Nucleolar variation in a pigeon pea intergeneric hybrid: evidence for allosyndetic recombination[1]

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Cytological study of a hybrid between *Cajanus cajan* and *Atylosia albicans* revealed regular bivalent formation and disjunction. Nevertheless, high pollen sterility and low seed set were evident. An examination of pollen mother cells revealed variation in nucleolar number at telophase-I (four to eight) and at telophase-II (zero to four in daughter nuclei), although each genome contained two nucleolar organizers. Variation was also recorded for nucleolar size and distribution at telophase-II. 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. Size variation is attributed to nucleolar dominance. These results explain the high degree of pollen sterility in the hybrid in spite of normal meiosis, and also suggest that the karyotypes of *C. cajan* and *A. albicans* have differentiated through structural heterozygosity.

Key words: *Cajanus cajan*, *Atylosia albicans*, nucleolar variation, structural heterozygosity, allosyndetic recombination.

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L'analyse cytologique d'un hybride entre *Cajanus cajan* et *Atylosia albicans* a démontré un processus de formation et de disjonction normal des bivalents. Cependant on a trouvé des indices de forte stérilité du pollen et de faible production de graines. Un examen des cellules mères du pollen a révélé une variation du nombre nucléolaire (quatre à huit) à la télophase-I et (zéro à quatre chez les noyaux soeurs) à la télophase-II, quoique chaque génome contient deux organisateurs nucléolaires. On a aussi noté une variation de la grosseur du nucléole et de sa distribution à la télophase-II. On attribue la variation du nombre et de la distribution des nucléoles à l'appariement et à la recombinaison entre (le(s) chromosome(s) d'organisateur nucléolaire d'une espèce parentale avec le(s) chromosome(s) d'organisateur non nucléolaire de l'autre. La variation de la grosseur est imputée à la dominance nucléolaire. Ces résultats expliquent le haut taux de stérilité du pollen chez l'hybride malgré une méiose normale, et suggèrent de plus que les caryotypes de *C. cajan* et de *A. albicans* se sont différentiés par hétérozygosité structurale.

Mots clés: *Cajanus cajan*, *Atylosia albicans*, variation nucléolaire, hétérozygosité structurale, recombinaison allo-syndétique.

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Introduction

Pigeon pea, *Cajanus cajan* ($n = x = 11$), is an important pulse crop of the tropics, and has several related wild species in the genus *Atylosia* which have the same chromosome number. Many of the *Atylosia* species possess several desirable characters such as disease and pest resistance, high protein content, and photoperiod insensitivity (Remanandan 1981). In spite of their potential, the use of *Atylosia* species in pigeon pea breeding programs has been limited partly because of poor understanding of the genome relationships between the cultivated and the wild species, and owing to a low rate of crossability and poor fertility of hybrids in many cross combinations. The elucidation of genome relationships in pigeon pea is hampered by difficulties in obtaining good cytological preparations. Investigations on *Cajanus* × *Atylosia* crosses were initiated to screen a wide range of crosses for haploid production through "selective chromosome elimination" as has been demonstrated in barley (Subrahmanyam and Kasha 1973), and also to gather information on the chromosomal relationships between the species of these genera.

During our studies we encountered in the F_1 of a cross between *C. cajan* and *A. albicans*, a wide variation for nucleolar number and distribution at both the meiotic

divisions. This suggested that the karyotypes of the two species have differentiated through chromosomal alterations which also explains the observed pollen sterility. This paper presents the evidence for recombination between genomes contributed by the parental species (allosyndetic recombination) in their hybrid.

Materials and methods

Seeds of *Cajanus cajan* cv. Pant-A2 and *Atylosia albicans* (NKR-177) were sown in the field at the International Crop Research Institute for the Semi-Arid Tropics. Seed of *A. albicans* was scarified before sowing to facilitate germination. For hybridization, buds on the female parent were emasculated between 0800 and 1200 during January to April, and the stigma was immediately dusted with the pollen of *A. albicans*. Dried pods were collected for raising the progeny. The F_1 was screened for true hybrids based on plant morphology.

For meiotic studies, flower buds were fixed in Carnoy's fluid (6:3:1) and the anthers were squashed in 1% acetocarmine. Pollen stainability with 1% acetocarmine was taken as an index for pollen fertility.

