinations where bud drop was delayed following hormone applications.

In all the reciprocal crosses there was variation in response to hormones (Fig.2b). *A. sericea* and *A. grandifolia* failed to respond to any of the three treatments. In *A. cajanifolia* GA3 delayed bud drop while GA3 or GA3 + kinetin treatments were ineffective, a trend which was similar to the one observed in unsuccessful *Cajanus* crosses. Kinetin at a low concentration (10 ppm) prolonged ovary development by 3 days in *A. platycarpa* and 4 days in *A. albicans*.

Fig. 2. Effect of gibberellic acid and/or kinetin on delaying bud abscission.

a. *Cajanus cajan* as female parent

b. *Cajanus cajan* as male parent



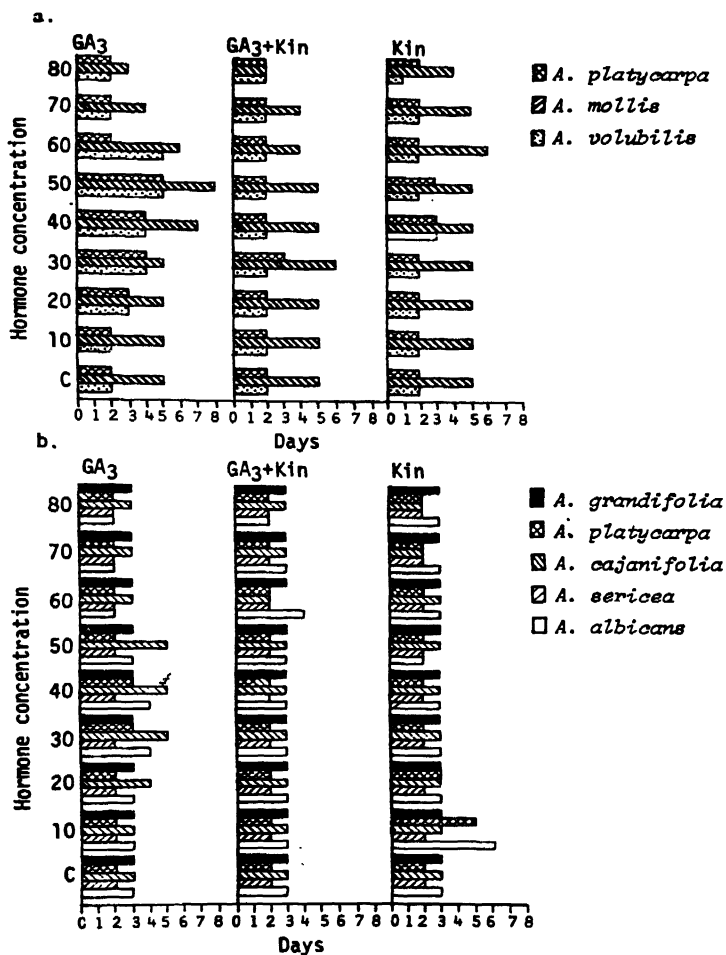


Figure 2.

### 3.1.3. HYBRID MORPHOLOGY

In gross morphology the  $F_1$  hybrids in general tended to be intermediate. *Atylosia* characters like seed strophiole, seed mottling, pod hairiness and persistence of petals were expressed in the  $F_1$ . A comparison of parents and their hybrids for qualitative and quantitative traits are presented in Tables VIII, IX and X, and a hybrid wise description is given below.

#### C. *cajan* x A. *albicans*

The hybrid was a twiner and had a leaflet shape intermediate between the parents (Fig.3B) in its initial stages of growth. The intermediate leaf shape in the hybrid continued for about 100 days. During this period the leaf tip was obtuse. The leaves in some of the branches (Fig.5a-leaf 2) which appeared after 100 days resembled the *Cajanus* parent, having an acute tip (Fig.5C:leaves 4-6). However, as the plant grew further the remaining branches also developed leaves similar to that in the *Cajanus* parent. Although the change in leaf shape was observed in all the three leaflets it was most pronounced in the terminal ones. The texture of the leaves produced after 100 days also resembled the *Cajanus* parent. Scanning electron microscopic studies of the upper surface of the terminal leaflets revealed similarities in the leaf surfaces of the *Cajanus* parent (Fig.5d) and the *Cajanus*-like leaves produced in the hybrid (Fig.5g). Both the leaf surfaces exhibited long trichomes with uniform spread. Leaves of the A. *albicans* parent had a very dense population of trichomes (Fig.5e), whereas the initial

Table VIII. Expression of qualitative traits in parental species and F<sub>1</sub> hybrid between *C. cajan* cv. Pant A2 and *Alysiia* species.

Species/Hybrid	Characters				
	Habit	Leaflet shape	Pod hairiness	Strophiole	Petals
<i>C. cajan</i> cv. Pant A2	Erect shrub	Lanceolate	Short hairs	Absent	Deciduous
<i>A. albicans</i>	Shrubby climber	Obovate	Short hairs	Present	Persistent
<i>C. cajan</i> × <i>A. albicans</i>	Climber	Intermediate initially and lanceolate later	Short hairs	Present	Persistent
<i>A. sericea</i>	Erect shrub	Oblanceolate	Long hairs	Present	Persistent
<i>C. cajan</i> × <i>A. sericea</i>	Erect shrub	Intermediate	Long hairs	Present	Persistent
<i>A. scarabaeoides</i>	Herbaceous twiner	Obovate	Long hairs	Present	Persistent
<i>C. cajan</i> × <i>A. scarabaeoides</i>	Twiner	Intermediate	Long hairs	Present	Persistent
<i>A. cajanifolia</i>	Erect shrub	Lanceolate	Short hairs	Present	Persistent
<i>C. cajan</i> × <i>A. cajanifolia</i>	Erect	Lanceolate	Short hairs	Present	Persistent
<i>A. lineata</i>	Erect shrub	Obovate to oblong	Long hairs	Present	Persistent
<i>C. cajan</i> × <i>A. lineata</i>	Erect shrub	Oblanceolate	Long hairs	Present	Persistent
<i>A. grandifolia</i>	Erect shrub	Ovate to rounded	Long hairs	Present	Persistent
<i>C. cajan</i> × <i>A. grandifolia</i>	Erect	Ovate to rounded	Long hairs	Present	Persistent
<i>A. latisepala</i>	Erect shrub	Ovate	Long hairs	Present	Persistent
<i>C. cajan</i> × <i>A. latisepala</i>	Erect	Ovate	Long hairs	Present	Persistent
<i>A. lanceolata</i>	Erect shrub	Lanceolate	Long hairs	Present	Persistent
<i>C. cajan</i> × <i>A. lanceolata</i>	Erect	Lanceolate			

Table IX. Expression of quantitative traits in parental species and F<sub>1</sub> hybrids between *C. cajan* cv. Pant A2 and *Atylosia* species.

Species/Hybrid	Character				
	Midleaf length (cm)	Midleaf width (cm)	Petiole length (cm)	Days to flower	Duration of flowering
<i>C. cajan</i> cv Pant A-2	7.13	2.85	4.1	<u>65</u>	<u>Sep-Jan</u>
<i>A. albicans</i>	4.41	3.82	4.3	155	Dec-Mar
<i>C. cajan</i> × <i>A. albicans</i>	5.3	3.3	6.0	140	Nov-Mar
<i>A. sericea</i>	2.51	0.63	1.52	126	Nov-Mar
<i>C. cajan</i> × <i>A. sericea</i>	5.34	1.85	3.55	115	Nov-Mar
<i>Atylosia scarabaeoides</i>	3.55	1.81	2.6	76	Sep-Mar
<i>C. cajan</i> × <i>A. scarabaeoides</i>	4.9	2.0	3.8	85	Oct-Feb
<i>A. cajanifolia</i>	5.9	2.35	4.3	118	Oct-Mar
<i>C. cajan</i> × <i>A. cajanifolia</i>	6.5	2.27	4.4	123	Oct-Mar
<i>A. lineata</i>	✓3.82	1.63	1.9	160	Dec-Mar
<i>C. cajan</i> × <i>A. lineata</i>	7.97	3.34	2.6	130	Nov-Apr
<i>A. grandifolia</i>	6.6	5.1	4.3	Maintained as perennial	
<i>C. cajan</i> × <i>A. grandifolia</i>	7.7	4.15	5.8	155	Dec-Apr
<i>A. latisepala</i>	5.83	4.21	5.7	205	Jan-Mar
<i>C. cajan</i> × <i>A. latisepala</i>	6.34	3.96	4.6	172	Dec-Mar
<i>A. lanceolata</i>	9.4	0.82	1.5	222	Feb-Apr
<i>C. cajan</i> × <i>A. lanceolata</i>	-	-	-	-	-

Table X. Expression of quantitative traits in parental species and F<sub>1</sub> hybrids between *G. cajan* cv. Pant A2 and *Atylosia* species.

Species/Hybrid	Character				
	Pod length (cm)	Chambers per pod (locules)	Seeds/pod	100-seed weight (g)	Seed protein (%)
<i>G. cajan</i> cv Pant A-2	5.93	3.8	3.7	6.65	23.95
<i>A. albicans</i>	3.52	4.8	4.5	2.52	30.09
<i>G. cajan</i> × <i>A. albicans</i>	3.91	4.2	2.4	4.90	28.95
<i>A. sericea</i>	1.35	1.5	1.5	2.28	29.87
<i>G. cajan</i> × <i>A. sericea</i>	3.83	2.0	1.2	4.75	28.42
<i>Atylosia</i> <i>scarabaeoides</i>	2.3	4.5	4.4	2.10	28.57
<i>G. cajan</i> × <i>A. scarabaeoides</i>	3.74	3.5	2.6	4.25	28.33
<i>A. cajanifolia</i>	4.31	3.4	3.3	4.4	33.80
<i>G. cajan</i> × <i>A. cajanifolia</i>	4.52	3.7	3.4	5.86	32.95
<i>A. lineata</i>	1.7	1.8	1.6	2.35	28.96
<i>G. cajan</i> × <i>A. lineata</i>	3.05	2.0	1.5	5.15	27.39
<i>A. grandifolia</i>	2.91	3.6	3.4	1.58	33.00
<i>G. cajan</i> × <i>A. grandifolia</i>	3.54	3.50	1.8	4.65	26.8
<i>A. latisejala</i>	2.79	3.3	3.0	2.81	24.60
<i>G. cajan</i> × <i>A. latisejala</i>	3.11	3.00	1.6	5.27	23.86
<i>A. lanceolata</i>	3.72	5.2	5.0	1.95	
<i>G. cajan</i> × <i>A. lanceolata</i>	-	-	-	-	-

intermediate type leaves had a sparse population of short trichomes (Fig. 5f) when compared to the leaves of the Cajanus parent and the Cajanus like leaves in the hybrid. Floral initiation took place only on the branches which had developed leaves similar in shape and texture to the Cajanus parent. The pods in the hybrid were flattened and glabrous with an average length of 3.91 cm. The chambers per pod averaged 4.2 and the seeds were strophiolated and mottled. The seed weight (4.92 g) was in between the parents with protein content (28.95%) close to the Atylosia parent (30.09%). Petals in the hybrid were persistent and the hybrid flowered from November-March with the first flush appearing 140 days after sowing.

#### C. cajan x A. lineata

The  $F_1$  hybrid was erect with a leaflet shape towards the Atylosia parent (Fig. 4D). The leaf length (7.97 cm) and the leaf width (3.34 cm) in the hybrid were higher than the better parent (Table IX). The first flush in the hybrid appeared about 130 days after sowing with the peak flowering period falling between November and April. Petals in the hybrid were persistent. Pod morphology was close to the Atylosia parent. The pods were reddish green in colour with dense hairs and had 2-3 chambers with an average of 1.6 seeds per pod. The pod length averaged 3.05 cm. The seeds were mottled and strophiolated. A 100-seed weight of 5.15 g, and a protein content of 27.39% was recorded (Table X)

Fig. 3. Twigs and leaflets of parents and hybrids.

- A) a. *Cajanus cajan*  
b. Hybrid between *Cajanus cajan* and *Atylosia scarabaeoides*  
c. *Atylosia scarabaeoides*
- B) a. *Cajanus cajan*  
b. Hybrid between *Cajanus cajan* and *Atylosia albicans*  
c. *Atylosia albicans*
- C) a. *Cajanus cajan*  
b. Hybrid between *Cajanus cajan* and *Atylosia sericea*  
c. *Atylosia sericea*
- D) a. *Cajanus cajan*  
b. Hybrid between *Cajanus cajan* and *Atylosia cajanifolia*  
c. *Atylosia cajanifolia*

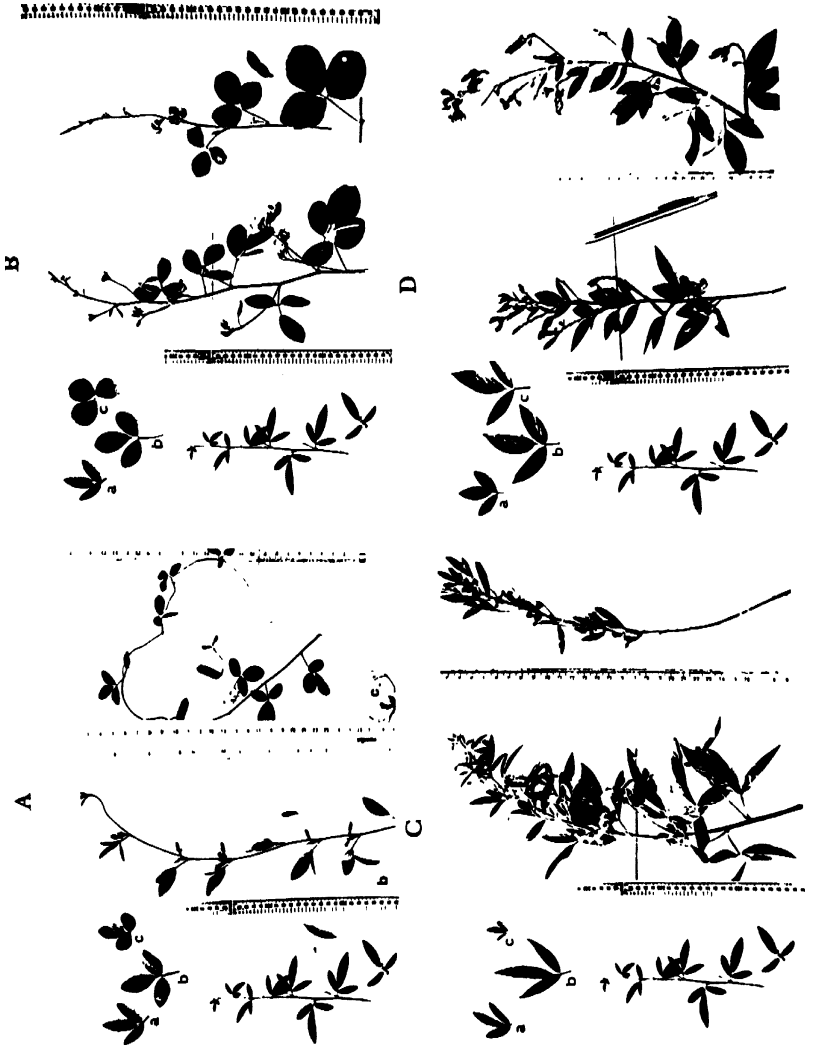




Fig. 4. Twigs and leaflets of parents and F<sub>1</sub> hybrids.

- A) a. *Cajanus cajan*  
b. Hybrid between *Cajanus cajan* and *Atylosia grandifolia*  
c. *Atylosia grandifolia*
- B) a. *Cajanus cajan*  
b. Hybrid between *Cajanus cajan* and *Atylosia latisepala*  
c. *Atylosia latisepala*
- C) a. *Cajanus cajan*  
b. Hybrid between *Cajanus cajan* and *Atylosia lanceolata*  
c. *Atylosia lanceolata*
- D) a. *Cajanus cajan*  
b. Hybrid between *Cajanus cajan* and *Atylosia lineata*  
c. *Atylosia lineata*

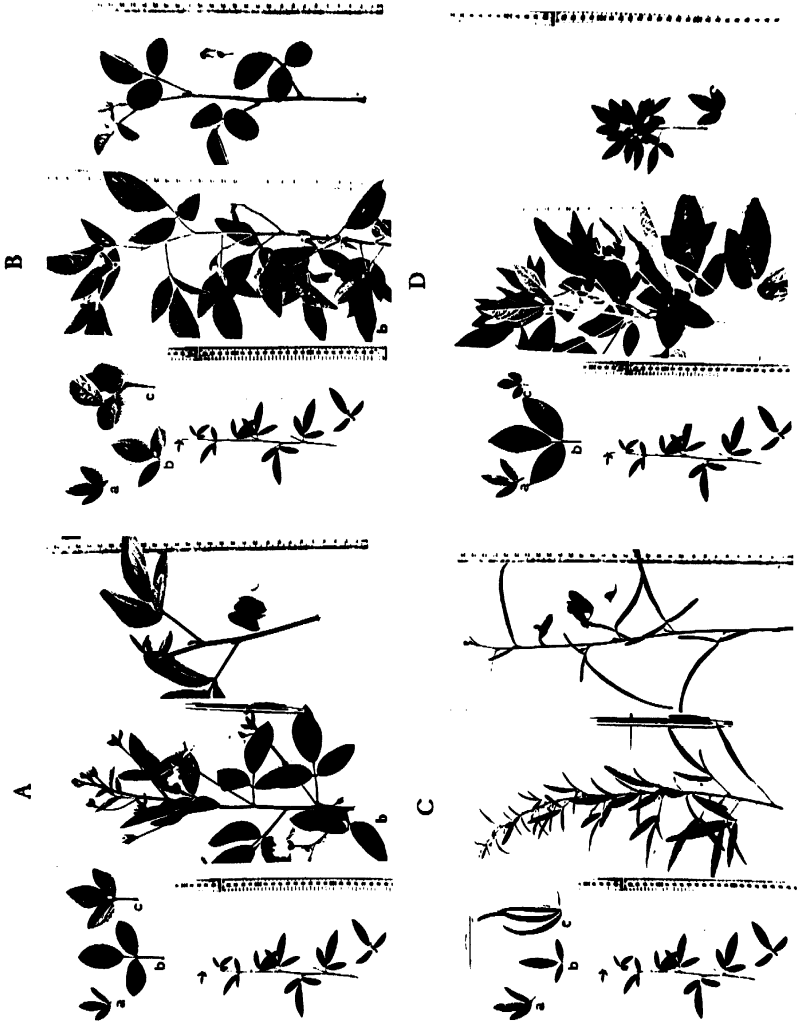
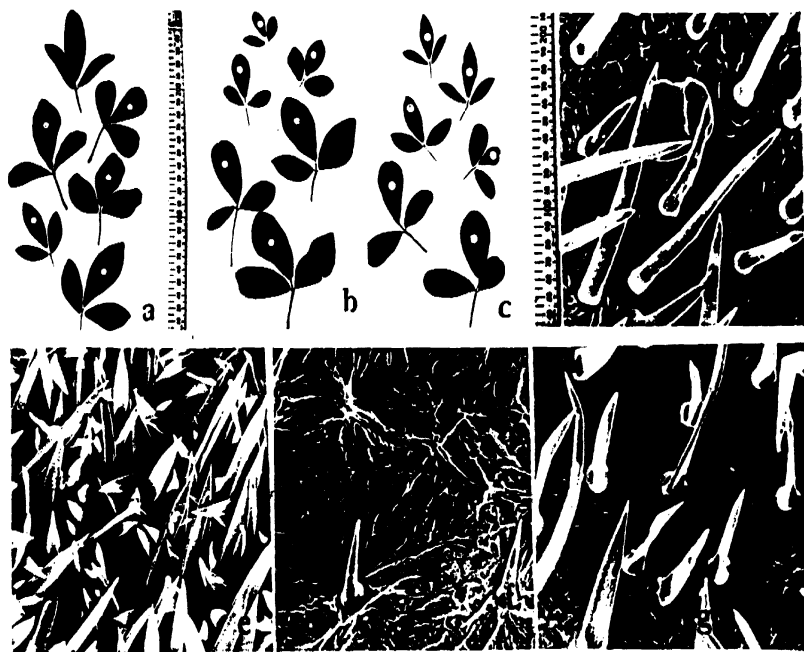


Fig. 5. Variation for leaf shape and ultra structure in hybrids between *Cajanus cajan* and *Atylosia albicans*.

- a. Leaves from different branches (1 to 6 from the bottom) of a typical *Cajanus cajan*-*Atylosia albicans* hybrid. (Note distinct leaf shape from the branch two).
- b. Leaves from bottom to top of a branch, which did not show discernible variation upto 5 months.
- c. Leaves from bottom to top of a branch which showed differently shaped leaves after 100 days.
- d. Leaf surface scan of *Cajanus cajan*.
- e. Leaf surface scan of *Atylosia albicans*.
- f. Intermediate type leaf surface scan.
- g. *Cajanus* type leaf surface scan.



