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Cytogenetics of F1 hybrids between *Cajanus* and *Atylosia* species and its phylogenetic implications

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Summary. Cytogenetic relationships between two cultivars of *Cajanus cajan* and six species of *Atylosia* were investigated. Of the 12 cross combinations obtained, only seven could be studied. Meiotic chromosome pairing, pollen and ovule fertility in parental species and four F1 hybrids were near normal. Some meiotic abnormalities were observed in the F1s: *A. lineata* × *A. scarabaeoides*, *A. scarabaeoides* × *A. sericea* and *C. cajan* (UPAS 120) × *A. trinervia*, indicating varying degrees of chromosomal and genic differences between these species. These observations suggested that *A. cajanifolia* is the closest wild relative of *C. cajan*, followed by *A. scarabaeoides*, *A. albicans* and *A. trinervia*. Among the *Atylosia* species, *A. sericea* was closer to *A. scarabaeoides* than to *A. lineata*.

Key words: Phylogenetic relationships – Meiosis – Fertility – *Cajanus cajan* – *Atylosia*

Introduction

Pigeonpea, *Cajanus cajan* (L) Millsp. is attacked by several diseases and insect pests although the wild species of this genus and related genera possess resistance against such biotic stresses. An understanding of cytogenetic affinities between these species is essential if transfer of resistance genes from wild relatives to the cultivated plant is to be accomplished. Further, the chromosomal associations of F1 hybrids provide information on the possibilities of gene transfer through meiotic recombinations.

Of the 13 genera that are closely related to *Cajanus*, *Atylosia* appears to be the closest based on morphological characters. Several workers have obtained intergeneric hybrids between *C. cajan* and *Atylosia* species. The meiotic behaviour of these F1 hybrids were studied and in most cases chromosomal affinities were found (Deodikar and Thakar 1956; Kumar et al. 1958; Sikdar and De 1967; De 1974; Pundir 1981; Reddy 1981 a, b, c; Reddy and De 1983). The present study reports on the meiotic chromosome pairing, pollen and ovule fertility and pod set of two cultivars of *C. cajan* ('Pant A 2' and 'UPAS 120'), five *Atylosia* species: *A. albicans* (JM 2356), *A. cajanifolia* (JM 2739), *A. lineata* (ICP 7469), *A. scarabaeoides* (ICP 7464), and *A. sericea* (ICP 7470) and seven F1 hybrids between these taxa. These results have been utilized in understanding the phylogenetic relationships among these species.

Materials and methods

Seeds of the materials used were obtained from the Genetic Resources Unit, ICRISAT, and the studies were conducted at the Banaras Hindu University, Varanasi during 1977–79. The two cultivars of *C. cajan*, seven *Atylosia* species and one *Rhynchosia* species were crossed in a diallel fashion. *Atylosia trinervia* failed to flower under Varanasi conditions so its pollen was collected from plants at a site in Ootacumund, Tamil Nadu, from where this accession was originally procured. However, the pollen was sufficient for making crosses with only one entry of *C. cajan* ('UPAS 120'). Pollinations were made in a total of 73 cross combinations of which only 12 were successful (Pundir 1981). Of the 12 successful hybrids, two combinations were lost in early growth stages and three did not flower under Varanasi conditions. Hence, the cytogenetic observations could be made only on the seven hybrids (Table 1). The observations included metaphase I (MI),

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Table 1. Mean and range (parentheses) of metaphase I configurations, anaphase I separations, and fertility of *C. cajan, Atylosia* species and their F1 hybrids