Results

The cross between *Cajanus cajan* and *A. albicans* was successful in 7% of the pollinations and yielded an unusually luxuriant hybrid with profuse branching and a thick leaf canopy. *Cajanus cajan* has an erect habit while *A. albicans* is a climber. The F_1 had a semi-spreading habit with a tendency to spread laterally. In its initial stages of growth the hybrid was intermediate for leaf shape and texture. Flower shape, flower colour, and pollen colour in the F_1 hybrid were similar to those in the *Cajanus* parent. The strophiole on the *C. cajan* seed is rudimentary, whereas in *A. albicans* it is prominent. The F_1 seeds had a prominent strophiole. The hybrid exhibited some interesting morphological variations as it grew and developed. These features will be published elsewhere.

At diplotene and diakinesis the hybrid showed regular pairing (Figs. 1A−1D). Diplotene cells displayed nucleoli with three or four bivalents attached, while at diakinesis 20 of 64 cells scored contained nucleoli associated with three or four bivalents. At metaphase-I, pollen mother cells (PMCs) showed 11 bivalents (Fig. 1E) with normal anaphase-I disjunction (Fig. 1F) and very rare precocious separation. We recorded differences in the staining intensities of partners in bivalents. Anaphase-II separation was also regular (Fig. 1G). In the hybrid we saw a wide variation in nucleolar number and distribution at telophase-I and telophase-II (Figs. 2A−2I). Each parental genome has two nucleolar organizing chromosomes (NOs).

The nucleolar number of the hybrid at telophase-I varied from four to eight. At telophase-II the percentage of PMCs with four nucleoli (Fig. 2B) was 40%. There
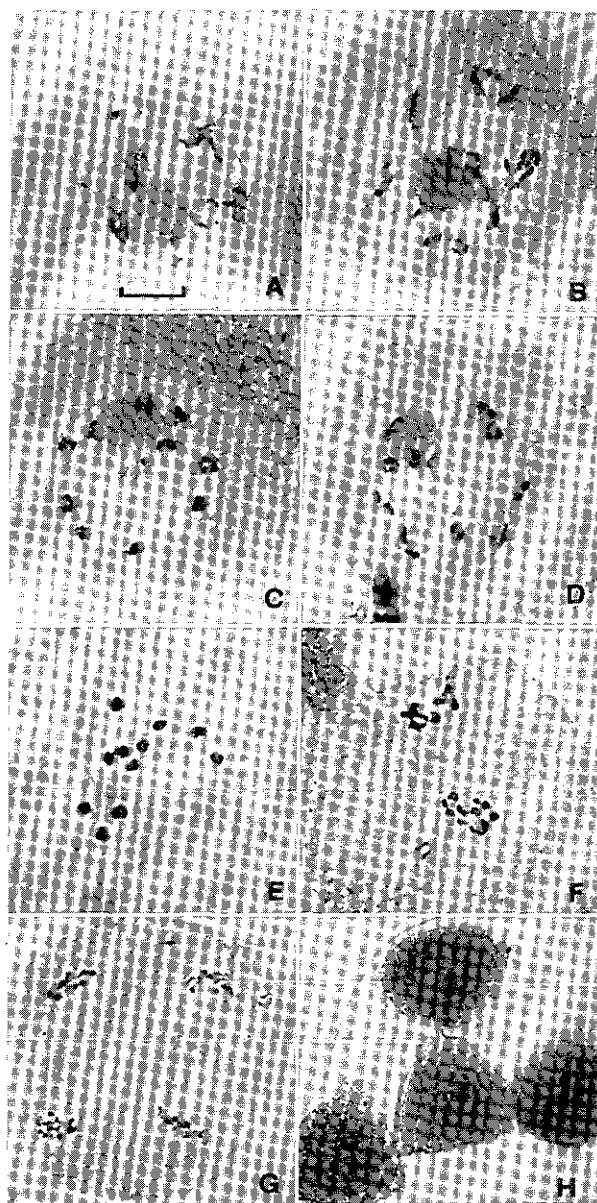