### C. cajan x A. sericea

The hybrid was erect in habit and close to the Cajanus parent in leaf shape (Fig.3C). The mid-leaf length (5.34 cm), mid-leaf width (1.85 cm) and the petiole length (3.55 cm) were all intermediate to the parents (Table IX). Floral initiation took place 126 days after sowing and the peak flowering was recorded between November and March. The petals were persistent and the pods were hairy. Pod length in this hybrid averaged 3.83 cm with an average of 1.8 chambers and 1.2 seeds per pod. The strophiolated and mottled seeds of the hybrid had a 100 seed weight of 4.75 g and a protein content of 28.42% (Table X).

### C. cajan x A. scarabaeoides

The hybrid was intermediate in its habit with the base being bushy and the upper portions showing a twining tendency. The leaflet shape was intermediate (Fig.3A) as were also the mid-leaf length (4.9 cm), mid-leaf width (2.0 cm) and the petiole length (3.8 cm) (Table IX). While the average pod length (3.74 cm) in the hybrid was in between the two parents the number of chambers per pod (3.5) and seeds per pod (2.6) were less than those of either parents (Table X). The seeds were mottled and strophiolated and the seed weight and protein content were 4.25 g and 28.33% respectively. The hybrid flowered 85 days after sowing (October to February) as against 75 days in Atylosia and 65 days in the Cajanus parent.

**C. cajan x A. cajanifolia**

This hybrid unlike others was indistinguishable from the parents. It has an erect habit and a lanceolate leaf shape (Fig.3D) similar to the parents. The mid-leaf length (6.5 cm), mid-leaf width (2.27 cm) and the petiole length (4.4 cm) were also close to the parental values (Table IX). The pod length in the hybrid averaged 4.5 cm with an average of 3.7 chambers and 3.4 seeds per pod. The seed was strophiolated and mottled with a 100-seed weight of 5.86 g and a protein content of 32.95%. The hybrid bloomed in 125 days after sowing and the flowering which initiated in October continued until March.

**C. cajan x A. grandifolia**

The hybrid resembled the Atyosia parent in gross morphology. The leaves were ovate to rounded in shape (Fig.4A) and the mid-leaf length (7.7 cm) was greater than that in either of the parents while the leaf width (4.15 cm) was intermediate between the parents (Table IX). Pods being hairy and yellowish resembled the Atylosia parent and the petals were persistent. The pods had an average of 3.5 chambers and 1.8 seeds with an average length of 3.54 cm. The seeds were mottled and strophiolated and had a 100 seed weight of 4.65 g as against 1.58 g in A. grandifolia and 26.8% protein (Table X). The hybrid which flowered between December and April had its first flush 155 days after sowing.

### C. cajan x A. latisepala

In gross morphology the hybrid was intermediate to the parents. The leaves were intermediate in shape but had a more pointed tip (Fig. 4B) than those in A. latisepala and were much less densely haired than the leaves of A. latisepala. The hybrid leaves were 6.34 cm long, 3.96 cm wide with a 4.6 cm long petiole. The pods in the hybrid, which were less densely haired than those in A. latisepala averaged 3.11 cm in length with an average of 3 chambers and 1.6 seeds per pod. The 100 seed weight (5.27 g) was intermediate to the parental values and the seed protein content was 23.86% (Table X). The hybrid flowered between December and March with the first flush appearing 175 days after sowing.

### C. cajan x A. lanceolata

The hybrid, which in general was intermediate in its gross morphology, had leaves shorter and broader than those of A. lanceolata (Fig. 4C). The hybrid did not flower even after 200 days of growth.

## 3.2. DISCUSSION

Eight species of Atylosia, A. albicans, A. sericea, A. scarabaeoides, A. cajanifolia, A. lineata, A. grandifolia, A. lanceolata and A. latisepala hybridized with C. cajan with varying degrees of success. The Australian species of Atylosia, A. grandifolia, A. lanceolata and A. latisepala have been crossed with cultivated pigeons for the first time. Studies on a few

other species of Australian Atylosias were conducted in a parallel study at the University of Queensland (Ian Dundas, pers. comm.). Earlier attempts at intergeneric hybridization of pigeonpea were mostly concerned with crosses involving A. lineata (Deodikar and Thakur, 1956; Kumar et al, 1958; Kumar and Thombre, 1958; Reddy and De, 1983). There have been a couple of successful attempts at crossing A. sericea and A. scarabaeoides (Sikdar and De, 1967; Reddy, 1981; Pundir, 1981) and Pundir (1981) used A. albicans, A. trinervea and A. cajanifolia in intergeneric studies.

Among eight successful Cajanus - Atylosia crosses in the present study the degree of species crossability was highest in crosses involving A. lineata as the pollen parent followed by those involving A. albicans. The crossability was relatively low with the other species. A. cajanifolia, which is morphologically indistinguishable from Cajanus except for the strophiole on the seed, did not hybridize with the cultivated species as freely as A. lineata or A. albicans. The rate of success in intervarietal crosses of pigeonpea varies from 6% to 70% depending upon the genotypes involved. Thus the degree of species crossability in itself is not an index of species relationship.

A wide variation in the crossability of a given Atylosia species with different cultivars of pigeonpea was evident. Similar genotypic variations for crossability are already known in intervarietal crosses of pigeonpea (Singh et al, 1980; Saxena et al, 1984). The complete failure of the cultivar ICP 102 to cross with any of the Atylosia species is not surprising in the



light of its poor performance in intervarietal crosses of pigeonpea (K.B. Saxena, pers. comm.). The narrow range of variation in the crossability of *Atylosia* species with four different genotypes (ICPL 32, ICPL 47, ICPL 59 and ICPL 95) of *C. cajan* is a reflection of a possible cytoplasmic/since each of these genotype originate from the same female parent.

Differences in the crossability of different varieties of wheat with rye (Rao, 1968; Zeven and Van Heemert, 1970; Lange and Wojciechowska, 1976; Moss and Jalani, 1980) and with *Hordeum bulbosum* (Snape et al, 1979) are known to be under the control of crossability genes (Moss and Jalani, 1980; Falk and Kasha, 1980). With the development of genetic stocks of pigeonpea, it should be possible in *Cajanus - Atylosia* crosses to elucidate the inheritance pattern and the nature of crossability barriers.

The failure of *Cajanus* as a male parent to cross with most of the *Atylosia* species might reflect a pre-fertilization barrier as known in some of the interspecific crosses of *Arachis* (Sastri and Moss, 1982). The differences in the reciprocal crosses might originate from the differences in the commencement and progress of division of endosperm and embryonic cells which has been shown in *Phaseolus* (Robakoarihanta et al, 1979; Shii et al, 1982) or as a result of the failure of the pollen tube to grow through the style (Muller, 1960).

In incompatible *Cajanus-Atylosia* crosses of the present study our preliminary observations on pollen germination and pollen tube growth with the aid of a light microscope using

aceto-carminic stain indicated that the pollen germinates and enters the style. Unfortunately this technique did not help in probing further the development of pollen tubes. Limited investigations on fluorescence microscopy using Aniline blue did not provide any further information because of the limited clearing of the stylar tissue. In the crosses *A. volubilis* x *C. cajan* and *A. mollis* x *C. cajan* pod development was normal but the seed was extremely shrivelled and was inviable. In these crosses the bud drop occurs 6 days after pollination as against the usual 2nd or 3rd day after pollination observed in the other crosses. These observations indicate that the barriers to hybrid production in these two cross combinations might be operating at or after fertilization. But the failure to recover any viable seed with hormone treatments or embryo culture in these crosses could be taken as an indication for pre-fertilization barriers. Development of mature pods with no seed development has also been reported in crosses involving *Pisum arvense* as female and *P. abyssinicum* and *P. jomardii* as pollen parents (Foujdar and Tandon, 1976).

The crossed pods were shorter in length with fewer seed per pod than selfed pods. The rate of pod development was also slower in the crossed pods. ICP 7035 consistently averaged a higher number of seeds per pod in crosses with all the *Alyosia* species. Seeds per pod in ICP 7035 is greater than that in any other cultivar used in this study implying the predisposition of a greater number of ovules per ovary for the alien pollen to fertilize leading to a higher seed set in the mature pods.

The results of hormone treatments indicate that in the intergeneric crosses of Cajanus and Atylosia the rate of success can be increased with hormone treatments which also increase the percentage of pod-set, pod length and number of seeds per pod in the cross combinations. The success rate in the untreated controls was low. Moreover, the onset of flower drop among the unsuccessful crosses was delayed following hormone treatments.

The increased pod set associated with most of the GA3 or GA3+kinetin treatments is likely due to enhanced post-fertilization development of both the ovary and ovule(s) since treatments commenced one day after pollination when fertilization would have been completed. The increase in pod length and number of seeds per pod following hormone treatments further substantiate such an interpretation. Higher concentrations of GA3 and GA3 + kinetin reduced the rate of success, but promoted pod length and the number of seeds per pod which suggests the role of hormones in post-fertilization development.

Kruse (1967) and Kasha et al (1978) attributed increased success in obtaining progeny from intergeneric or interspecific crosses to the check by exogenous GA3 supplied to the florets on post-fertilization breakdown. The increase in pod length in our Cajanus-Atylosia crosses may reflect increased cell number and/or cell elongation in the ovary wall after hormone treatments. Kasha et al (1978) observed increased mitotic activity following GA3 treatments in interspecific crosses of Hordeum. Sastri and Moss (1982) found increases in peg number as well as peg length following GA3 treatments in Arachis interspecific crosses.

Al-Yasiri and Coyne (1964) also recorded a positive influence of hormones on pod length and diameter in a *Phaseolus* species cross. Our findings in pigeonpea intergeneric crosses are consistent with these results.

The failure to increase further the number of successful cross combinations emphasises the hormonal role in the post-fertilization processes only. Since the hormones did not improve the size of the mature seeds, the increase in ovule size in the unsuccessful crosses is likely due to delay in bud drop thereby permitting the ovule to develop for a longer period. In the unsuccessful combinations GA3 treatments helped to delay the commencement of flower bud abscission. In crosses between *Phaseolus vulgaris* and *P. acutifolius*, a combination of GA3 and NAA was used to overcome pod abscission (Al-Yasiri and Coyne, 1964). Emsweller and Stuart (1948) reported the influence of growth regulators on retarding the senescence of the embryo sac, and on the length and diameter of capsules in *Lilium longiflorum*. Although kinetin treatments (10 to 80 ppm) did not show any improvement in pod-set or the number of seeds per pod in our study, treatment at the lowest concentration (10 ppm) was effective in delaying bud drop in the *A. albicans* x *C. cajan* and *A. platycarpa* x *C. cajan* crosses.

The data show that, among the various crosses studied, response to hormone treatments is determined by the female parent irrespective of the pollen parent used (Table VII, Figs. 1 and 2). The response of *A. cajanifolia* as a female parent was similar to that of *C. cajan*. These two species have more

morphological similarities than do the other species used in this study and are considered one genus by some taxonomists (van der Maesen, 1980). In the unsuccessful combinations in spite of increased ovule size following hormone treatments, our attempts to culture treated ovules aseptically have failed. Furthermore, cultures of premature (< 11 day old) selfed embryos on defined media have not developed into plants. (Details of embryo culture are presented in Chapter 7).

The present study shows that hormones can increase the percentage of success in the crosses where the degree of success is otherwise low. On the basis of the present results, it is suggested that it would be worthwhile to try other hormones and their combinations in an attempt to increase the number of successful crosses through further refinements in the embryo culture technique.

The hybrid nature of the  $F_1$  plants was very much evident from observing their plant morphology. *Atylosia* characters like seed strophiole, seed mottles, pod hairiness and twining nature are dominant and their expression in the hybrids permitted an unambiguous identification of hybrids and selfs. The leaf shape in the hybrids, which in most of the cases was either intermediate or tending to resemble the male parent, helped in easy identification of the hybrids. McComb (1975) raised doubts about the genuinity of intergeneric hybrids in leguminosae owing to apomictic development of seeds in some leguminous species but in the present material with the expression of the male parent traits the problem of identifying true hybrids is circumvented.

In the C. cajan x A. albicans hybrid an interesting feature was the variation for leaflet shape and texture (Fig.5). The development of leaves similar to the Cajanus parent after about 100 days led us to look for the possibility of selective chromosome elimination similar to that of Nicotiana (Gupta and Gupta, 1973) or Hordeum (Subrahmanyam and Kasha, 1973). But our cytological studies presented in Chapter 4 revealed eleven bivalents and regular disjunction. This could be taken as evidence to rule out the possibility of chromosome elimination or in support of chromosome doubling following chromosome elimination. A detailed meiotic analysis (Sateesh Kumar et al, 1984) revealed the hybrid nature of the material. Thus it was concluded that the variation in leaf morphology accompanied by flowering is a consequence of differential gene expression in different branches. Since floral initiation occurred only in the branches on which Cajanus like leaves started developing, it is likely that these two processes are temporal events in gene expression.

Somatic variation in C. cajan was reported to be chimeral (Rao and Reddy, 1975) which appeared from the seedling stage. Thus it is different from the type encountered in the present Cajanus-Atylosia hybrid.

#### **4. MEIOTIC BEHAVIOUR**

#### 4.1 PARENTS

##### 4.1.1. RESULTS

Chromosome number in *Cajanus cajan* and all the species of *Atylosia* and *Rhynchosia* used in this study was found to be  $n=11$  or  $2n=22$ . Meiosis in pigeonpea (Fig.6A-G) was highly organized with regular bivalent formation and normal disjunctions at both divisions irrespective of the cultivars used. Species of *Atylosia* and *Rhynchosia* also formed regular bivalents (Figs.7 and 8) with normal disjunction except for occasional asynchrony at division-II in *A. lanceolata* and *A. sericea*. Different cultivars showed minor variations in their chiasma frequency (Table XI). In all the nine cultivars of *Cajanus* the chiasma frequency was around 20 per cell ranging from 19.75 in C 11' to 20.75 in Baigani. The chiasmata per cell in *A. sericea* (20.85), *A. cajanifolia* (20.50), *A. latisejala* (20.50), *A. lanceolata* (20.30), *A. scarabaeoides* (20.52) and *A. lineata* (20.42) were close to those in *Cajanus* but different from *A. albicans* (17.75) and *A. grandifolia* (17.72).

In *Cajanus cajan* and *Atylosia* species studied, pollen mother cells (PMCs) containing nucleolus associated with two bivalents were common (Figs. 6B, 7G, 7J, 8D, 8J) at diakinesis while two nucleoli per PMC (Figs.7A and 7J) were less frequent at diakinesis and rare at pachytene (Fig.20A). In *C. cajan* the number of nucleoli per PMC varied from 2 to 4 at telophase-I (T-I) (Fig. 9A) and 4 to 8 at T-II (Fig. 9B-F) irrespective of the cultivar. Similar variations were evident in *A. albicans*,



Table XI. Metaphase-I configurations\* and pollen fertility in *G. cajan* cultivars and *Alysiia* species.

Cultivar/species	Bivalents Av. (Range)		x-ta/cell	Pollen fertility (%)
	Ring	Rod		
<i>G. cajan</i> cv. ICP 7035	9.4 (9-10)	1.6 (0-2)	20.40±0.09	96
<i>G. cajan</i> cv. Pant A2	9.13 (8-11)	1.86 (0-3)	20.12±0.13	97
<i>G. cajan</i> cv. Baigani	9.76 (9-11)	1.23 (0-2)	20.75±0.16	97
<i>G. cajan</i> cv. C 11	8.76 (8-9)	2.23 (2-3)	19.75±0.07	98
<i>G. cajan</i> cv. ICPL 32	9.33 (8-10)	1.66 (1-3)	20.32±0.12	94
<i>G. cajan</i> cv. ICPL 47	9.56 (9-11)	1.43 (0-2)	20.55±0.12	96
<i>G. cajan</i> cv. ICPL 59	9.13 (8-10)	1.86 (1-3)	20.12±0.15	95
<i>G. cajan</i> cv. ICPL 95	9.03 (8-10)	1.96 (1-3)	20.02±0.10	98
<i>G. cajan</i> cv. ICP 102	9.3 (8-10)	1.7 (1-3)	20.30±0.12	94
<i>A. albicans</i>	6.76 (6-9)	4.23 (2-5)	17.75±0.16	90
<i>A. lineata</i>	8.43 (7-10)	2.56 (1-4)	19.42±0.14	93
<i>A. scarabaeoides</i>	8.53 (7-9)	2.46 (2-4)	19.52±0.12	95
<i>A. sericea</i>	9.86 (8-11)	1.13 (0-3)	20.85±0.17	92
<i>A. cajanifolia</i>	9.5 (9-11)	1.5 (0-2)	20.50±0.12	96
<i>A. grandifolia</i>	6.73 (6-8)	4.26 (3-5)	17.72±0.13	89
<i>A. latisenala</i>	9.5 (8-11)	1.5 (0-3)	20.50±0.13	91
<i>A. lanceolata</i>	9.3 (9-10)	1.7 (1-2)	20.30±0.08	93

\*30 cells were scored for each species/cultivar.

Fig. 6. Meiosis in *C. cajan* cv. Pant A2 and metaphase I in cultivars of *C. cajan*. (Bar indicates 10  $\mu$ ).

A-G: Meiosis in *C. cajan* cv. Pant A2.

A) Pachytene showing regular pairing.

B) Diakinesis showing two bivalents attached to the nucleolus.

C) Metaphase I with 11 bivalents.

D) Equatorial view showing chromatic associations at late metaphase I.

E&F) Anaphase disjunctions.

G) Late anaphase II showing regular disjunction.

H-O: Metaphase I in cultivars of pigeonpea showing 11 bivalents.

H) Baigani. I) C 11. J) ICP 102. K) ICP 7035.

L) ICPL 32. M) ICPL 47. N) ICPL 59. O) ICPL 95.

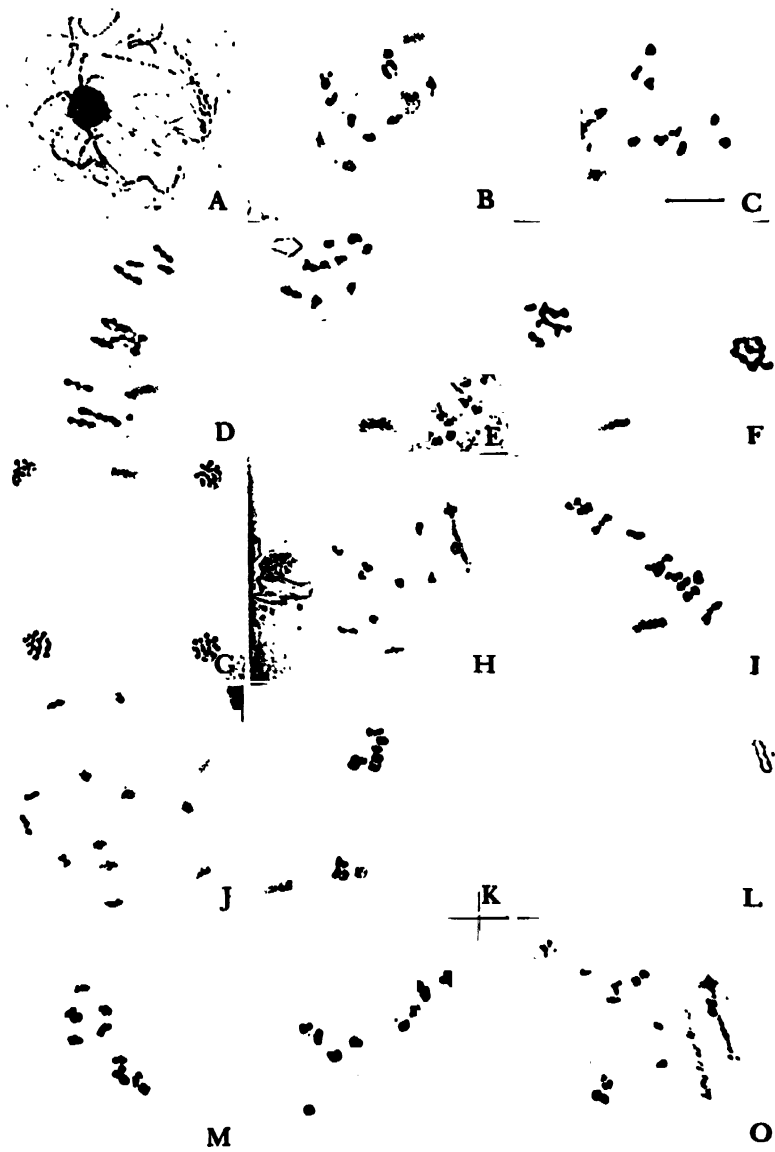


Fig. 7. Meiosis (Diakinesis, metaphase I and anaphase I) in *Atylosia* species. (Bar indicates 10  $\mu$ )

A-C: *Atylosia lanceolata*

D-F *Atylosia grandifolia*

G-I *Atylosia latisejala*

J-L *Atylosia albicans*

Note two nucleoli in A and J.



