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Interspecific hybridization between *Cajanus cajan* and *Cajanus cajanifolius*

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ABSTRACT - *Interspecific hybridization between five* Canajus cajan *genotypes* viz.*, ICPL 87, CORG 9302, CORG 5, TAT 93-47 and AS 46 and the wild species* C. cajanifolius *was attempted. Successful pod set was observed in direct crosses involving* C. cajan *as ovule parent and* C. cajanifolius *as pollen parent while the reciprocal crosses failed to set pods. The only surviving* F_1 *of ICPL 87 ×* C. cajanifolius *was vigorous in growth and exhibited intermediate leaf and flower morphology with predominance of* C. cajanifolius *plant type. Chromosome pairing was rather irregular in the* F_1 *hybrid with loose pairing, univalents, quadrivalents and abnormalities like laggards, stickiness, bridges, precocious separation and non-synchronization of the genomes. Pollen fertility of the hybrid was low and occurrence of triads was noticed. The partial homology coupled with seed set suggested the possibility of incorporating traits of economic importance such as pest resistance from* C. cajanifolius *into* C. cajan.

Key words: Interspecific hybridization, *C. cajan*, *C. cajanifolius*, crossability, cytology.

INTRODUCTION

Pigeonpea (*Cajanus cajan* (L.) Millsp.) (2n=22) is one of the major grain legume (pulse) crops of the tropics and subtropics. It belongs to the family Leguminosae, subfamily Papilionidae, tribe Phaseolae and subtribe Cajaninae. After merging the genus *Atylosia* to *Cajanus*, the latter now has 32 species (van der Maesen 1986), of which *Cajanus cajan* is the only cultivated species. It is grown for its protein- rich seeds since ancient days (De 1974). This crop is endowed with a rich germplasm base. According to van der Maesen (1990), the gene pool of pigeonpea is classified into three groups. The primary gene pool comprises cultivar collections, the secondary gene pool consists of closely related wild species and the tertiary gene pool holds wild species which are not crossable with pigeonpea.

Over the last few years, the yield potential of pigeonpea remained stagnant and there appears to be a limit to the seed yield *per se*. The restricted yield could be attributed to problems such as flower drop, disease and insect susceptibility and photosensitivity of the available cultivated germplasm. This could be overcome through introgression of useful characteristics from related wild forms into cultivated *C. cajan* by wide hybridization.

Wide hybridization is a classical method of expanding genetic variability. Since *C. cajan* is the only cultivated species (Reddy 1981), it is reasonable to seek additional variation in the wild and related species of genus *Cajanus*. Some gene transfer from wild relatives to the cultivated germplasm has been executed with at least 11 wild species that were successfully crossed

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with *C. cajan* (Pundir and Singh 1985a, Dundas et al. 1989, Kumar et al. 1990, Mallikarjuna and Moss 1995). Attempts to transfer high protein content to the cultivated type from *C. albicans*, *C. sericeus* and *C. scarabaeoides* have been reported (ICRISAT 1987). Among the 31 wild species reported in the genus *Cajanus*, *C. cajanifolius* has been mentioned as a valuable resistance source against podfly (Sithanathan et al. 1981) and *Alternaria* leaf spot disease (Singh et al. 1984), with a protein content of more than 30% (Singh and Jambunathan 1980). Though intercrossable, the crossability of *Cajanus* with pigeonpea is restricted in view of the problems of developing successful hybrids, of the varying degree of compatibility and of hybrid sterility.

The extent of crossability depends largely on the sporophytic compatibility supporting successful fertilization and the gametophytic compatibility as expressed in karyomorphology and chromosome pairing during meiosis. The information may also help to understand the phylogenetic relationship of different species and the probable mechanisms involved in the evolution of cultivated species (Reddy and De 1983). This study aimed to investigate the crossability of *Cajanus cajan* with *C. cajanifolius* and discuss the morphological and cytological performance of the F_1 hybrid.

MATERIAL AND METHODS

Seeds of the wild species *C. cajanifolius* (2n=22) and five cultivars of *C. cajan* (2n=22) *viz*. (ICPL 87, CORG 9302, CORG 5, TAT 93-47 and AS 46) were sown in a screen house of the Department of Pulses, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. Crosses were made both in direct and reciprocal directions. The crossability was determined as percentage of pod set. Normal crosses that produced interspecific hybrids were used for cytomorphological studies.

For meiotic observations, flower buds (1 to 2 mm) were fixed in modified Carnoy's fluid (ethanol, chloroform and glacial acetic acid, 6: 3: 2) between 8.30 A.M and 9.30 A.M. The plant material was maintained in the fixative for 24 hours, washed and preserved in 70% ethanol. The anthers were squashed in 2% acetocarmine and the slides observed under light microscope. The chromosomal associations at meiosis were studied for the hybrid ICPL 87x *C. cajanifolius* and its respective parents. Pollen mother cells (PMCs) at diakinesis and metaphase I were examined to obtain the frequencies of univalents, bivalents and multivalents. PMCs at later stages were analyzed for evidence of chromosomal abnormalities. For each stage, about 150 PMCs were scored.