FIG. 1. Meiosis in the hybrid between *Cajanus cajan* and *Atylosia albicans* (bar indicates 10 μm). (A) Diplotene nucleolus with four bivalents attached. (B) Diplotene nucleolus with three bivalents attached. (C) Diakinesis nucleolus with four bivalents attached. (D) Diakinesis nucleolus with three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

were more cells with an even number (six and eight) of nucleoli (29 and 16%, respectively) than with five and seven nucleoli (Fig. 3). At telophase-II, nucleoli in daughter nuclei varied from zero to four (Fig. 4). The
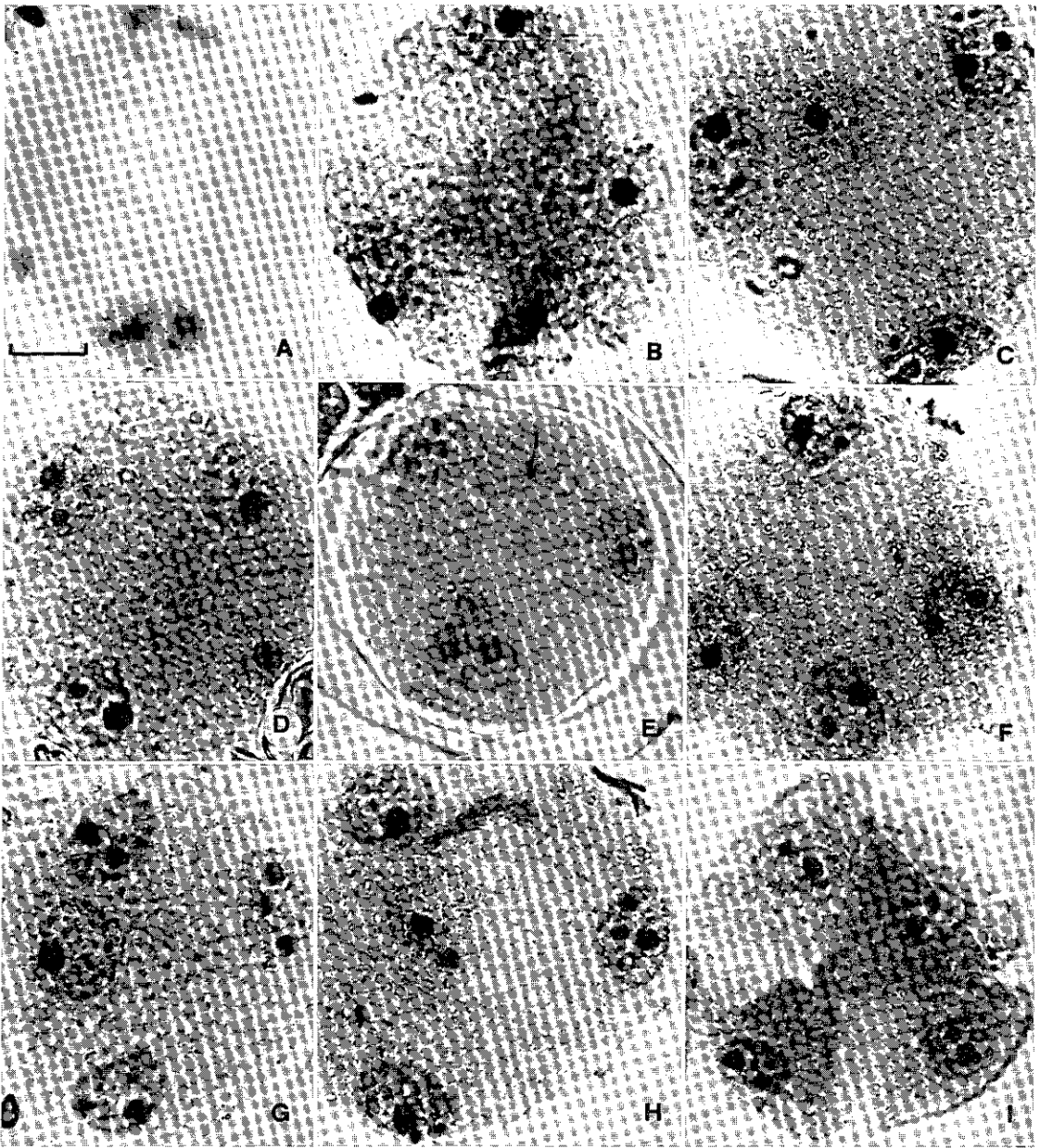
FIG. 2. Nucleolar variation in pollen mother cells of the hybrid between *Cajanus cajan* and *Atylosia albicans* (bar indicates 10 μm). (A) Telophase-I nucleolar distribution, 4−4. Telophase-II nucleolar distribution: (B) 1−1−1−1, (C) 2−1−1−1. (D) 2−2−1−1, (E) 4−2−1−0 (arrow indicates nucleus devoid of nucleoli), (F) 2−2−2−1, (G) 3−2−2−1, (H) 4−2−1−1, and (I) 2−2−2−2.