Fig. 8. Meiosis (Diakinesis, metaphase I and anaphase I) in *Atylosia* species. (Bar indicates 10  $\mu$ )

A-C: *Atylosia sericea*

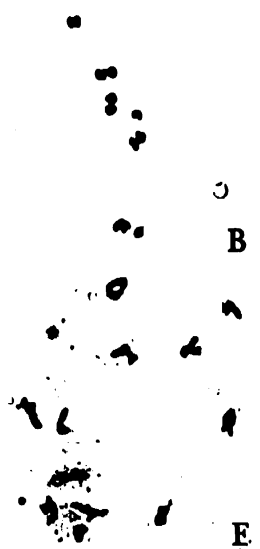
D-F: *Atylosia cajanifolia*

G-I: *Atylosia lineata*

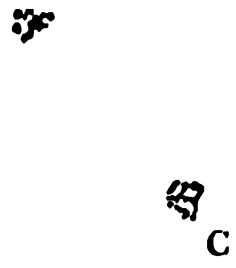
J-L: *Atylosia scarabaeoides*



A



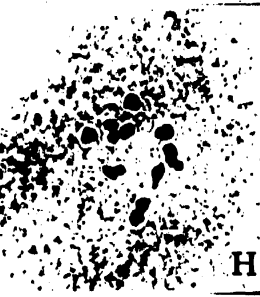
B



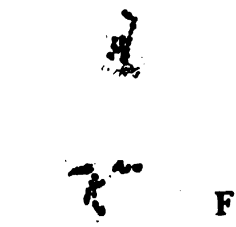
C



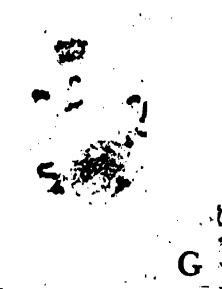
D



E



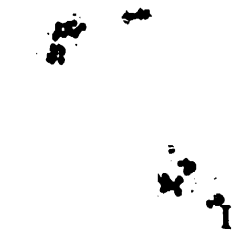
F



G



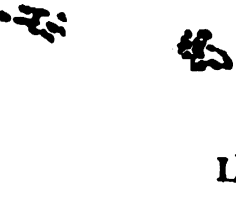
H



I



J



L

*A. lineata* and *A. grandifolia* while in *A. cajanifolia*, *A. lanceolata* and *A. scarabaeoides*, the maximum number of nucleoli per PMC was seven (Fig. 9G-L). The maximum number of nucleoli in each daughter nucleus at T-I or T-II never exceeded two (Fig. 9) one of which was always bigger than the other. The variations in frequencies of PMCs with different numbers of nucleoli at T-II in different species are presented in Fig. 10. The most frequent types were PMCs with four nucleoli while the least frequent ones were those with eight nucleoli.

#### 4.1.2. DISCUSSION:

The present study on the chromosome number, in *C. cajan*, *A. lineata*, *A. cajanifolia*, *A. sericea*, *A. scarabaeoides*, *A. volubilis* and *A. rugosa* confirms the earlier reports (Kumar *et al.*, 1958; Sikder and De, 1967; Shrivastava *et al.*, 1973; Reddy, 1973; Bir and Kumar, 1973; 1977; Pundir and Singh, 1978; Rao, 1978; Sanjappa and Satyananda, 1979; Pundir, 1981) as  $2n=22$ . The chromosome numbers of *A. grandifolia*, *A. latisepala*, *A. lanceolata*, *A. mollis*, *R. rothii* and *R. minima* which have not been reported earlier has been found to be  $2n=22$ .

The maximum number of nucleoli in each daughter nucleus at T-I or T-II, never exceeded two (Fig. 9) irrespective of the cultivar or species studied suggesting thereby the presence of two nucleolar organizers (N.O.) per genome in pigeonpea. Furthermore, the occurrence of a maximum of two nucleoli at



Fig. 9. Nucleolar distribution in pollen mother cells of *C. cajan* and species of *Atylosia*. (Bar indicates 10  $\mu$ m).

A-F: Nucleolar distribution in pollen mother cells of *Cajanus cajan*.

- A) Telophase-I nucleolar distribution, 2-2.  
Telophase-II nucleolar distribution. B) 2-2-2-2.  
C) 2-2-2-1. D) 2-2-1-1. E) 2-1-1-1. F) 1-1-1-1.

G-L: Nucleolar distribution in pollen mother cells of *Atylosia* species.

- G) Telophase-I nucleolar distribution in *A. grandifolia*, 2-2.  
H) Telophase-II nucleolar distribution in *A. grandifolia*, 2-2-1-1 (Arrow indicates two fusing nucleoli).  
I) Telophase-II nucleolar distribution in *A. cajaniifolia*, 2-2-1-1 (Note the difference in the size of fused and primary nucleoli).  
J) Telophase-II nucleolar distribution in *A. lineata*, 2-2-2-1.  
K) Telophase-II nucleolar distribution in *A. latisejala*, 2-2-1-1.  
L) Telophase-II nucleolar distribution in *A. albicans*, 2-2-2-1.

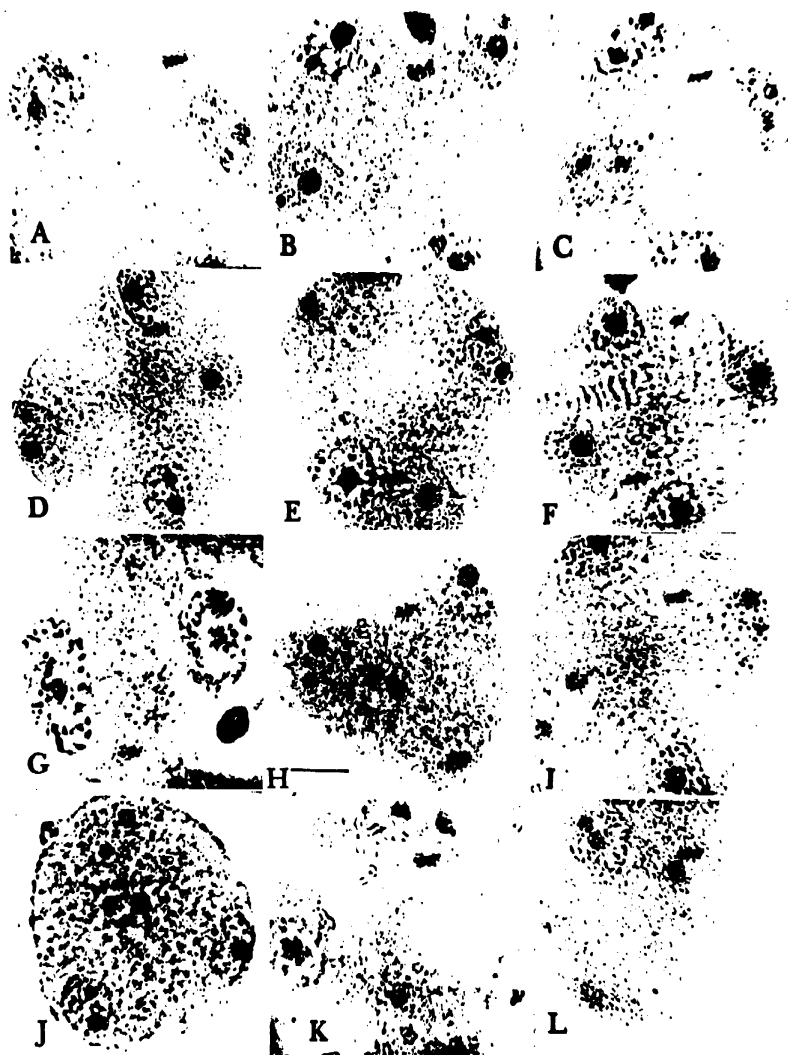
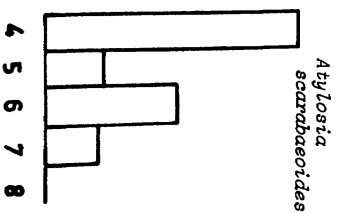
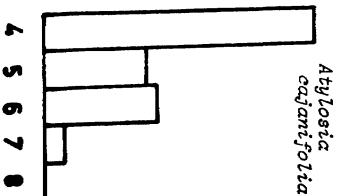
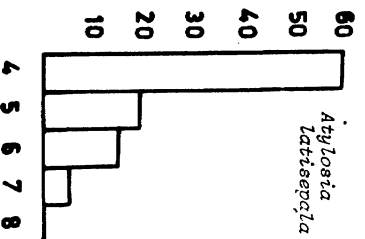
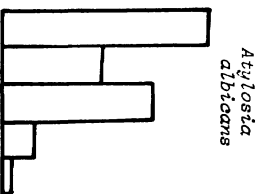
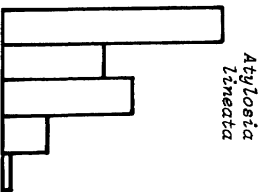
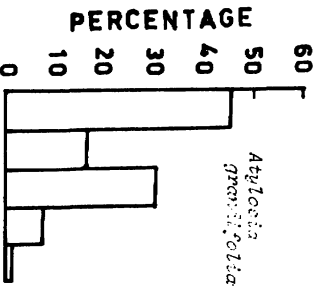
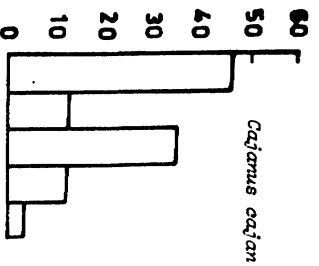


Fig. 10. Frequency distribution of nucleoli at telophase-II in pollen mother cells of *C. cajan* and *Atylosia* species.



NO. OF NUCLEOLI

pachytene and the presence of two nucleolar bivalents at diakinesis is also indicative of two pairs of nucleolar organizers in the *Cajanus* and *Atylosia* species studied.

The variation in the frequencies of PMC's at T-II with different numbers of nucleoli in different species are presented in Figure 10. Invariably the most frequent type were PMC's with four nucleoli while the least frequent ones were those with eight nucleoli. However, the frequency of PMC's with at least one daughter nucleus possessing the maximum number of nucleoli was >50% in all the species which showed up to 8 nucleoli per T-II PMC (Fig.10) while in the other species that had a maximum of 7 nucleoli, the frequencies of PMC's containing at least one daughter nucleus with two nucleoli was <50%. Such variation in the frequencies of PMC's with different numbers of nucleoli (4 to 8) is a reflection of the nucleolar fusion which is very common (Sybenga, 1972; Bennett *et al*, 1973; Darvey and Driscoll, 1972; Flavell and O'Dell, 1979). The preponderance of PMC's with 4 nucleoli (1 nucleolus per daughter nucleus) at T-II is indicative of the frequent occurrence of the fusion of two nucleoli in each daughter nucleus and the accumulation of cells with 4 nucleoli. Meiotic studies, in a spontaneous tetraploid of *C. cajan* (isolated by Dr. K.B. Saxena) revealed eight primary nucleoli at T-I (Fig.21 in chapter 4.2.2) further substantiating the presence of two nucleolar organizers per genome.

The size difference in the two nucleoli in each of the T-II nuclei (Fig. 9B) indicates that the two nucleolar organizers (in each of the genomes) differ in their activities. Cultivated barley is a well known example for such differences (Anastassova-Kristeva *et al.*, 1977; Subrahmanyam and Azad, 1978a; Linde-Laursen, 1984; Jessop and Subrahmanyam, 1984). According to Heitz (1931), the number of telophase nucleoli is constant, as well as the number of nucleolar organizers and SAT chromosomes for a given karyotype. These observations acquire a new meaning in the light of the evidence that genes coding for ribosomal RNA are located in the nucleolar organizer region (NOR) and the maximum number of nucleoli per nucleus correspond to the number of rRNA synthesizing loci (Anostassova-Kristeva, 1977; Nicoloff *et al.*, 1977; Subrahmanyam and Azad 1978a, b; Flavell and O'Dell, 1979; Linde-Laursen, 1984; Jessop and Subrahmanyam, 1984). For the karyotype of pigeonpea, there have been controversial reports on the number of SAT chromosomes. For instance, Sinha and Kumar (1979) reported lack of SAT chromosomes in certain cultivars of *C. cajan* and Shrivastava *et al.* (1973) recorded one SAT chromosome per genome in *Cajanus* while Pundir (1981) found two SAT chromosomes per genome in *Cajanus* and *Atylosia* species. The present data (Figs. 9 and 10) on the nucleolar behaviour in FMCs conclusively establish two nucleolar organizers per genome in *Cajanus* and the *Atylosia* species studied. The differences in the size of the two nucleoli in daughter nuclei may reflect differences in the degree of activity and/or differences in the multiplicity of rRNA cistrons in the two nucleolar organizers in each of the species.

Controversies in the nucleolar organizer number have often been resolved using the mitotic nucleolar number as an index of N.O. activity (Anastassova-Kristeva *et al.*, 1978; Jessop and Subrahmanyam, 1984; Linde-Laursen, 1984). In the present study nucleolar behaviour during meiosis has been used in demonstrating the number of nucleolar organizers in a wide range of species.

## 4.2. HYBRIDS

### 4.2.1. RESULTS

All the Cajanus-Atylosia hybrids that flowered were analyzed cytologically. Meiosis was studied in detail with emphasis on metaphase-I pairing, and anaphase disjunctions. The results show the following:

#### C. cajan x A. albicans:

FMCs of this hybrid exhibited regular formation of eleven bivalents which were predominantly rings (Fig. 11E). There were no significant differences in the chiasma frequency between cultivar combinations (Table XII), the maximum being 20.75 per cell in Pant A2 x A. albicans and the minimum 20.00 per cell in hybrids with ICP 7035. A low frequency of precocious separation was evident in all the cultivar combinations. Diplotene cells displayed nucleoli with three or four bivalents attached (Figs. 11A-B), while at diakinesis 20 of the 64 cells contained nucleoli with three or four bivalents (Fig. 11C-D). Anaphase-I and II separations in all the cultivar combinations were regular (Fig. 11F-G). Pollen sterility in these hybrids ranged from 27.3% in Baigani x A. albicans to 46.1% in ICP 7035 x A. albicans while ovule fertility ranged from 21.6% in Pant A2 x A. albicans to 32.2% in Baigani x A. albicans hybrids.



Fig. 11. Meiosis in the hybrid between *Cajanus cajan* and *Atylosia albicans* (Bar indicates 10  $\mu$ ): (A) Diplotene nucleolus with four bivalents attached. (B) Diplotene nucleolus with three bivalents attached. (C) Diakinesis nucleolus with four bivalents attached. (D) Diakinesis nucleolus with three bivalents attached. (E) Metaphase-I with eleven bivalents. (F) Regular disjunction at division-I. (G) Regular disjunction at division-II. (H) Quartet showing nucleolar variation, 3-2-1-1.

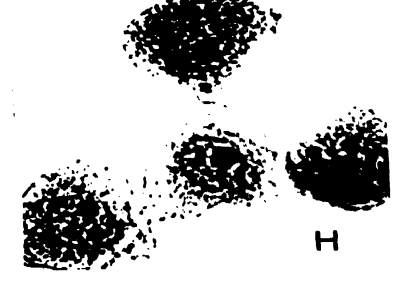
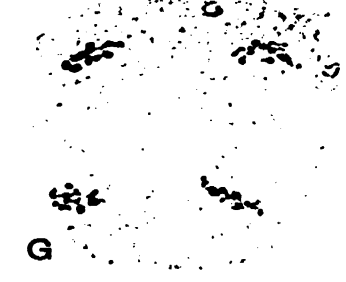
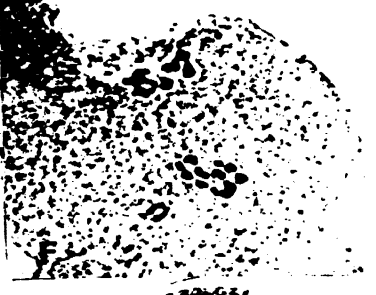
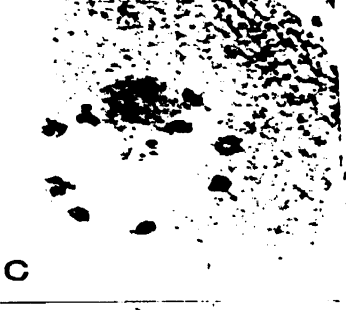
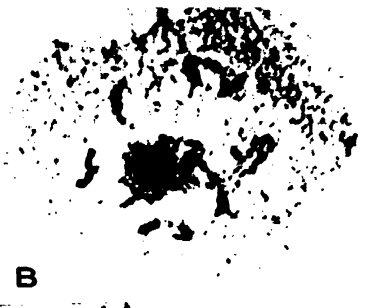


Table XII. Metaphase-I configurations, Anaphase-I abnormalities, pollen sterility and ovule fertility in  $F_1$  hybrids between *A. calian* and different species of *Alysiola*

$F_1$ Hybrids (♀ x ♂)	METAPHASE-I				ANAPHASE-I				Ovule ferti- lity (%)
	No. of cells	Bivalents Average (range)		Unive- lents Average (range)	X-ta/cell	Nor- mal	Abnor- mal (%)	Pollen ferti- lity (%)	
		Ring	Rod						
I. 1. Pant A2 x <i>A. albicans</i>	234	9.76 (9-11)	1.23 (0-2)	0	20.75 ± 0.05	98	0	39.4	21.6
2. Belgant x <i>A. albicans</i>	132	9.35 (9-11)	1.64 (0-2)	0	20.34 ± 0.052	58	0	27.3	32.2
3. ICP 7035 x <i>A. albicans</i>	56	9.01 (8-10)	1.98 (1-3)	0	20.00 ± 0.093	31	0	46.1	29.6
4. C 11 x <i>A. albicans</i>	116	9.68 (9-11)	1.31 (0-2)	0	20.67 ± 0.07	47	0	23.4	31.7
II. 1. Pant A2 x <i>A. saricca</i>	121	9.26 (8-11)	1.73 (0-3)	0	20.25 ± 0.085	81	1.2	29.7	54.3
2. Belgant x <i>A. saricca</i>	118	9.37 (9-10)	1.63 (1-2)	0	20.37 ± 0.044	67	0	33.6	61.9
3. ICP 7035 x <i>A. saricca</i>	79	8.82 (8-10)	1.97 (1-3)	0.40 (0-2)	19.61 ± 0.097	32	8.5	48.4	46.6
4. C 11 x <i>A. saricca</i>	130	8.39 (7-9)	2.60 (2-4)	0	19.38 ± 0.059	NA	-	36.1	52.5
III. 1. Pant A2 x <i>A. scarabaeoides</i>	104	8.66 (7-10)	2.16 (1-4)	0.34 (0-2)	19.48 ± 0.065	78	4.8	29.3	37.6
2. Belgant x <i>A. scarabaeoides</i>	68	8.75 (8-10)	2.13 (1-3)	0.26 (0-2)	19.63 ± 0.058	59	6.3	22.7	40.1
3. ICP 7035 x <i>A. scarabaeoides</i>	97	9.20 (8-11)	1.64 (0-3)	0.29 (0-2)	20.04 ± 0.087	32	8.5	41.5	29.3
4. C 11 x <i>A. scarabaeoides</i>	113	9.31 (8-11)	1.65 (0-3)	0.26 (0-2)	20.25 ± 0.072	52	3.7	31.3	52.8
IV. 1. Pant A2 x <i>A. calianifolia</i>	92	10.21 (10-11)	0.78 (0-1)	0	21.20 ± 0.043	24	0	14.6	71.4

Contd....

Table XII. Contd.