Identity	Metaphase I			Anaphase I		Fertility[a]		
	Cells observed	Bivalents	Univalents	Cells showing normal separation	Cells showing abnormal separation	Pollen	Ovule	Pod set
	(No.)	(No.)	(No.)	(No.)	(No.)	(%)	(%)	(%)
C. cajan cv. 'Pant A 2'	20	11	0	10	0	98.8	93.9	18.7
C. cajan cv. 'UPAS 120'	10	11	0	5	0	95.3	80.0	21.5
A. albicans	15	11	0	–	–	96.9	84.2	59.0
A. cajanifolia	20	11	0	5	0	94.6	80.8	14.0
A. lineata	15	11	0	20	0	97.6	79.3	66.7
A. scarabaeoides	20	11	0	–	–	98.3	94.6	74.2
A. sericea	10	11	0	–	–	96.9	96.7	66.7
C. cajan ('Pant A 2') × *A. albicans*	36	10.91 (10–11)	0.17 (0–2)	11	1	69.3	32.4	–
C. cajan ('Plant A 2') × *A. Scarabaeoides*	45	10.98 (10–11)	0.04 (0–2)	–	–	87.5	54.4	29.2
C. cajan ('UPAS 120') × *A. trinervia*	47	11	0	7	0	52.0	60.0	1.2
A. cajanifolia × *C. cajan* ('Pant A 2')	34	11	0	6	0	94.5	64.1	8.9
A. cajanifolia × *C. cajan* ('UPAS 120')	30	11	0	10	0	96.4	64.9	9.8
A. lineata × *A. scarabaeoides*	35	10.54 (9–11)	0.91 (0–4)	4	1	51.6	57.1	8.3
A. scarabaeoides × *A. sericea*	30	9.90 (8–11)	2.20 (0–6)	13	3	71.1	73.1	26.9

[a] Data were based on a study of more than 200 pollen grains, 20 pods and more than 200 bud scars for pollen, ovule and pod set, respectively

anaphase I (AI) separation, pollen and ovule fertility, and pod set.

For meiotic analysis, flower buds of 1–2 mm size were fixed between 10.00 to 13.00 h in modified Carnoys fluid (6 ethyl alcohol : 3 chloroform : 2 glacial acetic acid v/v) and stored in 70% alcohol at 10–15 °C. The anthers were squashed in 2% propiono carmine, covered with a coverslip, gently warmed and pressed to ensure the desired spread and staining. Destaining, if required, was done by putting a drop of 45% propionic acid along the sides of coverslip. Observations were recorded on pollen mother cells (PMCs) with discernable configurations. Microphotographs were made from temporary preparations under oil immersion at × 1,000 magnification. Pollen fertility was determined by staining mature pollen grains in 2% potassium iodide solution; stained pollen grains were counted as being fertile. Ovule fertility was determined on the number of fully formed seeds per 100 ovules. Pod set was calculated as percentage of fully developed pods to the total number of buds produced.

Results

Each species had 11 bivalents. The anaphase distribution was regular and the pollen fertility was above 94%. Ovule fertility however, was variable. The *Atylosia* species, especially *A. scarabaeoides* and *A. sericea,* had a higher score of ovule fertility than the other species. For pod setting, a greater variation was recorded between species but this trait was greatly affected by the extent of differential flower drop, the reasons for which are not yet known. Details of meiotic behaviour and fertility in the parental species and their F1 hybrids have been summarized in Table 1. Figure 1 shows chromosome associations in the different F1 hybrids.

Discussion

From a perusal of meiotic behaviour and fertility, it may be inferred that there are three patterns of differentiation between these taxa: (1) chromosome structural differences not enough to disturb the meiotic cycle and hence no hybrid sterility; (2) chromosomal differentiation to such an extent that there was univalent formation and unequal distribution, leading to hybrid sterility; (3) structural differences that do not affect pairing but significant enough to cause hybrid sterility.

The hybrids between *A. cajanifolia* and the two cultivars of *C. cajan* showed normal meiosis and fertility comparable to that found in the parental species. These observations suggest that both species are identical in their chromosomal complements. However, the reciprocal cross i.e. *C. cajan* (♀) × *A. cajanifolia* (♂) was