frequency of daughter nuclei devoid of nucleoli was about 3.5%. A majority (65%) of the daughter nuclei contained one nucleolus while the remaining daughter nuclei included 22% with two nucleoli, 6% with three, and 2% with four nucleoli. In 14% of the PMCs at telophase-II the nucleolar distribution was confined to only three daughter nuclei.

The cells which exhibited variation in the number of nucleoli also showed variation in nucleolar size. The nucleoli could be classed as large and small. In the PMCs with four nucleoli (at telophase-II) the nucleolar size was larger (Fig. 2B) than some of those with more than four nucleoli (Figs. 2C−2I). In the PMCs scored for nucleolar number and size there was not much vari-

		No. of nucleoli				
		4	5	6	7	8
PMCs	No.	62	14	46	11	26
	%	39	8.8	28.9	6.9	16.4

FIG. 3. Nucleolar variation at telophase-II in a hybrid between *C. Cajan* and *A. albicans*.

ation in the size of the larger nucleoli, while there was variation in the size of the smaller nucleoli.

At telophase-II we recorded variation in nucleolar distribution (Figs. 2B−2I and 3). We saw three types of distribution in cells with five nucleoli, four types in cells with six and seven nucleoli, and five types in those with eight nucleoli.

Flowering in the hybrid was profuse but there was heavy pod drop. Stainable pollen in the F_1 hybrid was 60% compared with 97% in *Cajanus cajan* and 89% in *A. albicans*. While the pollen size in both parents was uniform, we recorded a twofold variation in size in the hybrid. Seed set in the hybrid was 21%. Most of the seed was shrivelled and inviable.

Discussion

Meiosis in the hybrid was normal. The regular formation of 11 bivalents in the F_1 hybrid might be interpreted to indicate complete chromosome homology between the two genomes which can be misleading as will be evident from the following discussion.