F <sub>1</sub> Hybrids (♀ x ♂)	METAPHASE-I			ANAPHASE-I					
	No. of cells	Bivalents Average (range)	Univalent Average (range)	X-ta/cell	Mor- mal	Abnor- mal(%)	Pollen ferti- lity (%)	Ovule ferti- lity (%)	
2. Baiganj x <i>A. calanifolia</i>	104	9.5 (9-11)	1.49 (0-2)	0	20.49 ± 0.065	30	0	15.3	63.7
3. ICP 7035 x <i>A. calanifolia</i>	112	9.51 (9-11)	1.48 (0-2)	0	20.50 ± 0.058	49	0	29.1	70.4
4. C 11 x <i>A. calanifolia</i>	54	10.20 (10-11)	0.79 (0-1)	0	21.19 ± 0.055	NA	-	18.7	85.2
V. 1. Pant A2 x: <i>A. linata</i>	114	8.71 (8-10)	2.28 (1-3)	0	19.70 ± 0.072	67	0	31.2	54.5
2. Baiganj x <i>A. linata</i>	61	9.31 (9-10)	1.68 (1-2)	0	20.30 ± 0.059	44	0	37.5	67.7
3. ICP 7035 x <i>A. linata</i>	127	9.06 (8-10)	1.88 (1-3)	0.09 (0-2)	20.00 ± 0.062	22	4.3	46.2	31.3
4. C 11 x <i>A. linata</i>	112	9.35	1.64	0	20.34 ± 0.045	51	0	27.5	72.7
VI. 1. Pant A2 x <i>A. grandifolia</i>	131	7.52 (5-8)	3.06 (3-6)	0.61 (0-6)	18.10 ± 0.228	71	41.8	54.8	23.4
2. C 11 x <i>A. grandifolia</i>	68	7.57 (5-8)	2.91 (3-6)	1.02 (0-6)	18.05 ± 0.251	NA	-	48.3	19.9
VII. 1. Pant A2 x <i>A. latispala</i>	73	7.54 (7-8)	2.89 (3-4)	1.12 (0-4)	17.97 ± 0.24	70	27.8	43.9	24.5
2. C 11 x <i>A. latispala</i>	106	6.72 (5-7)	3.89 (4-6)	0.75 (0-4)	17.33 ± 0.20	58	33.3	50.6	21.7
VIII. 1. Pant A2 x <i>A. lanceolata</i>	The only plant died of wilt								
2. C 11 x <i>A. lanceolata</i>	Both the plants did not flower well								

**C. cajan x A. sericea:**

Bivalent formation was a general feature in these hybrids with the exception of the hybrids between ICP 7035 and A. sericea which displayed a low frequency (0.4 per PMC) of univalents. Chiasma frequency varied from 19.38 to 20.37 (Table XII). One or two bivalents were attached to the nucleolus (Fig.12A). Anaphase disjunctions were regular at both the divisions (Fig.12C,D) except in hybrids with ICP 7035. Laggards were observed in 8.6% of the PMCs. Pollen sterility of 29.7%, 33.6%, 48.4% and 36.1% and a ovule fertility of 54.3%, 61.5%, 46.6% and 52.5% were recorded in hybrids of A. sericea with Pant A2, Baigani, ICP 7035, and C 11 respectively (Table XII).

**C. cajan x A. scarabaeoides:**

Hybrids of Cajanus with A. scarabaeoides showed a low frequency of univalents (Fig.13C) (0.26-0.34/cell) with a maximum of two per cell. The chiasma frequency varied between cultivar combinations ranging from 19.48 in Pant A2 x A. scarabaeoides to 20.35 in hybrids with C 11 (Table XII). Diplotene/Diakinesis nucleoli were found associated with up to two bivalents. A low frequency (3.8 to 8.6%) of anaphase-I cells displayed laggards (Fig.13D) and irregular disjunction irrespective of the cultivar used. Pollen sterility ranged from 22.7% in hybrids of A. scarabaeoides with Baigani to 41.5% in hybrids with ICP 7035. Ovule fertility varied from 29.3% in hybrids with ICP 7035 to 52.8% in those with C 11 (Table XII).

Fig. 12. A-D: Meiosis in hybrids between *Cajanus cajan* and *Atylosia sericea*.

- A) Diakinesis showing 11 bivalents.
- B) Metaphase-I showing 11 bivalents.
- C) Anaphase-I showing regular disjunction.
- D) Anaphase-II showing regular disjunction.

E-H: Meiosis in hybrids between *Cajanus cajan* and *Atylosia cajanifolia*.

- E) Diakinesis showing 2 bivalents attached to the nucleolus.
- F) Metaphase-I showing 11 bivalents.
- G) Regular disjunction at division-I.
- H) Regular disjunction at division-II.

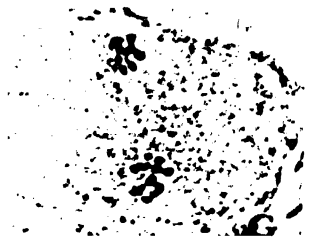
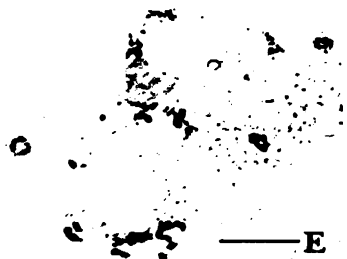
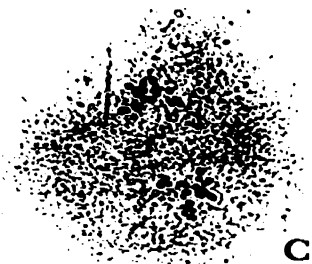
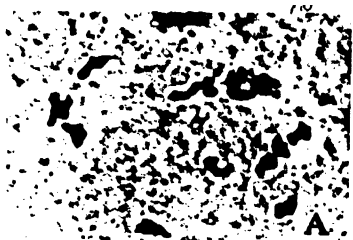


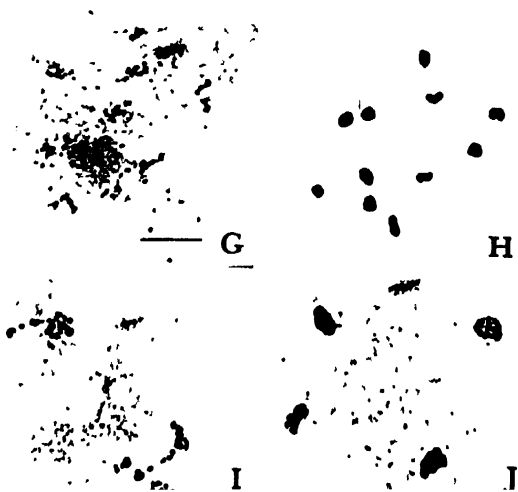
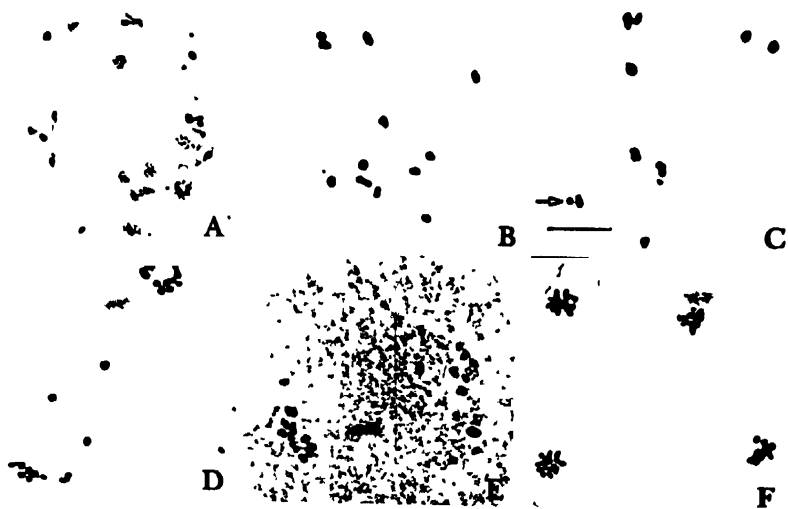
Fig. 13: A-F: Meiosis in hybrids between *Cajanus cajan* and *Atylosia scarabaeoides* (bar indicates 10  $\mu$ ).

- A) Diakinesis.
- B) Metaphase-I showing 11 bivalents.
- C) Metaphase-I showing 2 univalents (Arrow)
- D) Division-I showing laggards.
- E) Unequal chromosome distribution at division-I.
- F) Regular disjunction at division-II.

G-J: Meiosis in hybrids between *Cajanus cajan* and *Atylosia lineata*.

- G) Diakinesis showing 4 bivalents attached to the nucleolus.
- H) Metaphase-I showing 11 bivalents.
- I) Regular disjunction at division-I.
- J) Regular disjunction at division-II.





**C. cajan x A. cajanifolia:**

Hybrids with *A. cajanifolia* exhibited eleven bivalents in their PMC's (Fig.12F) with a relatively high chiasma frequency ranging from 20.49 to 21.20 depending on the cultivar involved in the cross (Table XII). Mostly two bivalents were seen associated with diplotene nucleolus. Anaphase separations at both the divisions were regular (Fig.12G,H). Pollen sterility in these hybrids was relatively low (14.6% to 29.1%) with high ovule fertility (63.7% to 85.2%) (Table XII).

**C. cajan x A. lineata:**

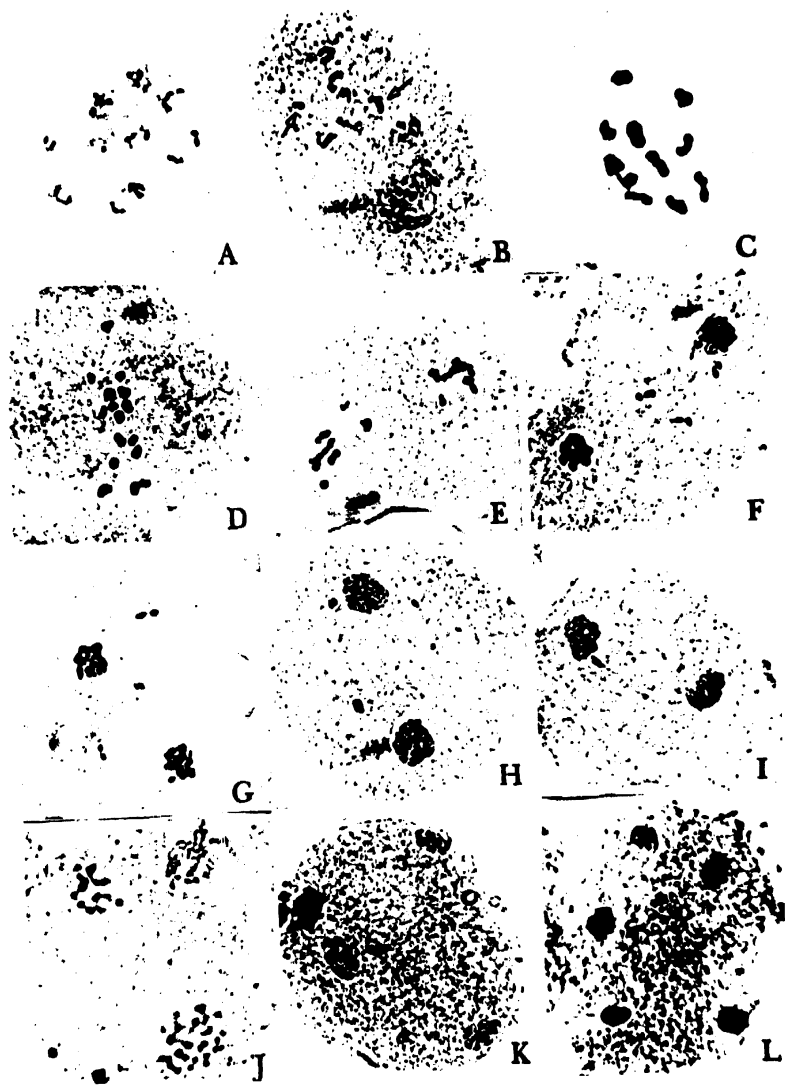
These hybrids showed regular bivalent formation (Fig 13H) except in combination with ICP 7035 where a low frequency (0.09/cell) of univalents were found. Chiasmata per cell varied from 19.70 to 20.34 depending on the *Cajanus* genotype (Table XII). A maximum of four bivalents attached to the nucleolus (Fig.13G) were found at diplotene/diakinesis. Anaphase segregation was regular at both the divisions (Fig.13I,J). Pollen sterility varied from 27.5% in hybrids with C 11 to 46.2% in those with ICP 7035 while ovule fertility ranged from 31.3% in hybrids with ICP 7035 against the maximum of 72.7% recorded in hybrids with C 11 (Table XII).

**C. cajan x A. grandifolia:**

A range of meiotic abnormalities were encountered in *C. cajan* x *A. grandifolia* hybrids (Fig.14). These hybrids displayed a relatively high frequency of univalents (up to (Fig.14D) and rod bivalents (3/cell). The chiasma frequency was

Fig. 14. Meiosis in hybrids between *Cajanus cajan* and *Atylosia grandifolia*.

- A) Diakinesis showing 4 bivalents attached to the nucleolus.
- B) Diakinesis showing univalents (Arrow).
- C) Metaphase-I showing 11 bivalents.
- D) Metaphase-I showing 6 univalents.
- E-H) Laggards at division-I.
- I) Bridge at division-I.
- J) Division-II showing chromatic separation but no disjunction in one of the telophase-II daughter nucleolus.
- K) Telophase-II.
- L) Five daughter nuclei at division-II.



low (18/cell) (Table XII). The diplotene nucleolus was often found with two, three or four bivalents attached (Fig 14A).

Bridges (Fig.14I) were found in 8.2% of the cells at anaphase-I. Bridges were rarely accompanied by fragments. Other Anaphase irregularities included laggards (Fig.14E,F), multipolar disjunctions (Fig.14L) and occasional restitution at division-II (Fig.14J). Lagging chromosomes were common in this combination (Fig.14E-H). Division-I laggards ranged from 2 to 6. At division-II chromosome segregation to five poles was frequent. Restitution in one of the T-I daughter nuclei was occasionally found (Fig.14J).

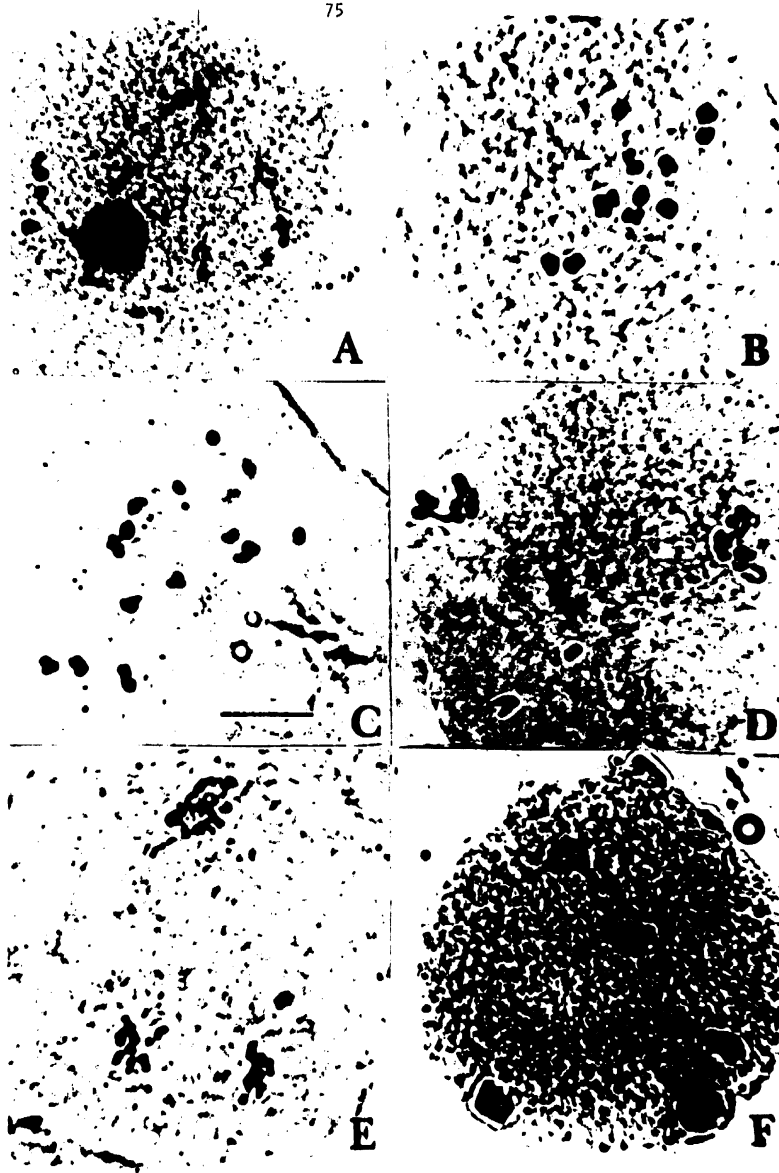
These hybrids exhibited a high degree of pollen sterility and poor ovule fertility. While the pollen sterility was 54.8% and 48.3% in hybrids with Pant A2 and C 11 the corresponding figures for ovule fertility were 23.4% and 19.9% (Table XII).

#### C. cajan x A. latisejala:

Univalents were more frequent (Fig.15B) than in hybrids with Asian Alyosias but the maximum number of univalents recorded was 4 with an average of 1.12 in hybrids with Pant A2 and 0.75 in those with C 11. Rod bivalents averaged 2.89 and 3.89 per cell in combination with Pant A2 and C 11 respectively. The chiasma frequencies were 17.97 and 17.33 involving crosses with Pant A2 and C 11 as female parents.

Fig. 15. Meiosis in hybrids between *Cujanus cajan* and *Atylosia latisepala* (bar indicates 10  $\mu$ ).

- A) Diakinesis.
- B) Metaphase-I showing 11 bivalents.
- C) Metaphase-I showing 4 univalents.
- D) Laggards at division-I.
- E) Three chromosome groups after division-I.
- F) Five daughter nuclei at division-II.



Anaphase-I laggards were frequent (Fig.15D). A common feature observed in these PMC's was congregation of the separated chromosomes at the center of the cell and subsequent segregation occasionally into more than two groups (Fig.15C). Five groups of chromosomes were often seen at division-II (Fig.15F).

In general precocious separation of bivalents was a general feature in all hybrids with all *Atylosia* species where ICP 7035 was the female parent and heteromorphic bivalents were common in all the hybrid combinations. Secondary chromosome associations were found in hybrids with the Australian *Atylosias*. Differences in staining intensities in partners of bivalents was detected in several hybrid combinations.

#### 4.2.1.1. NUCLEOLAR DISTRIBUTION AFTER TELOPHASE I AND II IN $F_1$ HYBRIDS

A wide variation in nucleolar number, size and distribution in PMC's at division-I and division-II (Fig.16,17) was recorded. Such a variation was detected in the hybrids between *C. cajan* and *A. albicans* (Fig.16), *C. cajan* and *A. lineata* (Fig.17A-D) and *C. cajan* and *A. grandifolia* (Fig.17E-H).

In these hybrids the nucleolar number varied from 4-8 at T-I. At T-II nucleoli in daughter nuclei varied from 0-4 (Fig.18) while the total number of nucleoli per PMC varied from 4-8. Consequently in 14% of the PMC's of *C. cajan* x *A. albicans*, 6% in the PMC's of *C. cajan* x *A. lineata* and 4% in the PMC's of *C. cajan* x *A. grandifolia* hybrids the nucleolar distribution at



Fig. 16. Nucleolar variation in pollen mother cells of the hybrid between *Cajanus cajan* and *Atylosia albicans* (Bar indicates 10  $\mu$ ).

A) Telophase-I nucleolar distributions, 4-4.

B-I: Telophase-II nucleolar distribution

B) 1-1-1-1. C) 2-1-1-1. D) 2-2-1-1. E) 4-2-1-0.

(Arrow indicates nucleus devoid of nucleoli).

F) 2-2-2-1. G) 3-2-2-1. H) 4-2-1-1. I) 2-2-2-2.

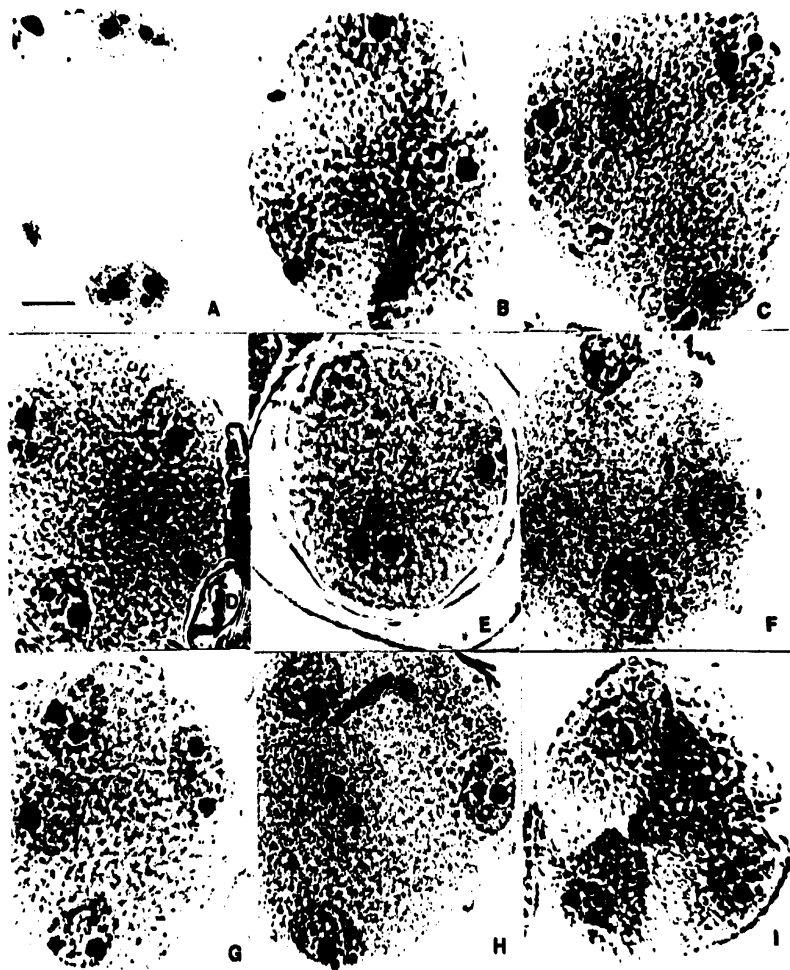


Fig. 17. Nucleolar variation in pollen mother cells of hybrids between *Cajanus cajan* and *Atylosia lineata* and *Cajanus cajan* and *Atylosia grandifolia*.