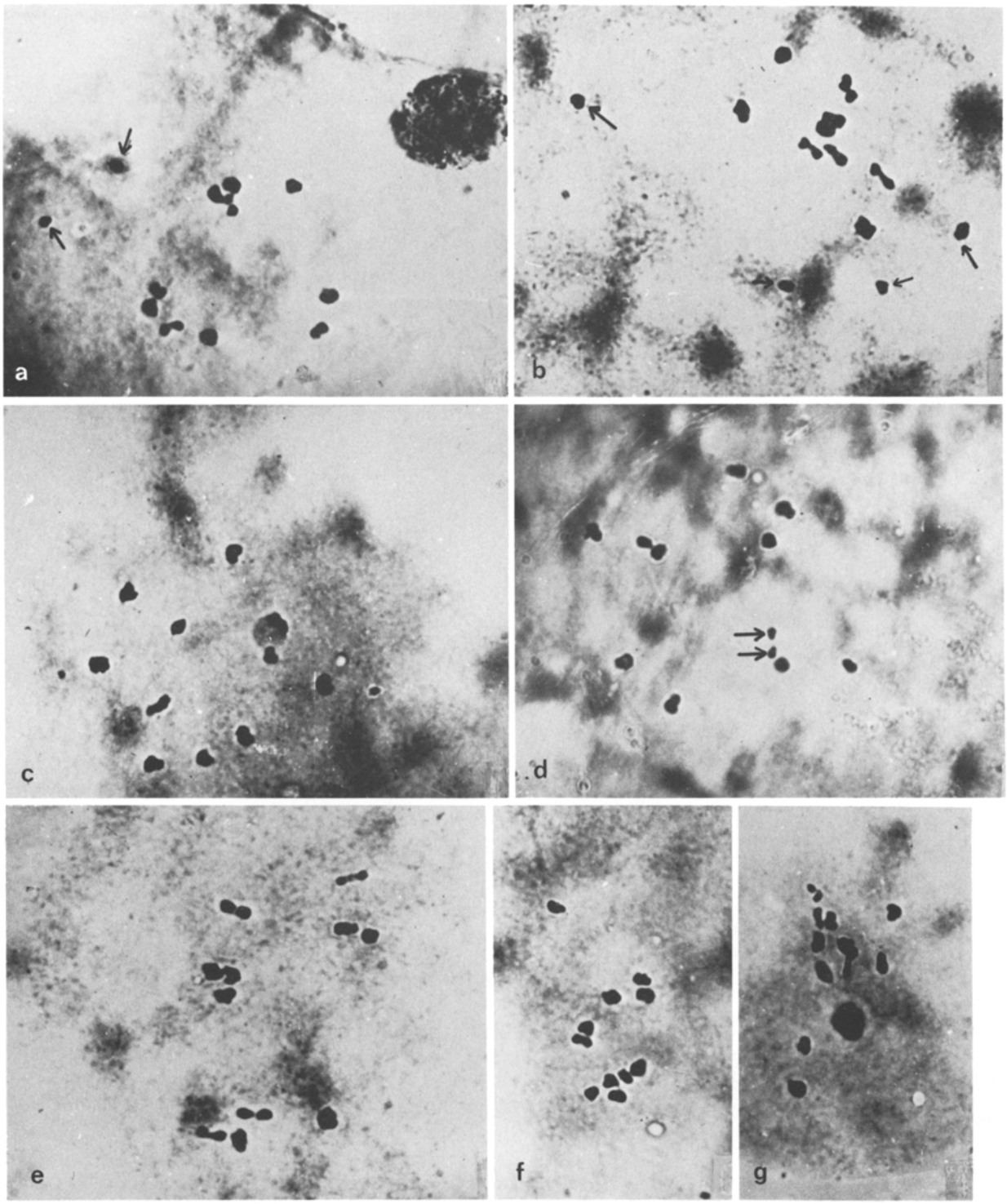

Fig. 1. **a** 'Pant A 2' × *A. albicans;* **b** *A. scarabaeoides* × *A. sericea;* **c** *A. cajanifolia* × 'Pant A 2'; **d** *A. lineata* × *A. scarabaeoides;* **e** 'UPAS 120' × *A. trinervia;* **f** 'Pant A 2' × *A. scarabaeoides;* **g** *A. cajanifolia* × 'UPAS 120', (*arrows* indicate the univalents)

not successful even though more than 1,500 pollinations were tried (Pundir 1981). This may be due to possible cytoplasmic differences between the two species that restrict their hybridization to only one direction despite chromosomal homology. Further studies on crossability involving a large number of genotypes/ecotypes of the two species and the F2 segregation patterns in all possible combinations may throw light on the extent of differentiation between the two species.