The most intriguing feature in the hybrid was the association of up to four bivalents with the nucleolus at diplotene/diakinesis, and the wide variation recorded
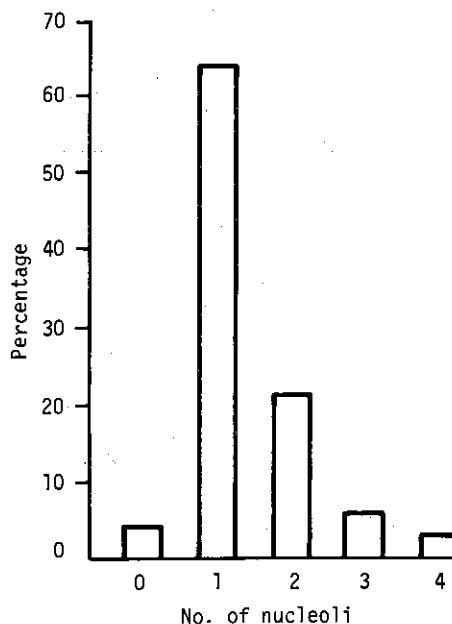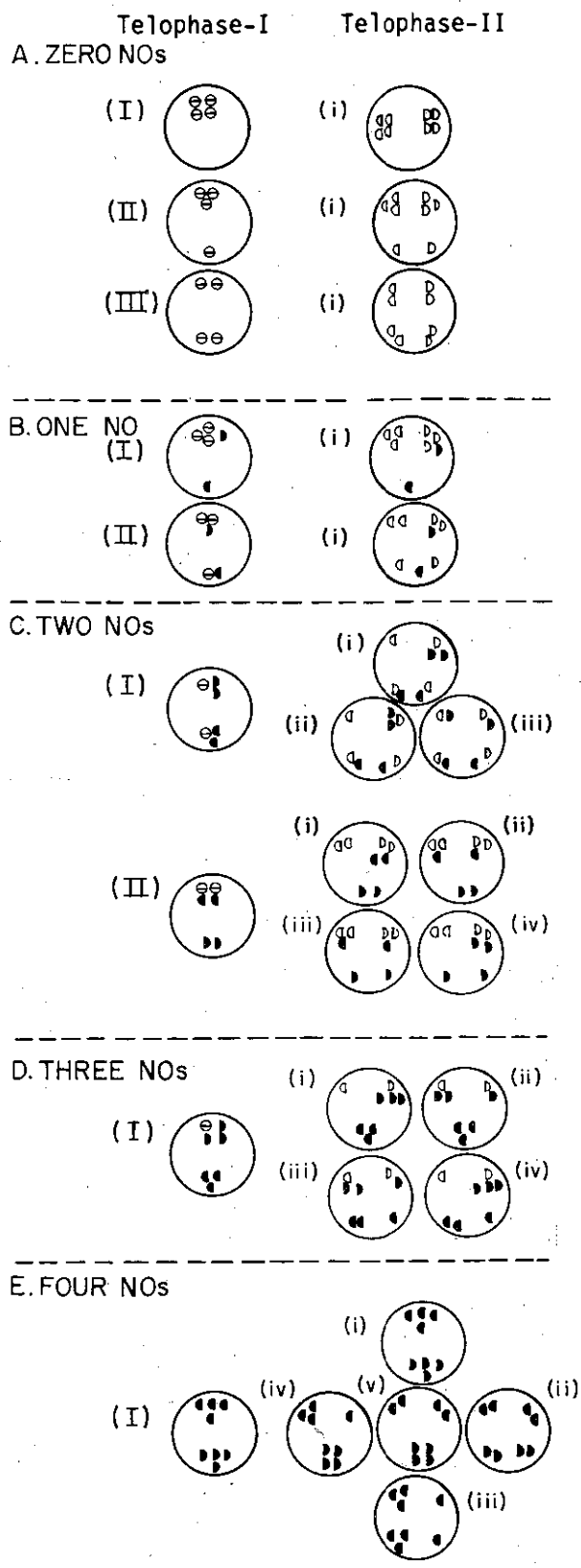

FIG. 4. Frequency distribution of nucleoli in telophase-II daughter nuclei of the hybrid between *Cajanus cajan* and *Atylosia albicans*.

for nucleolar number, distribution, and size. Karyotypes of *C. cajan* and *A. albicans* have two pairs of satellite chromosomes in each of their genomes (Pundir 1981). 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. The association of more than two bivalents with the nucleolus in the hybrid cells is suggestive of pairing between the NOs of *Cajanus cajan* and non-NOs of *Atylosia albicans* or vice versa and the variation in nucleolar number and distribution at telophase-I and telophase-II is interpreted to have originated from allosyndetic recombination.

The possible nucleolar distributions with four NOs, with and without recombination, are presented in Fig. 5. If one assumes no recombination between a NO and a non-NO (Fig. 5A), a maximum of four nucleoli can be expected at telophase-I. The presence of four to eight nucleoli at telophase-I compels us to suggest allosyndetic recombination. Furthermore, if the NOs 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 (i.e., assuming that crossing-over involves one, two, three, or four NOs with non-NOs). 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 NO of *C. cajan* and a non-NO of *A. albicans* or vice versa is supported by the fact that the nucleolar number never exceeded eight, which is the maximum that can be expected with four NOs (i.e., eight satellite chromatids). Nucleolar distribution to only three of the four daughter nuclei in a given PMC (Figs. 2E and 3) can occur only when the NOs are involved in recombination with non-NOs. Additional proof for recombination comes from 2% of the telophase-II nuclei which have four nucleoli (Fig. 2H). From Fig. 5 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 NOs of the parents; however, the presence of a total of eight nucleoli per PMC with a regular $2-2-2-2$ distribution (P. S. Kumar, unpublished data) in the parents and the absence of

FIG. 5. Possible nucleolar variation in pollen mother cells of the hybrid between *Cajanus cajan* and *Atylosia albicans* without recombination and following recombination between nucleolar organizing and nonnucleolar organizing chromosomes. ⊖, NO chromosome; ◁, NO chromatid; ◀, NO chromatid following recombination.