A-D: *Cajanus cajan* x *Atylosia lineata*.

A) Telophase-I nucleolar distribution: 4-4.  
Telophase-II nucleolar distribution.

B) 3-1-1-1.

C) 3-3-1-1.

D) 2-2-2-2.

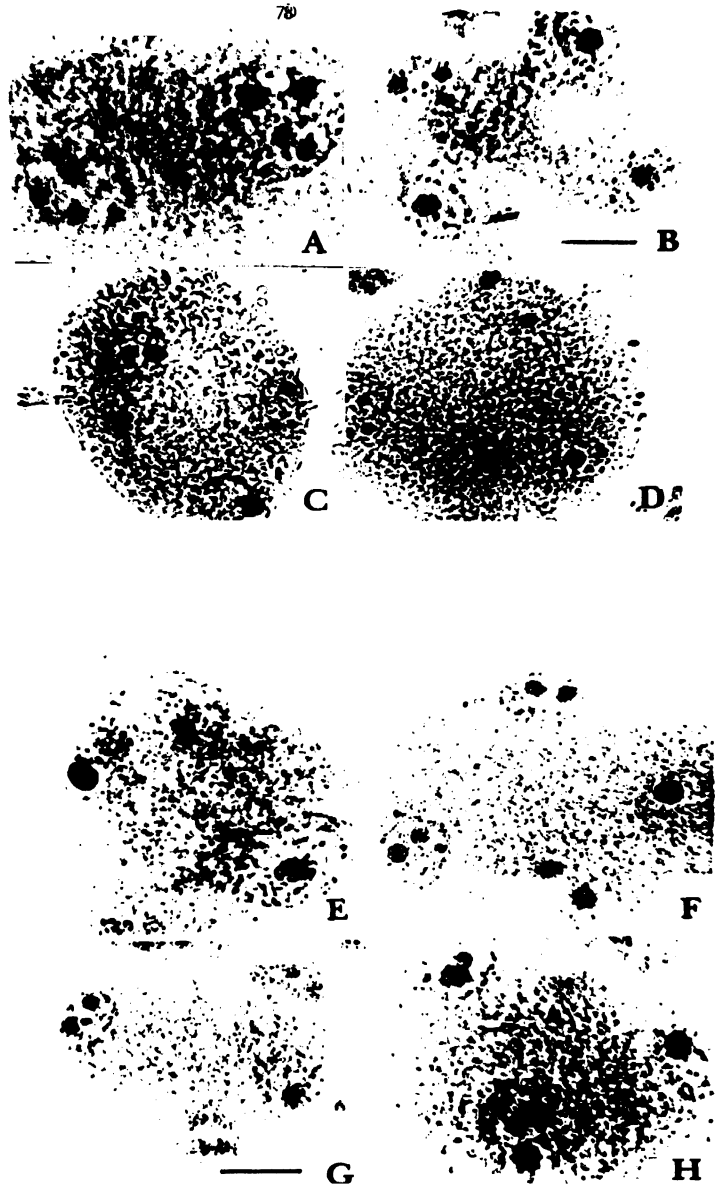
E-H: *Cajanus cajan* x *Atylosia grandifolia*.

E) Telophase-I nucleolar distribution: 3-1.  
Telophase-II nucleolar distribution.

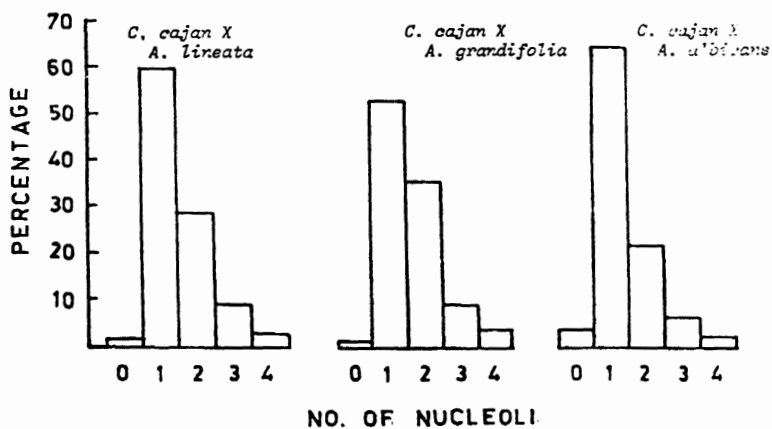
F) 3-2-1-1.

G) 2-2-2-1.

H) 4-2-1-0 (Arrow indicates nucleolus devoid of nucleolus).



**Fig. 18.** Frequency distribution of nucleoli in telophase-II daughter nuclei of hybrids between *Cajanus cajan* and *Atylosia* species.



T-II was confined to three out of the four daughter nuclei. The frequencies of daughter nuclei devoid of nucleoli were 3.5% in *C. cajan* x *A. albicans*, 1.5% in *C. cajan* x *A. lineata* and 1% in *C. cajan* x *A. grandifolia*. A majority (65% in *C. cajan* x *A. albicans*, 59% in *C. cajan* x *A. lineata* and 54% in *C. cajan* x *A. grandifolia*) of daughter nuclei contained one nucleolus while the remainder had 2, 3 or 4 nucleoli. The frequencies of daughter nuclei with two nucleoli were 22% in the hybrid *C. cajan* x *A. albicans*, 29% in that of *C. cajan* x *A. lineata* and 36% in *C. cajan* x *A. grandifolia*, while 6% in *C. cajan* x *A. albicans*, 8% in *C. cajan* x *A. lineata* and 9% in *C. cajan* x *A. grandifolia* included 3 nucleoli. Least frequent (2% in *C. cajan* x *A. albicans*, 3% in *C. cajan* x *A. lineata* and 3% in *C. cajan* x *A. grandifolia*) were the daughter nuclei with 4 nucleoli.

The cells with variation in nucleolar number also showed variation in nucleolar size. The nucleoli could be classified as large or small. In the FMCs with 4 nucleoli at T-II, (one in each daughter nucleus), the nucleoli were uniformly larger (Fig.16B) than those in the daughter nuclei with 2 or more nucleoli. There was no discernible variation in the size of the larger nucleoli, while variation was evident in the size of the smaller nucleoli. A wide variation was recorded for nucleolar distribution at T-II (Fig.16 and 17) as against the regular 2-2-2-2 distribution recorded in the parents.

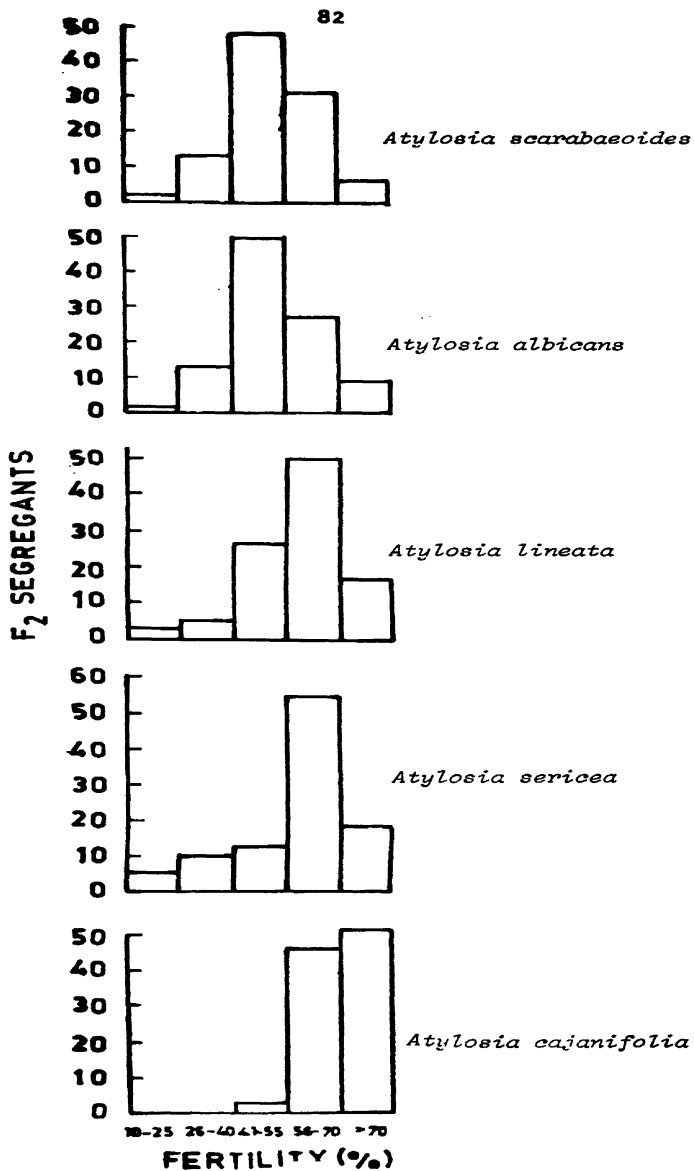
#### 4.2.1.2. CHROMOSOME BEHAVIOUR IN $F_2$ POPULATIONS

Fertility of the  $F_2$  segregants in the five populations, *C. cajan* x *A. albicans*, *C. cajan* x *A. sericea*, *C. cajan* x *A. scarabaeoides*, *C. cajan* x *A. lineata* and *C. cajan* x *A. cajanifolia* is presented in Fig.19. The fertility in the segregants ranged from 18 to 81% except in those of *C. cajan* x *A. cajanifolia* where the segregants were relatively more fertile with 106 plants showing >71% fertility, 95 plants with >56%. In the other four populations (Fig.19), the majority of plants showed 41-70% fertility with a few plants in the lower (10-25%) and the upper (>71%) ranges. Meiotic analysis, such as metaphase pairing studies, were conducted on randomly selected plants from the various groups of segregants.

In randomly analyzed plants in the higher fertility group (>56% fertility), chromosome pairing was mostly normal with the formation of eleven bivalents which predominantly were of the ring type. Unpaired chromosomes were noticed occasionally in very few PMCs. Unpaired chromosomes were more frequent in the lower fertility groups. Some multivalent configurations were found in some of the segregants of the *C. cajan* x *A. sericea* population while secondary chromosome associations were common in the segregants of *C. cajan* x *A. scarabaeoides* and also to a less extent in other populations. Chromosome pairing was regular in all the analyzed segregants of the *C. cajan* x *A. cajanifolia* population. In general pairing was normal in the segregants of the populations of *C. cajan* x *A. albicans* and *C. cajan* x *A. lineata* except in their lower fertility group. Heteromorphic bivalents were common in several cases.



Fig. 19. Frequency distribution of  $F_2$  segregants in different fertility groups.



#### 4.2.2. DISCUSSION

Meiotic studies in the intergeneric hybrids revealed regular chromosome pairing in hybrids of Cajanus with Asian Atylosias. In these hybrids eleven bivalents were common at metaphase-I. As in the parents ring bivalents predominated in these hybrids. Chiasma frequencies in hybrids with Asian Atylosias was high and comparable to the values in the parental species. Fedak (1982) has shown significant variation for chiasma frequency between cultivar combinations in Barley-wheat hybrids but such differences were not evident in the present Cajanus-Atylosia hybrids.

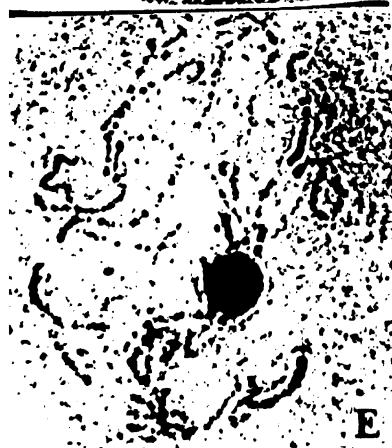
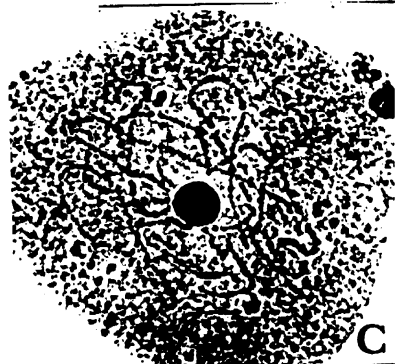
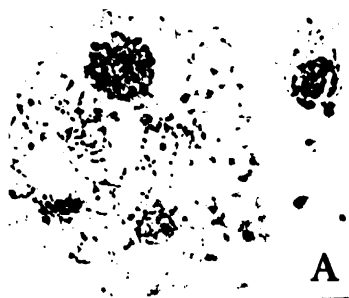
Another interesting feature in these studies has been the behaviour of the Cajanus cultivar ICP 7035. Invariably in hybrids with all the Atylosia species where it was used as the female parent the pollen sterility was consistently higher. Chiasma frequency was also found to be lower in hybrids involving this cultivar and the occasional univalents and anaphase irregularities encountered were also in hybrids with this genotype. In Cajanus intervarietal crosses also the behaviour of ICP 7035 is reported to be distinct (K.B. Saxena, Pers. comm.). Shrivastava *et al.*, (1973) and Sinha and Kumar (1979) found significant differences in karyotypes of Cajanus cultivars and Shrivastava *et al.*, (1973) who compared the karyotype of A. lineata with 15 cultivars of Cajanus found that the karyotype of A. lineata was closer to karyotypes of some Cajanus cultivars than to others. It is likely that some differences in the chromosome complement of ICP 7035 are responsible for the

distinct behaviour of this cultivar. The differences between cultivar combinations for pollen sterility and ovule fertility are expected and attributed to the effect of the genotype. A parentage dependent pollen sterility was also reported in interspecific crosses of Glycine (Kiazuma and Ono, 1980).

In general chromosome pairing and disjunction is regular in hybrids between Cajanus and species of Asian Atylosias. Meiotic studies suggest that the Asian Atylosias share a common genome with C. cajan. Absence of univalents in hybrids is an indication of chromosome to chromosome homology between the parental genomes. Pairing at diakinesis/metaphase gives only an indication of the gross-chromosome homology but does not provide information on the extent of homology. Pachytene PMC's of the hybrids (Fig. 20B-F) also did not show univalents indicating a homology of a high degree between C. cajan and species of Atylosia. However, intercalary unpaired regions (Fig. 20B,C,E) were evident in the pachytene chromosomes. Similar observations were made by Reddy (1981, a,b,c).

It has been shown in some cases that chromosome homology is not a prerequisite for pachytene pairing. For example in mono-haploids of barley the seven chromosomes participate in pairing at pachytene thereby implying the occurrence of "Non-Homologous pairing" that does not persist until metaphase-I (Sadasivaiah and Kasha, 1971). Such non-homologous associations at pachytene have also been reported in monoploids of rice (Chu, 1967), tomato (Ecochord et al, 1969) and maize (Ford, 1970; Weber and Alexander 1972). In polyploids, a substantial

- Fig. 20. A) Two nucleoli at pachytene in *Cajanus cajan*.  
B&C) Pachytene in hybrids between *Cajanus cajan* and *Atylosia grandifolia* showing inversion loops (solid arrow) and unpaired regions (dotted arrow).  
D) Pachytene in hybrids between *Cajanus cajan* and *Atylosia latiseptala* showing cross shaped configuration (Arrow).  
E) Pachytene in hybrids between *Cajanus cajan* and *Atylosia lineata*.  
F) Pachytene in hybrids between *Cajanus cajan* and *Atylosia cajanifolia*.



reduction in chromosomal pairing from prophase-I to metaphase-I is seen and such a reduction in pairing has been attributed to non-homologous pairing (Subrahmanyam, 1978).

In species hybrids univalents and other higher chromosome associations are usually frequent and these impairments in pairing are taken as an index of genome affinities or the lack of it. Meiotic studies in distant hybrids have helped in establishing evolutionary and species relationships in several crop species which include many leguminous species (Foujdar and Tandon, 1976; Machado *et al.*, 1982; Newell and Hymowitz, 1983).

In the hybrids of *Cajanus* and the Asian *Atylosias*, chromosome pairing at metaphase has been perfect implying a very high degree of genome homology. *A. cajanifolia* which is morphologically very close to *Cajanus* shares a close genome relationship as well with the cultivated species. *A. albicans*, *A. lineata* and *A. sericea* though morphologically distinct from pigeonpea also have a close genome relationship with *Cajanus*. It was only in the hybrids of *C. cajan* x *A. scarabaeoides* that consistently univalents were found irrespective of cultivar combination. This distinctly suggests that one chromosome of *A. scarabaeoides* has diverged to a relatively greater extent from its counterpart in the *Cajanus* complement. The differences in staining intensities between partners in a bivalent and also the appearance of heteromorphic bivalents in the hybrid PMCs is an indication of intergenomic homology.

Anaphase separations were highly organized in all the Cajanus cultivar combinations involving A. albicans (Fig. 11F), A. sericea (Fig. 12C), A. lineata (Fig. 13I) and A. cajanifolia (Fig. 12G) with the hybrids with ICP 7035 background being the exception where a low frequency of anaphase abnormalities mostly laggards were observed (Table XII). Anaphase irregularities at a low frequency were encountered in hybrids with A. scarabaeoides in all the cultivar combinations. Apparently the sparse homology between one of the chromosomes of C. cajan and A. scarabaeoides as evidenced by univalents at metaphase contributes to these irregularities at anaphase-I. Anaphase-II separations were almost regular (Fig. 11G, 12D, 12H, 13F, 13J), with occasional asynchrony in disjunction in a few cases. These findings, i.e. regular chromosome pairing and disjunction in the hybrids between C. cajan and Asian Atylosias, are in line with those of earlier studies on hybrids of Cajanus-Atylosia hybrids (Kumar et al, 1958; Pundir, 1981).

As against the regular meiosis encountered in the hybrids with Asian Atylosias the hybrids of Cajanus with the Australian species of Atylosia for which no previous reference was found exhibited a relatively high degree of pairing and disjunctional abnormalities. Univalents in hybrids with both A. grandifolia and A. latisejala were far more frequent. As many as 6 univalents were recorded in hybrids with A. grandifolia and up to 4 in those with A. latisejala. These findings suggest that at least 3 chromosomes of A. grandifolia and 2 of A. latisejala have diverged from their counterparts in the Cajanus genome.



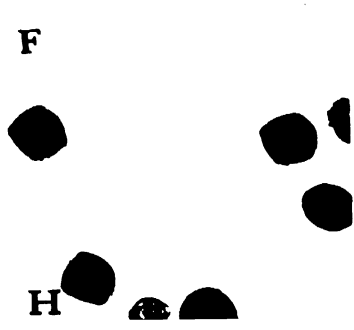
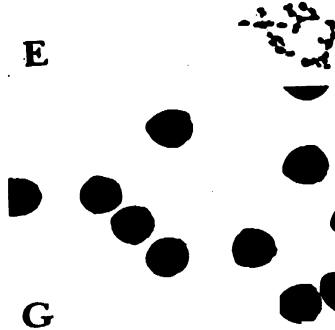
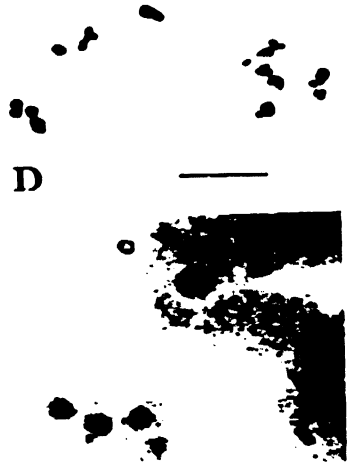
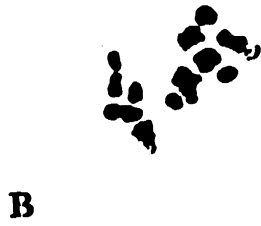
Apparently less effective crossing over due to lack of adequate homology has contributed to the higher frequency of univalents. However, from the present findings it is difficult to ascertain whether the chromosomes of *A. latisepala* and *A. grandifolia* that have diverged from *C. cajan* are similar or different from each other. Hybrids between these species of *Atylosia* can help in resolving this question.