Comparing the intergeneric hybrids with the interspecific *Atylosia* hybrids, it was seen that in crosses, *C. cajan* ('Pant A 2')×*A. scarabaeoides; C. cajan* ('Pant A 2')× *A. albicans; A. lineata*× *A. scarabaeoides;* and *A. scarabaeoides*×*A. sericea,* the univalents ranged from 0 to 2 in the intergeneric hybrids whereas in interspecific *Atylosia* hybrids the number ranged from 0–6. This suggests that the chromosome homology between the *C. cajan* and *Atylosia* species is not less than the chromosome homology between the species of the genus *Atylosia.* The *Cajanus*×*A. albicans* and *Cajanus*×*A. scarabaeoides* hybrids have a similar univalent frequency although the latter had considerably higher pollen and ovule fertility than the former. Thus, it is plausible to suggest that there is greater genetic divergence between *C. cajan* and *A. albicans* than between *C. cajan* and *A. scarabaeoides.*

In the two intra-*Atylosia* hybrids, the univalent frequency and the proportion of cells showing aberrant anaphase separation were almost similar. However, the fertility of pollen and ovule was higher in *A. scarabaeoides*×*A. sericea* compared to *A. lineata*×*A. scarabaeoides.* This may be indicative of the closer relationship of *A. scarabaeoides* with *A. sericea* than with *A. lineata.* This is strengthened by the fact that *A. lineata* has two pairs of satellite chromosomes whereas the other two species have one pair each (Pundir 1981).

The hybrids showing univalents (though in low frequency) have lower pollen stainability than other combinations with the exception of *C. cajan* ('UPAS 120') ×*A. trinervia* (Table 1). These results suggest that the chromosomal and genetic differences between the genomes of these taxa are expressed by the nonpairing of some chromosomes resulting in the occurrence of univalents, unequal segregation of chromosomes and lower fertility of the hybrids.

In hybrids such as *C. cajan* ('UPAS 120')×*A. trinervia,* where even after normal pairing the fertility was very low, the suggestion is that either the cryptic structural differences between the two genomes or disharmonious gene combinations probably caused disturbances in the meiotic cycle in subsequent stages, causing abortion of gametes. It is also possible that since the distribution of *A. trinervia* is limited to Nilgiri Hills (India), and the plants did not establish and flower in the Varanasi habitat, this narrow adaptation might have contributed to gametic and embryo abortion resulting in the poor fertility of the hybrid.

Kumar et al. (1984) observed a low fertility in spite of normal meiosis in *Cajanus*×*A. albicans* hybrids and ascribed it to the effect of recombination between nucleolar organizer chromosomes of the two parental species. It is likely that similar phenomena also occur in the hybrids *C. cajan* ('UPAS 120')×*A. trinervia,* and *A. lineata*×*A. scarabaeoides* of the present study.

Phylogenetic implications

Based on the present cytological evidence and the results from morphological and biochemical investigations on *Atylosia* and *Cajanus* species (Pundir 1981), *A. cajanifolia* appears phylogenetically very close to *C. cajan.* Further, strophiolated seeds of *A. cajanifolia,* regarded as the distinguishing feature between *Cajanus* and *Atylosia,* is no more tenable as its occurrence has been reported in several accessions of *C. cajan* (van der Maesen 1980 a). The two species crossed readily and the F1 plants grew normal. Considering these facts it is inferred that *A. cajanifolia* is a wild form of *C. cajan.*

Cajanus cajan and *A. scarabaeoides* are the two most widely distributed species of Cajaninae (van der Maesen 1980 b). That their hybrid plants grow successfully and show near normal meiosis and fertility, implies that the two species have a close relationship.

Atylosia albicans and *A. trinervia,* both have limited geographic distributions. Morphologically they are different from each other, they also differ from *C. cajan.* However, both species are crossable and show cytogenetic affinity with *C. cajan.* It is likely that these species have deviated at an early stage of evolution through the accumulation of modifying genes over a period of time and have adapted to a narrow habitat.

A collective evaluation of the cytogenetical data suggests that *A. cajanifolia* is the closest wild relative of *C. cajan,* followed by *A. scarabaeoides, A. albicans* and *A. trinervia.* Among the *Atylosia* species, *A. sericea* is closer to *A. scarabaeoides* than to *A. lineata.*

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