PMCs with more than eight nucleoli in the present hybrid rules out such a possibility.

A majority (about 40%) of the PMCs in the hybrid had only four nucleoli, one in each daughter nucleus at telophase-II. Hence it can be presumed that the normal NO disjunction without recombination is $2-2$ (Fig. 5A, III-i) resulting in two nucleoli in each daughter nucleus, which on fusion give rise to one nucleolus. Nucleolar fusion is common in several plant species especially in meiosis (Bennett *et al*. 1973; Darvey and Driscoll 1972; Darvey *et al*. 1973; Sybenga 1972; Flavell and O'Dell 1979; Jessop and Subrahmanyam 1984). The remaining (60%) PMCs that undergo allosyndetic recombination with respect to the NO involve a minimum of one NO 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 NOs involved in recombination. Although we obtained a variation ranging form four to eight nucleoli at telophase-I, it was not possible to score enough cells at this stage to enable us to arrive at the frequency of recombination products. This was not possible because it is difficult to get a PMC at telophase-I with primary (unfused) nucleoli, owing to the short meiotic cycle in pigeon pea. In the cells ($>16\%$) containing eight nucleoli at telophase-II the distribution pattern of $4-2-2-0$ (Fig. 3) is only possible when all the four NOs are involved in recombination with non-NOs.

The presence of five, six, and seven nucleoli at telophase-II, many a time with variable size, is due to nucleolar fusion. In the light of the different possibilities (Fig. 5) the pattern of nucleolar fusion could not be assessed. Although in the daughter nuclei nucleolar variation of zero to four existed at telophase-II (Fig. 4), most of the daughter nuclei at the quartet stage showed a single nucleolus with a few quartets (Fig. 1H) showing two or three distinct nucleoli. Because the overall variation of nucleolar number can be attributed to both fusion and 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 NOs involved in allosyndetic recombination could be given.

Another interesting feature in the hybrid was the variation in nucleolar size. Variation in the nucleolar size, especially the presence of smaller nucleoli than the normal (Fig. 2I) formed by a single nucleolar organizing chromatid, can be attributed to either a crossing-over involving a break within the secondary constriction or crossing-over between two NOs resulting in the formation of a chromatid with two nucleolar organizing termini leading to nucleolar dominance (differential

amphiplasty). If the first alternative is assumed, one would expect more than eight nucleoli. Absence of PMCs with more than eight 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 (Navashin 1934; Keep 1962; Subrahmanyam and Azad 1978a, 1978b) in interspecific hybrids, wherein nucleolar expression of one of the species involved in the cross is suppressed. Partial suppression of a NO leading to a reduction in nucleolar size has been reported by Nicoloff *et al*. (1979) in their barley translocation lines.

Differences in the staining intensities between the partners of a bivalent is indicative of possible differences in chromomere distribution between the chromosomes contributed by the parental species, further substantiating intergenomic pairing in the hybrid.

Regular meiosis in the hybrid indicates a close relationship between the cultivated and the wild species. The high frequency of recombination in the hybrid further confirms the proximity of the two parental species and suggests that the two species have differentiated through structural alterations of chromosomes (probably translocations). Our findings support the conclusions of some workers (McComb 1975; van der Maesen 1981) that the taxonomic separation of *Cajanus* and *Atylosia* should not be maintained.

In spite of the profuse flowering and regular meiosis, the high degree of pollen sterility and low seed set in the hybrid is surprising. Structural heterozygosity in the chromosomes of the two parents as evidenced by the allosyndetic recombination in the present hybrid could be the major reason for the sterility, because the pollen fertility was much lower than expected on the basis of normal bivalent formation and disjuction, suggesting that 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. Kumar and Thombre (1958) observed 49% pollen sterility in an F_1 hybrid between *C. cajan* and *A. lineata* in spite of regular meiosis. Structural differences between the chromosomes of the two species involved were suggested as the possible cause of sterility. Morphologically and cytologically *A. lineata* is more closely related to *C. cajan* than *A. albicans* (Reddy 1973). 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 structural differences in the parental chromosomes. Our results provide a conclusive evidence for that possibility.

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