A significant observation has been that the univalents in the hybrids of *Cajanus* with *A. scarabaeoides*, *A. grandifolia* or *A. latisepala* were always even numbered (2, 4 or 6) which is indicative of a low number of pairing initiation sites [(Zygomeres) Sybenga, 1966]. Studies on pairing in tetraploid *C. cajan* revealed a high degree of bivalent formation (16.54 per cell) and a fertility of around 60%. Quadrivalents averaged only 2.73 per cell with the maximum being 4 per cell. The selfed progeny of the tetraploid exhibited regular disjunction (Fig. 21E) and high fertility (70%). The high frequency of bivalent formation in the tetraploid also implies a few pairing initiation sites in pigeonpea similar to that found in *Medicago sativa* (Bingham and Gillies, 1971).

An interesting feature in the PMCs of *C. cajan* x *A. grandifolia* hybrids is the formation of chromatin bridges at division-I with or without fragments. Meiotic bridges are mostly a consequence of recombination within a paracentric inversion (McClintock, 1933; 1938) or alternatively in very rare instances due to prophase breakage of chromosomes often referred to as 'U' type exchanges (Lewis and John, 1966). The distinguishing

Fig. 21. Meiosis in tetraploid *Cajanus cajan*. (bar indicates 10  $\mu$ )

- A) Diakinesis showing quadrivalents (Arrow).
- B) Metaphase-I showing quadrivalents.
- C) Irregular disjunction at division-I.
- D) Progeny of tetraploid showing regular bivalent formation.
- E) Regular disjunction in the progeny of tetraploid.
- F) Eight nucleoli at telophase-I.
- G) Pollen grains of diploid *Cajanus cajan*.
- H) Pollen grains of tetraploid *Cajanus cajan*.



feature of the 'U' type exchanges from those arising out of crossing over within a paracentric inversion is the presence of fragments of variable size following 'U' type exchanges.

Observations on pachytene FMCs of the *C. cajanus* x *A. grandifolia* hybrid (Fig. 20B,C) also revealed loops which indicate that bridges at anaphase are a consequence of inversions. Further both the parents involved in this cross have exhibited regular bivalent formation and disjunction. The limitations in chromosome size and the inconsistent appearance of the fragments in the present material did not permit precise fragment size measurements. Chromatin bridges are common in species hybrids (Ahmed *et al.*, 1977; 1979; Malik and Mary, 1975; Chaudhuri *et al.*, 1976; Reddy and Subrahmanyam, 1985; Sprirger and Buckner, 1982) but this is the first report in the *Cajanus* - *Atylosia* hybrids.

These results indicate that structural variation in the form of inversions have played a role in species differentiation between *C. cajan* and *A. grandifolia*. Meiotic bridges are only a qualitative index of inversions but not quantitative. Not all paracentric inversions manifest themselves in the form of bridges. A minimum of one crossover in the inverted segment is a prerequisite for bridge formation and the possibility of a crossover is directly proportional to the size of the inverted segment.

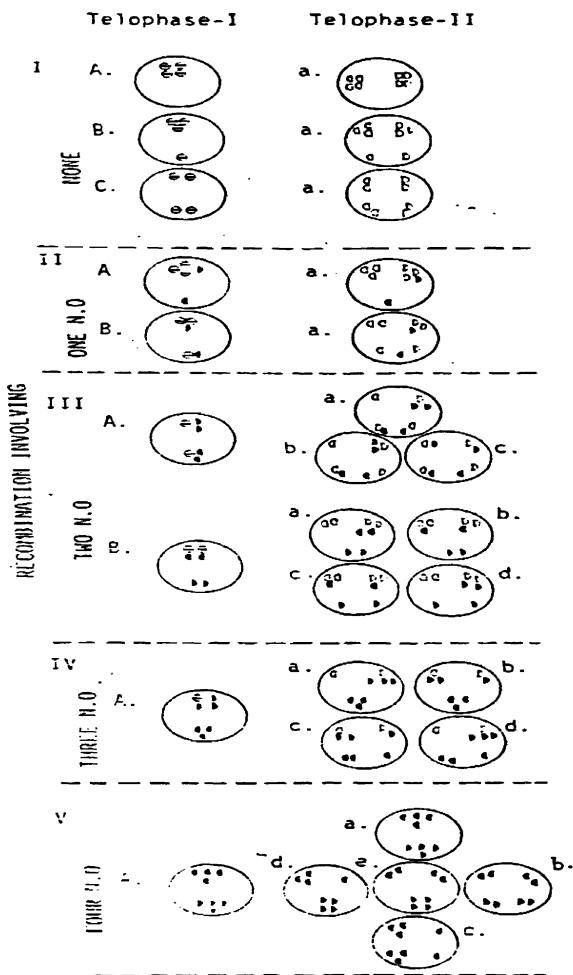
The structural differences between the chromosomes of *C. cajan* and *A. grandifolia* as evidenced by the anaphase bridges and the relative lack of homology between *C. cajan* and *A. grandifolia* and *A. latisejala* which is apparent from the higher frequency of univalents and anaphase abnormalities in hybrids between *C. cajan* and these species explain the relatively high degree of sterility encountered in these hybrids. This suggests that the genomes of the Australian species of *Atylosia* are more diverged from *Cajanus* than those of Asian *Atylosias*. The regular formation of eleven bivalents in the hybrids of *Cajanus* with Asian *Atylosias* without any detectable conventional meiotic irregularities might be interpreted to indicate complete genome homology between these species which can be misleading as will be evident from the following discussion of our results on nucleolar variation.

The most intriguing feature in the hybrids of *Cajanus* with *A. albicans*, *A. lineata*, and *A. grandifolia* was the association of up to four bivalents with the nucleolus at diplotene/diakinesis and the wide variation in nucleolar number, distribution and size. Karyotypes of *C. cajan* and those of *A. albicans* and *A. lineata* have two pairs of satellite chromosomes in each of their genomes (Pundir, 1981). Our studies on nucleolar numbers at telophase have confirmed this finding and have revealed a similar situation for *A. grandifolia*. Thus an association of nucleolus with a maximum of two bivalents at prophase-I and a maximum of four nucleoli per PMC at telophase-I are expected in these hybrids. The association of more than two

bivalents with the nucleolus in the hybrid cells is suggestive of pairing between the N.O.s of *Cajanus cajan* and non-N.O.s of *Atylosia* species or *vice versa* and the variation in nucleolar number and distribution at telophase-I and II is interpreted to have originated from allosyndetic recombination.

The possible nucleolar distributions with 4 N.O.s, with and without recombination, are presented in Fig.22. If one assumes no recombination between N.O. and a non-N.O. (Fig.22:I), a maximum of 4 nucleoli can be expected at telophase-I. The presence of 4 to 8 nucleoli at telophase-I compels us to suggest allosyndetic recombination. Furthermore, if the N.O. chromosomes are not involved in recombination there are only three possible telophase-II distribution patterns. However, the observed deviation in nucleolar distribution at telophase-II fits well with the theoretical expectations assuming crossing over involving 1, 2, 3 or 4 N.O.s with non-N.O.s (Fig.22:II-V). Our interpretation that variation in nucleolar number at telophase-I and telophase-II, and the variation in nucleolar distribution at telophase-II result from recombination between a N.O. of *C. cajan* and non-N.O. of *Atylosia* species or *vice-versa* is supported by the fact that the nucleolar number never exceeded eight, which is the maximum that can be expected with 4 N.O.s (i.e. 8 satellite chromatids). Nucleolar distribution to only three of the four daughter nuclei in a given PMC (Fig.16E; 17C, H and Table XIII) can occur only when the N.O. chromosomes are involved in recombination with non-N.O.s. Additional proof for recombination comes from the telophase-II nuclei which have 4

Fig. 22. Possible nucleolar variation in pollen mother cells of the hybrids between *Cajanus cajan* and *Atylosia* species without recombination and following recombination between nucleolar organizing and non-nucleolar organizing chromosomes.



- : N.O. Chromosome;
- ◐: N.O. chromatid;
- ◑: N.O. chromatid following recombination



nucleoli (Figs. 16E, H and 17H). From Fig. 22 it is evident that without recombination there never exists a possibility for more than three nucleoli per nucleus at telophase-II when there is nucleolar distribution to either three or four nuclei. The occurrence of three or more nucleoli per telophase-II nucleus might suggest a possible expression of otherwise latent N.O.s of the parents. But the presence of a total of eight nucleoli per PMC with a regular 2-2-2-2 distribution in the parents and the absence of PMCs with more than eight nucleoli in the present hybrid rules out such a possibility.


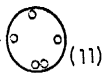
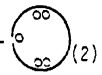
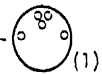
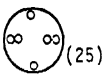
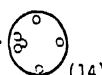


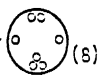



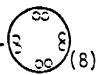
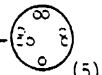


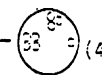
A majority of the PMCs in the hybrids exhibiting nucleolar variation had only 4 nucleoli, one in each daughter nucleus at telophase-II. Hence it can be presumed that the normal N.O. chromosome disjunction without recombination is 2:2 (Fig. 22:I-C) resulting in 2 nucleoli in each daughter nucleus that on fusion give rise to one nucleolus. Nucleolar fusion is common in several plant species especially in meiosis (Darvey and Driscoll, 1972; Sybenga, 1972; Bennett *et al.*, 1973; Darvey *et al.*, 1973; Flavell and O'Dell, 1979; Jessop and Subrahmanyam, 1984). The remaining P.M.C.s undergo allosyndetic recombination with respect to the N.O. involving a minimum of one N.O.-chromosome and thus deviate from the expected nucleolar number at telophase-I and distribution pattern at telophase-II assuming no recombination. The total number of nucleoli at telophase-I in a PMC is a direct indication of the number of N.O. chromosomes involved in recombination. Although we obtained a variation ranging from 4-8 nucleoli at telophase-I (Fig. 16A, J) it was not possible to

score enough cells at this stage to enable us to arrive at the frequency of recombination products. This was because it is difficult to get a PMC at telophase-I with primary (unfused) nucleoli owing to the short meiotic cycle in pigeonpea. In the cells containing 8 nucleoli at telophase-II the distribution pattern of 4-2-2-0 (Table XIII) is only possible when all the four N.O.s are involved in recombination with non-N.O.s.

The presence of 5, 6 and 7 nucleoli at telophase-II many a time with variable size is due to nucleolar fusion. In the light of the different possibilities (Fig. 22) the pattern of nucleolar fusion could not be assessed. Although in the daughter nuclei nucleolar variation of 0-4 existed at telophase-II (Figs 16 and 17), most of the daughter nuclei at the quartet stage showed a single nucleolus with a few quartets (Fig. 11H) showing 2 or 3 distinct nucleoli. Since the overall variation of nucleolar number can be attributed to both fusion and/or allosyndetic recombination, an estimate of such recombination could not be obtained. Nevertheless, for any given number of nucleoli at telophase-I and number and distribution of nucleoli at telophase-II, the minimum number of N.O.s involved in allosyndetic recombination could be given.

Another interesting feature in these hybrids was the variation in nucleolar size. Variation in the nucleolar size, especially the presence of smaller nucleoli than the normal formed by a single N.O. chromatid (Fig. 16I), can be attributed to either a crossover involving a break within the secondary constriction or a crossover between two N.O.s resulting in the

Table XIII Nucleolar variation at telophase-II in a hybrid between *C. Cajan* and *A. albicans*.

		No. of nucleoli				
		4	5	6	7	8
P.M.C's	No.	62	14	46	11	26
	%	39	8.8	28.9	6.9	16.4
			  	   	   	    

formation of a chromatid with two N.O. termini leading to nucleolar dominance (differential amphiplasty). If the first alternative is assumed one would expect more than 8 nucleoli. Absence of PMC's with more than 8 nucleoli rules out such a possibility. Thus we are inclined to suggest that nucleolar dominance is operative in the present hybrid. There have been several reports on differential amphiplasty in interspecific hybrids, wherein nucleolar expression of one of the species involved in the cross is suppressed (Navashin, 1934; Keep, 1962; Subrahmanyam and Azad, 1978a,b). Partial suppression of a N.O. leading to a reduction in nucleolar size has been reported by Nicoloff *et al.*, (1979) in their barley translocation lines.

As indicated earlier in spite of the profuse flowering and regular meiosis the high degree of pollen sterility and low seed set in the hybrids of *Cajanus* with Asian *Alyosias* is surprising. Structural heterozygosity in the chromosomes of the parents as evidenced by the allosyndetic recombination in the present hybrids could be the major reason for the sterility, since the pollen fertility was much lower than expected on the basis of normal bivalent formation and disjunction suggesting thereby, complete homology is not a prerequisite for regular bivalent formation. The absence of nucleoli in some of the daughter nuclei clearly indicate deficiency for the nucleolar organizer while the presence of four nucleoli in a daughter nucleus is suggestive of duplications for the nucleolar organizer. Although these two are the exclusive conditions which indicate deficiency and duplications, the range of variation recorded also includes

such possibilities. It has already been demonstrated (Burnham, 1950) that translocations involving satellite chromosomes result in duplication-deficiency aberrations leading to pollen sterility. Stebbins (1971) discussed the causes of sterility in distant hybrids and proposed that "cryptic structural hybridity" could be the major contributing factor for sterility in the hybrids assuming that the parental species differ with respect to many small chromosomal rearrangements such as interstitial translocations. Since then several workers on hybridization have attributed sterility in spite of normal bivalent formation and disjunction in their hybrids to a series of small structural differences in the parental chromosomes. Our results provide a conclusive evidence for that possibility.

The cryptic structural alterations involving the satellite chromosomes and chromosomes that pair with the satellite chromosomes were discernible in view of the advantage of the nucleolus acting as a marker for the products of recombination, while recombination arising out of structural alterations in other chromosomes is not traceable at later stages in the absence of any such cytological marker and hence the structural alterations traced with the aid of the nucleolus form only a part of the total.

Regular meiosis in the Cajanus-Atylosia hybrids indicate a close relationship between the cultivated and the wild species. The high frequency of recombination in the hybrids further confirms the proximity of the parental species and suggests that the species have differentiated through structural alterations of

chromosomes. Our findings support the conclusions of some other workers that the taxonomic separation of Cajanus and Atylosia should not be maintained (McComb, 1975; van der Maesen, 1981).

Our findings on the chromosome pairing and recombination in the Cajanus-Atylosia hybrids clearly indicate that A. cajanifolia is the closest relative of C. cajan. The regular chromosome pairing in the hybrids of Cajanus with A. cajanifolia and a relatively high degree of fertility implies a close genome relationship between these two species. "Cryptic structural differences" if any between these genomes are less than in the other species. On the other hand the relatively high degree of pollen sterility in the hybrids of Cajanus with the other species of Asian Atylosias, which is unaccountable on the basis of meiotic pairing and disjunction in their hybrids, is a consequence of gametic duplications and deficiencies resulting from structural differences in the chromosomes of the species involved and consequent recombination which has found clear cytological evidence in the pairing of N.O. chromosomes with non N.O. chromosomes and the data on nucleolar variation. Hence the four Atylosia species, A. albicans, A. lineata, A. scarabaeoides and A. sericea which have differentiated through structural heterozygosity are closely related to Cajanus but more distantly than A. cajanifolia. Among these four species, all of which differ from Cajanus on the structural basis, it is difficult to establish the hierarchy of relationship with respect to Cajanus since in this material the limitations in chromosome size makes it impossible to quantify the structural differences. Also the

differences in sterility between hybrid combinations are not different enough to delineate their closeness of relationship. Since univalents though at a low frequency, accompanied by a low frequency of anaphase abnormalities were found in all the cultivar combinations of Cajanus - A. scaraboeides hybrid it is possible that the genome of A. scaraboeides is relatively more distantly related to Cajanus than those of the other three species. A. albicans and A. lineata share a common feature i.e. structural heterozygosity with respect to the nucleolar organizing chromosomes. Both the Australian Atylosias used in our study are cytologically distinct from Cajanus with respect to the Asian Atylosia species. The structural heterozygosity between the genomes of Cajanus and the Australian Atylosias discussed earlier in this chapter contribute to the high degree of sterility in these hybrids.

Our results clearly indicate that pollen sterility and ovule fertility in the hybrids is a reliable index of genome affinities. For example the hybrids with A. cajanifolia which were cytologically most stable had the highest pollen and ovule fertility whereas hybrids with A. grandifolia and A. latisepala exhibited the highest degree of meiotic abnormalities and the highest pollen sterility thereby implying that the levels of gametic sterility reflect the degree of divergence of the Atylosia species from C. cajan.

Apart from the above major general trends the hybrids also exhibited a few cytological features which merit discussion in some detail. Occasional meiotic restitution was observed at division-II in the hybrid *C. cajan* x *A. grandifolia*. Restitution has been reported in several species hybrids (Li *et al.*, 1964; Wagenarr, 1968a, b; Mann and Sasakuma, 1977; Riley and Chapman, 1958; Islam and Shepherd, 1980). Meiotic restitution could be a result of delay in spindle formation or due to spindle fusion. Restitution is also possible when the time required to reach univalent accumulation exceeds the period of activity of kinetochores resulting in failure of anaphase movements as has been suggested by Islam and Shepherd (1980). In the present instance the occasional restitution might be due to either spindle fusion or delayed spindle formation. If the restitution nucleus happens to form a fertile gamete as has been reported in species hybrids of *Arachis* (Singh and Moss, 1984) a few triploids can be expected in the F<sub>2</sub> progeny of *C. cajan* x *A. grandifolia*. Chromosome movement to five poles in hybrids of *Cajanus* with the Australian *Atylosias* is also a consequence of spindle abnormalities. Formation of chromosome sub groups resulting in more than four sporads has been reported in allopolyploids, for example *Rubus* (Bammi, 1965) and *Globba* (Lim, 1973). Such a situation has also been reported in a trihaploid of *Hordeum procerum* (Reddy and Subrahmanyam, 1985). In this case the polyads were a consequence of genome specific organization at division-I. In the present hybrids the occurrence of more than four sporads result from altered spindle organization.



#### 4.2.2.1 F<sub>2</sub> GENERATION

In all the five Cajanus-Atylosia hybrids from which a F<sub>2</sub> population was raised, a majority of the segregants showed an improved level of fertility over the F<sub>1</sub> hybrids while a few segregants did show fertility less than that in the F<sub>1</sub>.

Recovery of fertile segregants from the progeny of partially sterile interspecific hybrids has been accomplished in genera like Nicotiana, Triticum, Bromus (Stebbins, 1950) and also in species crosses of melilotus (Webster, 1950) and Helianthus (Stebbins, 1958). Such results have also been reported in the segregants of hybrids between Phaseolus vulgaris x P. coccineus (Haq et al, 1980) and in crosses involving Avena longiglumis and Avena strigosa (Yamaguchi et al, 1976).

Stebbins (1958) suggested that the F<sub>2</sub> segregants which show improved fertility over the F<sub>1</sub> have apparently recovered a relatively higher degree of genic balance than the one existing in the F<sub>1</sub>. A fallow-up of this interpretation would be whether the optimal genic balance corresponds to the balance that existed in the parents or whether it denotes a new balance. In the present case several of the F<sub>2</sub> segregants which showed improved fertility were morphologically distinct from the parents, with the F<sub>2</sub> populations showing a high degree of variability for different characters, a point which will be discussed in more detail in the chapter 6. The recovery of fertility in the segregants without recovery of parental morphology suggests that the recovered genic balance is a new one. This can be confirmed

by crossing the fertile derivatives to the original parents i.e. back crossing and in case the derivatives have acquired a new balance, the progeny of the back cross will again be expected to be partially sterile.

Secondary association of bivalents was evident in some  $F_1$  hybrids and was prevalent in some  $F_2$  segregants. Secondary associations appear due to the presence of genetically and structurally similar chromosomes (Riley, 1960) and the bivalents involved in such associations have "residual homology" (Darlington and Moffett, 1930; Thomas and Revell, 1946). Kempanna and Riley (1964) with the aid of telocentric chromosomes demonstrated that secondary associations occur between genetically related bivalents and that they are independent of similarities in the size of bivalents. In the present material the secondary associations further reflect the presence of structural alterations in the related genomes.

The appearance of multivalents in some of the segregants could be an artefact of chromosome stickiness since never could we observe any clear configurations of higher associations.

As is evident from the results and the above discussion there is no chromosome elimination in any *Cajanus-Atylosia* crosses. Kasha and Kao (1970) reported recovery of haploids from the cross *Hordeum vulgare* x *H. bulbosum* and Subrahmanyam and Kasha (1973) reported that the haploids from this cross are a consequence of gradual and selective elimination of the *bulbosum* chromosomes from the hybrid embryos. In the last 15 years since

the selective chromosome elimination method of haploid production commonly known as the "Bulbosum method" has come to light there has been no report of haploid production by this method without the involvement of a *Hordeum* species. In both wheat (Barclay, 1975) and *Psathyrostachys* (Bothmer et al, 1984) two other species where haploid have been recovered via the Bulbosum method, species of *Hordeum* are involved. In the genus *Nicotiana* sectorial elimination was recorded in interspecific hybrids (Gupta and Gupta, 1973). A systematic screening for selective chromosome elimination both in the present study in pigeonpea and also in the genus *Arachis* (Subrahmanyam, unpublished and Singh and Moss, pers. comm.) there has been no evidence of elimination. Thus selective chromosome elimination appears to be of limited application in haploid production.

## **5. INHERITANCE OF QUALITATIVE TRAITS**

## 5.1. RESULTS

The segregation and inheritance of the following qualitative traits was studied in the hybrids of *C. cajan* x *A. albicans*, *C. cajan* x *A. lineata*, *C. cajan* x *A. scarabaeoides*, *C. cajan* x *A. sericea* and *C. cajan* x *A. cajanifolia*. The female *Cajanus* genotype in all the cases was Pant A2.

### SEED STROPHIOLE:

Strophiole on the seed is rudimentary in *C. cajan* cv Pant A2 while a prominent strophiole is present on the seed of all *Atylosia* species. In all the five hybrids the seed on the  $F_1$  plants invariably had a prominent strophiole. In the  $F_2$  generation the segregation for seed strophiole found a good fit for the ratio 13:3 (Table XIV) except in *C. cajan* x *A. cajanifolia* where the segregation found a good fit for 15:1 ratio.

### SEED MOTTLING:

While the seeds of *Atylosia* species are mottled, in cv Pant A2 the seeds are not mottled. Seeds produced on the five  $F_1$  hybrids were mottled.  $F_2$  segregation in *C. cajan* x *A. albicans*, *C. cajan* x *A. lineata*, *C. cajan* x *A. scarabaeoides* and *C. cajan* x *A. lineata* found a good fit for 9:7 ratio (Table XV). Whereas the segregation pattern in the  $F_2$ 's of *C. cajan* x *A. sericea* did not fit into any of the standard genetic ratios.

Table XIV. Inheritance pattern of seed strophiole in *C. cajan* × *Atylosia* species hybrids.

Cross	♀	♂	F <sub>1</sub>	F <sub>2</sub> segregation			χ <sup>2</sup>	Probability
				Pre-sent	Ab-sent	Ratio		
<i>C. cajan</i> × <i>A. albicans</i>	Absent	Present	Present	154	28	13:3	1.35	0.5-0.25
<i>C. cajan</i> × <i>A. cainifolia</i>	Absent	Present	Present	189	18	15:1	2.11	0.25-0.1
<i>C. cajan</i> × <i>A. sericea</i>	Absent	Present	Present	140	23	13:3	2.30	0.25-0.1
<i>C. cajan</i> × <i>A. scarabaeoides</i>	Absent	Present	Present	165	46	13:3	1.28	0.5-0.25
<i>C. cajan</i> × <i>A. lineata</i>	Absent	Present	Present	245	46	13:3	1.65	0.25-0.1

Table XV. Inheritance pattern of seed mottles in *C. cajan* x *Alyosia* species hybrids.

Cross	♀	♂	F <sub>1</sub>	F <sub>2</sub> segregation			χ <sup>2</sup>	Proba- bility
				Pre- sent	Ab- sent	Ratio		
<i>C. cajan</i> x <i>A. albicans</i>	Absent	Present	Present	101	81	9:7	0.04	0.9-0.75
<i>C. cajan</i> x <i>A. cajanifolia</i>	Absent	Present	Present	127	80	9:7	2.19	0.25-0.1
<i>C. cajan</i> x <i>A. sericea</i>	Absent	Present	Present	110	53	9:7	8.36	< 0.005
<i>C. cajan</i> x <i>A. scarabaeoides</i>	Absent	Present	Present	118	93	9:7	0.009	> 0.9
<i>C. cajan</i> x <i>A. lineata</i>	Absent	Present	Present	170	121	9:7	0.55	0.5-0.25

## POD HAIRINESS:

Although the pods of Cajanus have hairs, the hair being very short the pods appear almost glabrous, while the pods of A. scarabaeoides, A. lineata and A. sericea have very prominent and dense hairs. In the  $F_1$ 's involving all these three Atylosia species, pods had dense and long hairs similar to those in the Atylosia parents and in all the three cases the  $F_2$  segregation found a good fit for the simple 3:1 ratio (Table XVI).

## TWINING NATURE:

Two (A. albicans and A. scarabaeoides) of the five Atylosia species under study are climbers while the rest are erect types as in Cajanus, hence the inheritance of twining nature was studied in the hybrids of C. cajan x A. albicans and C. cajan x A. scarabaeoides. Hybrids with A. albicans were twiners in their gross morphology. In the  $F_2$  generation although minor variations were recorded for the erect or twining nature, basically the segregants were either twiners or erect types and the segregation found a good fit for 13:3 ratio (Table XVII). The hybrids of Cajanus with A. sericea were intermediate with a bushy base and twiny nature in the upper portions and the  $F_2$  segregation in this case did not fit into any simple ratio.



Table XVI. Inheritance pattern of pod hairiness in C. cajan x Atylosia species hybrids.

Cross	♀	♂	F <sub>1</sub>	F <sub>2</sub> segregation		χ <sup>2</sup>	Probabi- lity	
				Long hairs	Short hairs			
<u>C. cajan</u> x <u>A. sericea</u>	Short hairs	Long hairs	Long hairs	131	32	3:1	2.50	0.25-0.1
<u>C. cajan</u> x <u>A. scarabaeoides</u>	Short hairs	Long hairs	Long hairs	157	54	3:1	0.04	0.9-0.75
<u>C. cajan</u> x <u>A. lineata</u>	Short hairs	Long hairs	Long hairs	210	81	3:1	1.24	0.5-0.25

Table XVII. Inheritance pattern of twining nature in *C. cajan* x *Atylosia* spect hybrids.

Cross	♀	♂	F <sub>1</sub>	F <sub>2</sub> generation				χ <sup>2</sup>	Probability
				Twining	Erect	Inter-mediate	Ratio		
<i>C. cajan</i> x <i>A. albicans</i>	Erect	Twining	Twining	150	32	-	13:3	0.16	0.75-0
<i>C. cajan</i> x <i>A. scarabaeoides</i>	Erect	Twining	Twining (Inter-mediate)	35	74	102	-	-	-

Table XVIII. Inheritance pattern of leaflet shape in *C. cajan* x *Atylosia* spect hybrids.

Cross	♀	♂	F <sub>1</sub>	F <sub>2</sub> generation			χ <sup>2</sup>	Probability	
				Inter-type	Inter-mediate	Ratio			
<i>C. cajan</i> x <i>A. albicans</i>	Lanceolate	Obovate	Inter-mediate	39	90	53	1:2:1	2.16	0.5-0.1
<i>C. cajan</i> x <i>A. scarabaeoides</i>	Lanceolate	Obovate	Inter-mediate	48	104	59	1:2:1	1.18	0.75-0.

**LEAFLET SHAPE:**

The leaflet shape in *C. cajan* is lanceolate while that in *A. albicans* and *A. scarabaeoides* is obovate. The leaflet shape in the hybrids involving *A. albicans* or *A. scarabaeoides* was intermediate to the parents. The  $F_2$  generation segregated for a 1:2:1 ratio for leaflet shape in both the populations (Table XVIII).

## 5.2. DISCUSSION

### SEED STROPHIOLE:

Our results clearly indicate that the seed strophiole in the *Atylosia* species is a dominant trait and is controlled by two genes. Even in the hybrids with the two Australian species where genetic studies were not conducted the hybrid seed had a strophiole, unequivocally establishing the dominant nature of this trait. Reddy *et al* (1980) reports three ratio's, 3:1, 9:7 and 13:3 for seed strophiole expression in crosses involving species of *A. sericea* and *A. scarabaeoides* while Pundir (1981) indicates that two genes with a duplicate gene action (15:1) govern this character. In the present study four out of the five hybrids segregated for a 13:3 ratio indicating a inhibitory gene action while the hybrids of *C. cajan* x *A. cajanifolia* segregated in to 15 strophiolated to 1 nonstrophiolated types. A significant observation is that the seed germinability in the F<sub>2</sub> populations of *C. cajan* x *A. cajanifolia* which has deviated from the 13:3 ratio has been poor (Table III) when compared to others. This implies that the genetic factors responsible for the absence of strophiole are linked with certain lethal factors affecting seed germinability.

**SEED MOTTLING:**

Seed mottling being invariably expressed in all the hybrids is a dominant trait. Reddy *et al* (1980) in hybrids of *Cajanus* with *A. scarabaeoides* reported a 9:7 ratio when ICP 6915 and ICP 7035 were used as female parents and a 15:1 ratio when ICP 6997 was the female parent. In our studies 4 out of the 5 populations found a good fit for 9:7 ratio indicating a complementary gene action. The segregation in the hybrids with *A. sericea* did not fit into any of the standard genetic ratio's although it was nearest to the 9:7 ratio but it finds a good fit for 2:1 ratio suggesting a close linkage with a semidominant lethal of the complimentary gene(s) responsible for the expression of this trait wherein the homozygous dominant genotypes do not survive.

**POD HAIRINESS:**

Pod hairiness of the *Atylosia* species is dominant over the relative pubescence of *C. cajan*. Reddy (1973) and Pundir (1981) found that pod hairiness of *Atylosia* species is a monogenic dominant trait. Our results for this character agree with the two earlier reports since in all the three hybrids involving species of *Atylosia* with hairy pods the pods in the  $F_1$ 's were hairy and in the  $F_2$  generation they segregated in a simple 3:1 ratio.

**TWINING NATURE:**

The segregation pattern of twining nature in hybrids involving *A. albicans* indicated that the character is governed by two genes with a inhibitory gene action. In *C. cajan* x *A. scarabaeoides* the  $F_1$  was intermediate to the parents in its habit and in the  $F_2$  generation all the three types i.e. twining, intermediate and erect types were recovered and the pattern of segregation did not fit into any of the genetic ratio's. Accurate classification of segregants in to distinct groups was not possible for this character due to overlapping of characters between the groups.

**LEAFLET SHAPE:**

Leaflet shape in both the hybrids studied was found to be controlled by a single gene with a partially dominant gene action. Reports on genetic studies for leaflet shape in *Cajanus* are variable. Deshpande and Jeswani (1956) reported both 15:1 and 3:1 ratio for lanceolate and obcordate types and the studies of Jeswani and Deshpande (1962) and D'cruz and Deokar (1970) indicate a 3:1 ratio, while the studies of Patil and Deokar (1980) and Chaudhary and Thombre (1977) indicated that more than one gene controls leaflet shape.

In intergeneric crosses involving *C. cajan* and *A. scarabaeoides*, Reddy (1973) and Pundir (1981) reported a 1:2:1 ratio which is in agreement with our results.

## 6.1. RESULTS

Variation for pod length (Table XIX), 100-seed weight (Table XX), seeds per pod (Table XXI), mid-leaf length (Table XXIII) and mid-leaf width (Table XXII) were studied in the  $F_2$  generation of the hybrids of *C. cajan* (Pant A2) with *A. albicans*, *A. sericea*, *A. scarabaeoides*, *A. lineata*, and *A. cajanifolia*.

### POD LENGTH:

The mean pod length of the  $F_2$  segregants in all the five populations was close to the mid parental value (Table XIX). The mean for the population of *C. cajan* x *A. albicans* was 4.71 cm with a range of 2.8-6.3 cm and a coefficient of variation (c.v.) of 19.5% as against the parental values of 5.93 cm (*C. cajan*) and 3.52 cm (*A. albicans*). The difference between the parental values was minimum between *C. cajan* (5.93 cm) and *A. cajanifolia* (4.31 cm). The  $F_2$  mean in the population derived from the cross between these species was 4.82 cm, the  $F_2$  range being 3.8-6.1 cm with a c.v. of 13.9%. The difference between parental values was maximum in *C. cajan* (5.93 cm) x *A. sericea* (1.35 cm). The variation for pod length in the  $F_2$  population of this hybrid was 29.2% with the length ranging from 1.2 to 5.4 cm with an average of 3.59 cm. Almost identical results were obtained in the population of *C. cajan* x *A. lineata* where the average pod length was 3.35 cm with a range of 1.3-5.1 cm and a c.v. of 29.9%. A variation of 22.4% was recorded in the  $F_2$ 's of *C. cajan* x *A. scarabaeoides* with the pod length ranging from

Table XIX. Variation for pod length (cm) in the F<sub>2</sub> population of hybrids between *C. cajan* and *Atylosia* species.

Cross	♀	Mid-parental value	F <sub>2</sub> population				
			Mean	Range	S.D.	C.V.	
<i>C. cajan</i> × <i>A. albicans</i>	5.93	3.52	4.72	4.71	2.8-6.3	0.92	19.5
<i>C. cajan</i> × <i>A. cajanifolia</i>	5.93	4.31	5.12	4.82	3.8-6.1	0.67	13.9
<i>C. cajan</i> × <i>A. sericea</i>	5.93	1.35	3.64	3.59	1.2-5.4	1.05	29.2
<i>C. cajan</i> × <i>A. scarabaeoides</i>	5.93	2.3	4.11	3.62	2.5-5.5	0.81	22.4
<i>C. cajan</i> × <i>A. lineata</i>	5.93	1.7	3.81	3.35	1.3-5.1	1.00	29.9

Table XX. Variation for 100-seed weight (g) in the F<sub>2</sub> population of hybrids between *C. cajan* and *Atylosia* species.

Cross	♀	Mid-parental value	F <sub>2</sub> population				
			Mean	Range	S.D.	C.V.	
<i>C. cajan</i> × <i>A. albicans</i>	6.65	2.52	4.58	4.16	3.00-7.18	0.98	23.6
<i>C. cajan</i> × <i>A. cajanifolia</i>	6.65	4.40	5.52	5.4	4.71-6.77	1.13	21.1
<i>C. cajan</i> × <i>A. sericea</i>	6.65	2.28	4.46	4.3	2.13-6.45	1.43	33.3
<i>C. cajan</i> × <i>A. scarabaeoides</i>	6.65	2.10	4.37	3.7	2.15-6.13	1.13	30.5
<i>C. cajan</i> × <i>A. lineata</i>	6.65	2.35	4.5	4.09	2.23-6.19	1.19	29.1



Table XXI. Variation for seeds per pod in the F<sub>2</sub> population of hybrids between C. cajan and Atylosia species.

Cross	♀	♂	Mid-parental value	F <sub>2</sub> population			
				Mean	Range	S.D.	C.V.
<u>C. cajan</u> × <u>A. albicans</u>	3.7	4.5	4.1	2.61	1.5-4.0	0.85	32.6
<u>C. cajan</u> × <u>A. cajanifolia</u>	3.7	3.2	3.45	3.31	3.0-3.7	0.21	06.3
<u>C. cajan</u> × <u>A. sericea</u>	3.7	1.5	2.6	2.51	0.8-3.5	0.77	30.7
<u>C. cajan</u> × <u>A. scarabaeoides</u>	3.7	4.4	4.05	3.05	2.1-4.2	0.68	22.3
<u>C. cajan</u> × <u>A. lineata</u>	3.7	1.6	2.65	2.71	1.2-3.5	0.58	21.4

Table XXII. Variation for mid-leaf width (cm) in the F<sub>2</sub> population of hybrids between C. cajan and Atylosia species.

Cross.	♀	♂	Mid-parental value	F <sub>2</sub> population			
				Mean	Range	S.D.	C.V.
<u>C. cajan</u> × <u>A. albicans</u>	2.85	3.82	3.33	3.64	2.4-5.3	0.41	11.3
<u>C. cajan</u> × <u>A. cajanifolia</u>	2.85	2.35	2.6	2.55	2.4-2.8	0.13	5.1
<u>C. cajan</u> × <u>A. sericea</u>	2.85	0.63	1.74	1.83	0.7-2.9	0.57	31.1
<u>C. cajan</u> × <u>A. scarabaeoides</u>	2.85	1.81	2.33	2.57	1.4-3.3	0.44	17.1
<u>C. cajan</u> × <u>A. lineata</u>	2.85	1.63	2.24	2.08	1.4-3.2	0.34	16.3

Table XXIII. Variation for mid-leaf length (cm) in the F<sub>2</sub> population of hybrids between *C. cajan* and *Atylosia* species.

Cross	Mid-parental			F <sub>2</sub> population			
	value	Mean	Range	S.D.	C.V.		
<i>C. cajan</i> × <i>A. albicans</i>	7.13	4.41	5.77	5.9	4.2-8.5	0.74	12.5
<i>C. cajan</i> × <i>A. cajanifolia</i>	7.13	5.9	6.51	6.31	5.7-7.4	0.45	07.1
<i>C. cajan</i> × <i>A. sericea</i>	7.13	2.51	4.82	5.17	2.4-7.1	1.34	25.9
<i>C. cajan</i> × <i>A. scarabaeoides</i>	7.13	3.55	5.34	5.14	3.3-7.0	1.02	19.8
<i>C. cajan</i> × <i>A. lineata</i>	7.13	3.82	5.47	5.84	3.8-8.2	1.26	21.6

2.5-5.5 cm with an average of 3.62 cm.

SEED WEIGHT:

A much greater variation ranging from 21.1-33.3% was recorded for seed weight (Table XX) in the five populations but the  $F_2$  mean was again mostly close to the mid parental value. Seed weight in Pant A2 is 6.65 g. The 100 seed weight in the four *Alyosia* species, *A. albicans* (2.52 g), *A. sericea* (2.28 g), *A. scarabaeoides* (2.10 g) and *A. lineata* (2.35 g) were close to each other while that in the other species, *A. cajanifolia* was 4.46 g. In the  $F_2$  generation the 100-seed weight ranged from 3.00-7.18 g in the populations of *C. cajan* x *A. albicans*, 4.71-6.77 in the population of *C. cajan* x *A. cajanifolia*, 2.13-6.45 g in that of *C. cajan* x *A. sericea*, 2.15-6.13 g in *C. cajan* x *A. scarabaeoides* and 2.23-6.19 g in *C. cajan* x *A. lineata* with 4.16 g, 5.4 g, 4.3 g, 3.7 g and 4.09 g being the respective means.

SEEDS PER POD:

None of the segregants in the five  $F_2$  populations exceeded the better parent value for seeds per pod (Table XXI). Segregates with fewer seeds per pod than the poor parent were common as is evident from the range (Table XXI). The mean in the  $F_2$  population of *C. cajan* x *A. cajanifolia* (3.31), *C. cajan* x *A. sericea* (2.51) and *C. cajan* x *A. lineata* (2.71) were close to the mid parental value while those of *C. cajan* x *A. albicans* (2.61) and *C. cajan* x *A. scarabaeoides* (3.05) were less than the

mid parental value. The coefficient of variation for this character was maximum (32.6%) in the populations of *C. cajan* x *A. albicans* and minimum (6.3%) in that of *C. cajan* x *A. cajanifolia*, while in the other populations the variation was 21.4% in *C. cajan* x *A. lineata*, 22.3% in *C. cajan* x *A. scarabaeoides* and 30.7% in *C. cajan* x *A. sericea*.

#### MID-LEAF WIDTH:

$F_2$  variation for mid-leaf width was maximum (31.1%) in the population of *C. cajan* x *A. sericea*, while the other four populations, *C. cajan* x *A. cajanifolia* (5.1%), *C. cajan* x *A. albicans* (11.4%), *C. cajan* x *A. scarabaeoides* (17.1%) and *C. cajan* x *A. lineata* (16.3%) showed much less variation (Table XXII). The  $F_2$  means for mid-leaf width although close to the mid-parental value, fractionally exceeded that value in *C. cajan* x *A. albicans*, *C. cajan* x *A. scarabaeoides* and *C. cajan* x *A. sericea*. The width ranged from 2.4-5.3 cm in the population of *C. cajan* x *A. albicans*, 0.7-2.9 cm in *C. cajan* x *A. sericea*, 1.4-3.3 cm in *C. cajan* x *A. scarabaeoides* and 1.4-3.2 cm in the population of *C. cajan* x *A. lineata* while the range (2.4-2.8 cm) was very narrow in the population of *C. cajan* x *A. cajanifolia*.

## 6.2. DISCUSSION

Studies on  $F_2$  variation for the characters pod length, seeds per pod, 100-seed weight, mid-leaf width and mid-leaf length have shown interesting trends. In general the  $F_2$  means with some exceptions have been close to the mid-parental value but transgressive segregants, i.e. those exceeding the better parent values have been recorded for all the characters except seeds per pod. Transgressive segregation has been most pronounced for the leaf characters especially in the population of *C. cajan* x *A. albicans*. Transgression of a marginal magnitude has also been recorded for pod length and 100-seed weight in the  $F_2$  's of *C. cajan* x *A. albicans* and *C. cajan* x *A. cajanifolia*. A few segregants with leaves wider than the better parent have been obtained from the population of *C. cajan* x *A. sericea*. As is evident from Tables XXII and XXIII leaf length and width in some of the  $F_2$  segregants of *C. cajan* x *A. albicans* is considerably higher than that of the better parent.

Another striking feature in these studies is that the variation among the five  $F_2$  populations is invariably minimum for all the characters studied in the cross *C. cajan* x *A. cajanifolia*, the variation being as low as 5.1% for leaf width, 7.1% for leaf length, 6.3% for seeds per pod, 13.9% for pod length and 21.1% for 100-seed weight. For 100-seed weight and the leaf characters the variation has been maximum in the population of *C. cajan* x *A. sericea* while for seeds per pod it is highest in the population of *C. cajan* x *A. albicans*. In the  $F_2$  population of *C. cajan* x *A. albicans* segregants with values

better than the better parent have been recorded for all traits except seeds per pod.  $F_2$  segregants poorer than the poorer parent have been recorded in several cases.

Transgressive segregation for most of the characters revealed the potential of the *Atylosia* species for pigeonpea improvement. BarBacki *et al* (1976) has emphasized the role of transgressive segregation in crop improvement. Stebbins (1977) and BarBacki *et al* (1976) have shown that transgressive segregation from distant hybrids can give rise to extreme characters and a range of new forms. The exploitation of the transgressive segregants would be a viable method for the use of wild species in the improvement of the cultivated species especially when the cultivated and the wild forms share a common chromosome number as in pigeonpea. Such studies i.e. those aimed at the exploitation of transgressive segregants have been successfully attempted in other crop species (Reeves, 1950; Reeves and Bockholt, 1964; Efron and Everett, 1969; Harlan and Dewet, 1976; Lawrence and Frey, 1976; Frey, 1976; Frey *et al*, 1983).

In the present study as indicated earlier transgressive segregants of considerable significance especially for leaf characters have been recorded in the *Cajanus-Atylosia*  $F_2$  populations. Such segregants for leaf characters assume greater significance in the light of the findings of Sharma and Saxena (1982) who have shown from intervarietal cross of pigeonpea that leaf characters are highly heritable with a positive influence on grain yield. The marginal improvement over the better parent in

the seed weight in some of the  $F_2$  populations is also a point of significance. In a nutshell these findings indicate that the species of Atylosia can be exploited for the improvement of certain quantitative traits of Cajanus apart from their utility in incorporation of specific traits like disease and insect resistance.

Further the wide range of variation recorded for the characters studied with segregants better than the better parent and poorer than the poor parent is indicative of the substantial degree of recombination between the genomes of Cajanus and Atylosia. In these studies the variation recorded has been least in the population of Cajanus x A. cajanifolia which further substantiates the morphological and cytological evidence for the relative genetic proximity of these two genomes/species.

The limited investigations made on seed protein content reveal a distinct trend. In all the  $F_1$  hybrids the seed protein content is close to the values recorded in the better parent (Atylosia) with the lone exception of C. cajan x A. grandifolia where the protein content was intermediate to the parental values. These results i.e. the proximity of the protein percentage values in the hybrids to the values in the Atylosia parents indicate that the high protein content in the Atylosia parents is a dominant trait. Weber (1950) reported partial dominance for high protein content in crosses involving wild and cultivated soybeans. It has not been possible to analyse the protein content in all the  $F_2$  segregants due to limitations in seed sample but analysis in some segregates showed values

marginally better than the better parent values. However, without sufficient data it is not appropriate to conclude that these are transgressive segregants since environment exerts a strong effect on protein percentage.

Certain characteristic and interesting segregants were observed in some of the  $F_2$  populations. In the population of *C. cajan* x *A. albicans* pods in one of the segregants were with and without streaks on the same plant. A few segregants in the populations of *C. cajan* x *A. sericea* and *C. cajan* x *A. scarabaeoides* were found to have seeds of different colours. A segregant each in the population of *C. cajan* x *A. lineata* and *C. cajan* x *A. albicans* was found to be non branching.

The occurrence of pods and seeds of different types on the same plant is a possible case of chimera. Such chimeral individuals especially for pod colour were also observed in the progenies of *Cajanus* intervarietal crosses (K.B. Saxena, 1984). A non branching single stem mutant of the type observed in some of the  $F_2$  populations was earlier reported (Dahiya and Sidhu, 1979) as a spontaneous mutation in the  $F_2$  generation of a *Cajanus* intervarietal hybrid. These aberrant types in the  $F_2$  populations might also be a consequence of some freak genic constitutions as a result of recombination and segregation in the  $F_1$  hybrids.



## 7. IN VITRO REGNERATION

## 7.1. RESULTS

### 7.1.1. TISSUE CULTURE

Attempts to standardize culture conditions for regeneration of *Cajanus* and *Atylosia* plants from different explants such as cotyledons from mature seeds and leaf and epicotyl segments from one week old seedlings resulted in different degrees of success. Cotyledon tissue was found to be the best responding explant in our initial exploratory attempts and further detailed studies were conducted on that explant. The initial attempts also revealed the superiority of MS medium over the others hence, the response of cotyledons was studied on MS medium.

Basal (MS) medium supplemented with 2, 4-D (2 mg/l) induced copious amounts of healthy callus from all explants irrespective of the region of the cotyledon used. Whole cotyledons and nodal halves of the cotyledons on MS medium supplemented with 2, 4-D (0.5 mg/l) and BA (2 mg/l) developed multiple shoots (3-7) in addition to small amounts of callus (Fig.23C) in 2-46% of the cultures depending on the cultivar (Table XXIV). Among the wild relatives, multiple shoots developed from 21% of *A. cajanifolia* cultures, while in the cultures of *A. albicans* and *A. sericea* mainly single shoots developed (Fig.23B) and multiple shoots were rare. In the cultures of the distal segments of the cotyledons, only shoot bud initiation was observed in low frequencies after profuse callusing. From the explants with multiple shoots, rooting was obtained on MS medium supplemented with NAA (2 mg/l) and BA (0.5 mg/l).

Fig. 23. In vitro regeneration of *Cajanus* and *Atylosia* plants from cotyledons and immature embryos.

- A) Sequence of plantlet regeneration from whole cotyledons of *Cajanus cajan*.
- B) Shoot regeneration from whole cotyledons of *Atylosia sericea*.
- C) Multiple shoot formation in whole cotyledons of *Cajanus cajan*.
- D) Sequence of plantlet regeneration from immature embryos (15 day old) of *Cajanus cajan*.

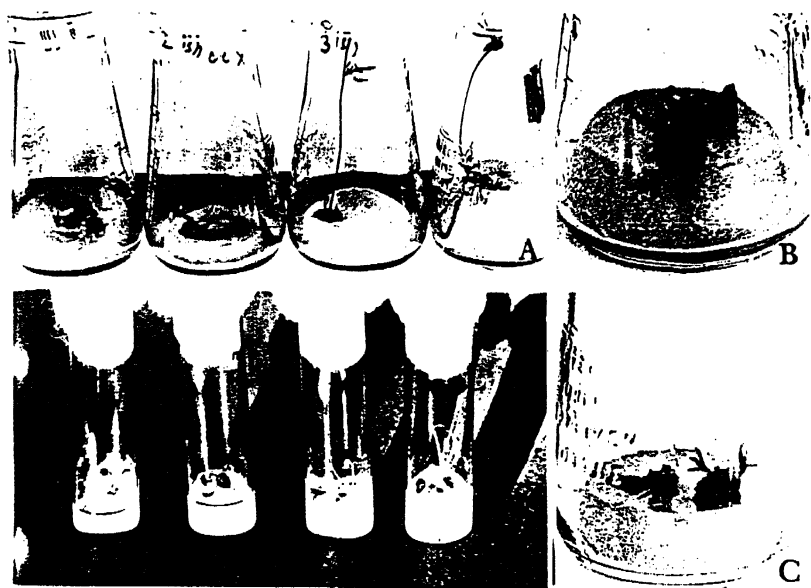


Table XXIV. Percentage shoot regeneration from cotyledon cultures of different cultivars of *Cajanus* and species of *Alysicia* on MS medium supplemented with 2,4-D (0.5 mg/l) and BA (2 mg/l).

Species/cultivars	Whole cotyledon	Cotyledon segments	
		Nodal	Distal
<i>C. cajan</i> cv ICP 4726	38	33	7
<i>C. cajan</i> cv ICP 7035	46	31	13
<i>C. cajan</i> cv Pant-A2	27	29	6
<i>C. cajan</i> cv GS-4	2	-	-
<i>A. cajanifolia</i>	21	14	10
<i>A. albicans</i>	19	16	-
<i>A. sericea</i>	11	10	-

Basal medium supplemented with 2, 4-D (0.5 mg/l), BA (2 mg/l) and NAA (1 mg/l) also induced multiple shoots from whole cotyledons and nodal cotyledonary segments. In this hormone combination both shoots and roots developed from 2 to 14% of whole-cotyledon explants of *C. cajan* and from about 2% of *A. cajanifolia* (Table XXV). A low frequency of plant regeneration was obtained from cultures of nodal cotyledonary segments in *C. cajan*. In the cultures of *A. albicans* and *A. sericea* only shoot regeneration was obtained.

Of the four *C. cajan* cultivars tested, three cultivars showed varying levels of regeneration, while the fourth cultivar GS 4, which had the smallest seed size, almost completely failed to respond. The response of *A. cajanifolia*, which is morphologically quite similar to *C. cajan*, compared well with the response of *C. cajan* cultivars.

A general observation in cotyledon cultures has been that, wherever there was multiple shoot formation, only one or two shoots developed into full shoots while the others remained suppressed.

#### 7.1.2. EMBRYO CULTURE

Response of embryos from four genotypes of pigeonpea (Pant A2, ICP 7035, Prathat and C 11) was evaluated on B5 medium supplemented with 2, 4-D (1 mg/l). An age dependent embryo response was evident (Table XXVI). Eleven to fourteen day old embryos developed callus from which a low frequency of plantlet

Table XXV. Percentage plantlet and shoot regeneration from cotyledon cultures of different *Cajanus* cultivars and species of *Arylosia* on MS medium supplemented with 2,4-D (0.5 mg/l), BA (2 mg/l) and NAA (1 mg/l).

Cultivar/species	Whole cotyledon		Cotyledon segments			
	Shoots	Plant-lets	Nodal		Distal	
			Shoots	Plant-lets	Shoots	Plant-lets
<i>C. cajan</i> cv ICP4726	32	8	31	3	17	-
<i>C. cajan</i> cv ICP7035	39	14	22	2	13	-
<i>C. cajan</i> cv Part-A2	26	2	23	-	12	-
<i>C. cajan</i> cv GS-4	-	-	-	-	-	-
<i>A. cajanifolia</i>	29	2	25	1	8	-
<i>A. allispii</i>	21	-	9	-	-	-
<i>A. sativae</i>	14	-	11	-	-	-

regeneration was obtained. In 15-19 day old embryos plantlets were recovered directly accompanied by small amounts of callus at the base (Fig. 23D). Direct plantlet recovery and occasional callus formation was observed in embryos older than 19 days. Embryos younger than 11 days failed to respond on both the media and basal media supplemented with several hormone combinations also failed to extract any response from such embryos.

Though there was no striking influence of genotype on plantlet recovery, ICP 7035 performed marginally better in all treatments (Table XXVI). ICP 7035 has the largest seed size of the cultivars used, in this study.

### 7.1.3. ANTHER CULTURE

Callus was obtained from the anthers of *C. cajan* and *A. albicans* on MS medium supplemented with 2 mg/l of 2, 4-D. Potato starch extract medium promoted callus development from the anthers of *A. grandifolia* and *A. volubilis*. Callusing was more profuse from the anthers of *A. albicans* and *A. youbilis* than from the other species. Attempts to induce differentiation by subculturing the callus on basal media supplemented with various hormones and their combinations did not meet with success. On subculturing the callus turned brown and degenerated.



Table XXVI. Percent plantlet recovery in embryo cultures of pigeonpea on MS and B5 media supplemented with 1/1 mg of 2,4-D.

Embryo age		<u>Cajanus cultivars</u>			
		Pant A2	Prabhat	ICP-7035	C-11
<11 days	MS	-	-	-	-
	B5	-	-	-	-
11-14 days	MS	11	8	10	7
	B5	17	7	19	13
15-19 days	MS	73	69	81	67
	B5	74	88	93	84
>19 days	MS	57	66	71	55
	B5	87	90	89	84

## 7.2. DISCUSSION

In cotyledon cultures basal medium supplemented with 2, 4-D induced only callus while the medium supplemented with both 2, 4-D and BA induced shoots. Rooting of such explants was achieved on the medium when it was supplemented with NAA. When the basal medium was supplemented with both BA and NAA in addition to 2, 4-D a low frequency of whole plants were recovered. These findings suggest that while BA plays a role in shoot regeneration while NAA helps in root regeneration. Cultivar differences with respect to the percentage of regeneration (Table XXIV) is a possible consequence of the genotypic effect and such results have been reported in other species. This conclusion is strengthened by the fact that the response of *A. cajanifolia* which is genetically close to *C. cajan* was similar to the response obtained in the cultures of *C. cajan* while the performance of *A. albicans* and *A. sericea*, species which are more distantly related to *Cajanus* was distinct.

Variation in response was recorded with respect to the cotyledonary segment cultured. The response of nodal segments was close to the response obtained from the whole cotyledons while the response from the distal segments was poor. The improved response of the nodal segments might be due to the presence of embryonic cells in the nodal half.

Culture conditions for the regeneration of immature pigeonpea embryos have been standardized for the first time. An age dependent embryonic response was obtained in the embryo cultures.

It was not possible to regenerate plants from embryos younger than 11 day old. This has been a common feature in other leguminous species (Gosal and Bajaj, 1983; Newell and Hymowitz, 1982; Cubero, 1981). Genotypic effect on percentage of regeneration in embryo cultures was less pronounced than in cotyledon cultures.

Anther culture studies were not successful beyond the stage of callus induction inspite of attempts to induce regeneration by suppling the basal media with several hormone combinations. Anther cultures have been successful to a large extent in cereals, solanaceous crops etc. but have not made an impact on any of the leguminous crops. An earlier attempt on pigeonpea anther cultures also did not progress beyond the callus induction stage (Bajaj et al, 1980).

## **8. GENERAL DISCUSSION AND CONCLUSION**

The present investigation was initiated in an attempt to understand the genome relationships between *C. cajan* and *Atylosia* species, screen *Cajanus-Atylosia* crosses for selective chromosome elimination, develop methods to overcome barriers to species crossability, study the inheritance and variation for important traits and develop *in vitro* regeneration techniques for pigeonpea. All these objectives have been met.

Out of the twelve species of *Atylosia* and two species of *Rhynchosia* used in the study eight species of *Atylosia* hybridized with the cultivated species. The present study has shown that the species crossability is significantly influenced by the genotype of the *Cajanus* parent. Studies on hormone treatments have revealed that post-pollination hormone applications improve species crossability by checking the post-fertilization barriers. Reciprocal success in these crosses is very difficult. Four of the twelve *Atylosia* species failed to cross with *Cajanus* in any direction. In the unsuccessful crosses post-pollination hormone treatments delayed bud/pod drop but it was not sufficient to get ovules/embryos responding to *in vitro* regeneration. *In vitro* rescue of embryos could be achieved only in embryos older than 11 days. Both hormone applications and embryo culture techniques in pigeonpea have been developed for the first time. The results indicate that hormone applications in conjunction with embryo culture technique offer potential for obtaining new *Cajanus-Atylosia* hybrids with further refinements of these techniques.

The present study has resolved the longstanding controversy regarding number of nucleolar organizer chromosomes in pigeonpea. In spite of the limitations of short meiotic cycle and difficulties in obtaining good cytological preparations in pigeonpea a detailed information on the number, size and distribution of nucleoli at T-I and T-II was obtained. Evidence in the form of nucleolar number at T-I and T-II in diploid and a spontaneous tetraploid of pigeonpea established beyond doubt the presence of two nucleolar organizer chromosomes in pigeonpea. A similar situation exists in the species of *Atylosia*. This is the first study to use meiotic activity of the nucleolar chromosomes as an index of the nucleolar organizer number. The present study has also shown the attachment of two bivalents to the diplotene/diakinesis nucleoli and also the presence of two primary nucleoli at pachytene/ diplotene/diakinesis to substantiate the presence of two nucleolar organizing chromosomes. The chromosome number in the Australian species of *Atylosia* is being reported for the first time.

The major accomplishment of this investigation is the detailed insight provided into the genome relationships between *C. cajan* and the species <sup>of</sup> *Atylosia*. These investigations suggest that *C. cajan* and the species in the genus *Atylosia* basically have a similar genome or in other words they have evolved from a common gene pool and the differentiation has occurred as a result of structural alterations. The closest relative of pigeonpea among the species studied is *A. cajanifolia*. The major distinguishing feature between these two species is the presence

of seed strophiole. Some of the recent collections of Cajanus cajan by the Genetic Resources Unit at ICRISAT show a prominent strophiole (van der Maesen, pers. comm.). In view of these findings it is likely that C. cajan might differ from A. cajanifolia by only a few gene mutations. This was further substantiated from the data on variation for quantitative traits from the  $F_2$  populations. For all the characters studied the variation has been the lowest in the populations of C. cajan x A. cajanifolia. The other species of Atylosia that have hybridized with C. cajan are morphologically more distinct. Meiotic studies of their hybrids reveal that they are genomically close to pigeonpea and have differentiated through structural alterations and the results have shown that the levels of gametic sterility reflect the degree of species divergence. Australian species of Atylosia are more diverged from Cajanus than the Asian species.

The predominantly one way success of the Cajanus-Atylosia crosses suggest that the structural alterations and/or gene mutations that have led to the species differentiation are accompanied by the differentiation of plasmon. There is no evidence for chromosome elimination in the intergeneric hybrids of pigeonpea. The information gathered in the present study is not sufficient to comment upon the relationships of C. cajan with the species of Rhynchosia except that they share the same chromosome number.

The study on inheritance of qualitative traits has shown that these traits are controlled by one or two genes. Several novel segregants were recorded in the  $F_2$  generation and transgressive segregants of considerable value have been recovered in the  $F_2$  generation for characters like leaf length, leaf width, seed protein content etc underlining the importance of Atylosia species for the improvement of the cultivated species.

This study for the first time has provided direct cytological evidence for recombination between genomes by way of studying the pairing of nucleolar organizer chromosomes and tracing the products of recombination using the nucleolus as a marker. This technique can be a potential tool in recombination studies in distant hybrids. These findings have also provided the first cytological evidence for the structural hybridity between Cajanus and Atylosia species.

The present investigation has provided ample cytological and genetical evidence for recombination between the genomes of Cajanus and Atylosia species and has proved that the species of Atylosia possess all the requisites (close genome relationship with the cultivated species, a high degree of recombination between the genomes and recovery of transgressive segregants from the progeny of hybrids with the cultivated species) for effective introgression.



In an overview the present investigation has shown that the species of *Atylosia* can be of potential in pigeonpea improvement programmes and these species deserve more attention than they have received so far.

In view of these findings future attempts must be directed at obtaining hybrids between *Cajanus* and species of *Atylosia* which have not been used so far in intergeneric studies. Further refinements in unconventional techniques like embryo culture and hormone treatments and development of new techniques like recognition and mentor pollen techniques should be given importance to achieve success in otherwise incompatible crosses in an attempt to widen the genetic base. There is also a need to obtain hybrids between *Atylosia* species to be able to gain more knowledge about the divergence of these species from *Cajanus*.

There is a need to screen the progeny from the intergeneric hybrids and select the desirable segregants, evaluate them and incorporate them into pigeonpea improvement programmes to achieve introgression of the desired character by adapting the relevant breeding methodologies.

The present study has shown that tetraploid pigeonpea is cytologically stable and hence long term attempts must also be directed at crosses between tetraploid *Cajanus* and species of *Atylosia* to obtain addition and/or substitution lines of pigeonpea.

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**PUBLICATION FROM THE THESIS**



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