Pollen fertility was determined by staining fresh pollen in a drop of Alexander's stain (Alexander 1969). Fertile pollen grains were stained pink while sterile grains lacking functional cytoplasm appeared greenish. Readings were taken from five microscopic fields and replicated three times. Pollen fertility was estimated on the basis of percentage of stainable pollen.

RESULTS AND DISCUSSION

Crossability and hybrid survival

Successful pod set was obtained in direct crosses involving *C. cajan* as ovule parent and *C. cajanifolius* as pollen parent. However, the reciprocal crosses using the parents failed to set pods. From 528 *C. cajan* buds pollinated with *C. cajanifolius* pollen, 4 F_1 plants were obtained of which only one survived until maturity. The percentage of pod set in the *C. cajan* x *C. cajanifolius* cross ranged from 0 to 8.4% and two out of five crosses of this combination did not produce F_1 hybrids (Table 1). This finding confirms earlier reports of Dundas et al. (1989) and Kumar et al. (1990). However, Pundir and Singh (1985a) obtained successful crosses between *C. cajan* and *C. cajanifolius* using the latter as female parent. This unidirectional success might be due to cytoplasmic incompatibility with the nuclear genome of the respective male parent species that restricted hybridization in one direction only (Pundir and Singh 1985b).

Though only five *C. cajan* genotypes were used in the present investigation, a marked difference between them was observed in their crossability with *C. cajanifolius*. These results clearly demonstrate that the success of the interspecific crosses depends not only on the species and the direction of the cross but also on the genotypes of the species involved in the hybridization. Seed setting in interspecific and intergeneric crosses is a product of the interaction between genotypes used in the hybridization

Table 1. Crossability relationship between *C. cajan* and *C. cajanifolius*

Cross combinations	Number of buds pollinated	Number of pods set	Percentage of pod set	Number of seeds sown	Number of surviving hybrid plants
Direct					
ICPL 87 x *C. cajanifolius*	49	3	6.1	2	1
CORG 5 x *C. cajanifolius*	85	5	5.9	3	-
CORG9302 x *C. cajanifolius*	149	-	-	-	-
TAT 93-47 x *C. cajanifolius*	78	-	-	-	-
AS 46 x *C. cajanifolius*	167	14	8.4	11	-
Total	528	22	-		
Mean	105.6	4.4	4.1		
Reciprocal					
C. cajanifolius x *C. cajan*	365	-	-		

(Bozorgipour and Snape 1990). This explains the wide range of variation amongst *C. cajan* genotypes with respect to their ability to set seeds in the interspecific crosses. Further studies on crossability involving a large number of genotypes are therefore needed which can yield hybrids by chance recombination between appropriate crossability genes.

In this study, out of four F_1 hybrids obtained, only one hybrid ICPL 87 x *C. cajanifolius* survived and grew to maturity. The hybrid inviability may be attributed to the genomic imbalance or cytoplasmic incompatibility between two parental species. Hybrid weakness can be overcome by embryo rescue *in vivo* or embryo implantation *in vitro* and, regarding lethality, reciprocal crosses or hybrid grafting can be attempted to obtain successful interspecific hybrids. Khush and Brar (1992) elaborately described post-zygotic barriers in terms of hybrid inviability and hybrid lethality in interspecific crosses.

Morphology of parents and F_1 hybrid

A detailed comparative account of the morphological features of *C. cajan* cv. ICPL 87, *C. cajanifolius* and their F_1 hybrid revealed that the parents differed in some of the morphological characters, especially in pod hairiness and the presence of strophile (Table 2). ICPL 87 has hairy pods and no strophile while the wild species *C. cajanifolius* has glabrous pods and seeds with strophile. The F_1 hybrid was vigorous in growth, intermediate in height, profusely branched and abundantly flowered (Figure 1). For most of the flower, pod and seed characters the hybrid showed intermediate nature with predominance of the characters of *C. cajanifolius* (Figures 2 and 3). Some traits such as oblique leaflet base, green petiole, yellow standard petal, glabrous pods, color of the immature pod, light gray seed color, presence of strophile, and prominent beak in the pod were *C. cajanifolius* traits. Characteristics of the male parent expressed in the F_1 hybrid progeny (either dominant or incomplete dominant) could be useful genetic markers to verify the authenticity of hybrids. A few characteristics like shape of first leaflet pair, petiole length, size of the standard petal, pod length and number of seeds per pod were intermediate between the two parents. The occurrence of characteristics from both species in the hybrid indicated that the F_1 had a gene combination of both parents. These observations are in conformity with those reported by Mohanty and Patnaik (1989) for most characters and Reddy et al. (1980) regarding the dominance relationship with respect to the traits seeds with strophile versus seeds without strophile. Occasionally, seed set was achieved by open pollination in the hybrid plant, while no seed set was obtained with self-pollination. The F_1 plant was found to be free of podfly and *Alternaria* leaf spot disease under field conditions although no artificial screening was done.

Cytology of parents and F_1 hybrid

The chromosome associations observed in PMCs of F_1 hybrid ICPL 87 x *C. cajanifolius* are presented in Table 3 and some representative cells are shown in Figures 5-13. In the *Cajanus* species, it is difficult to produce excellent chromosomal preparations due to the

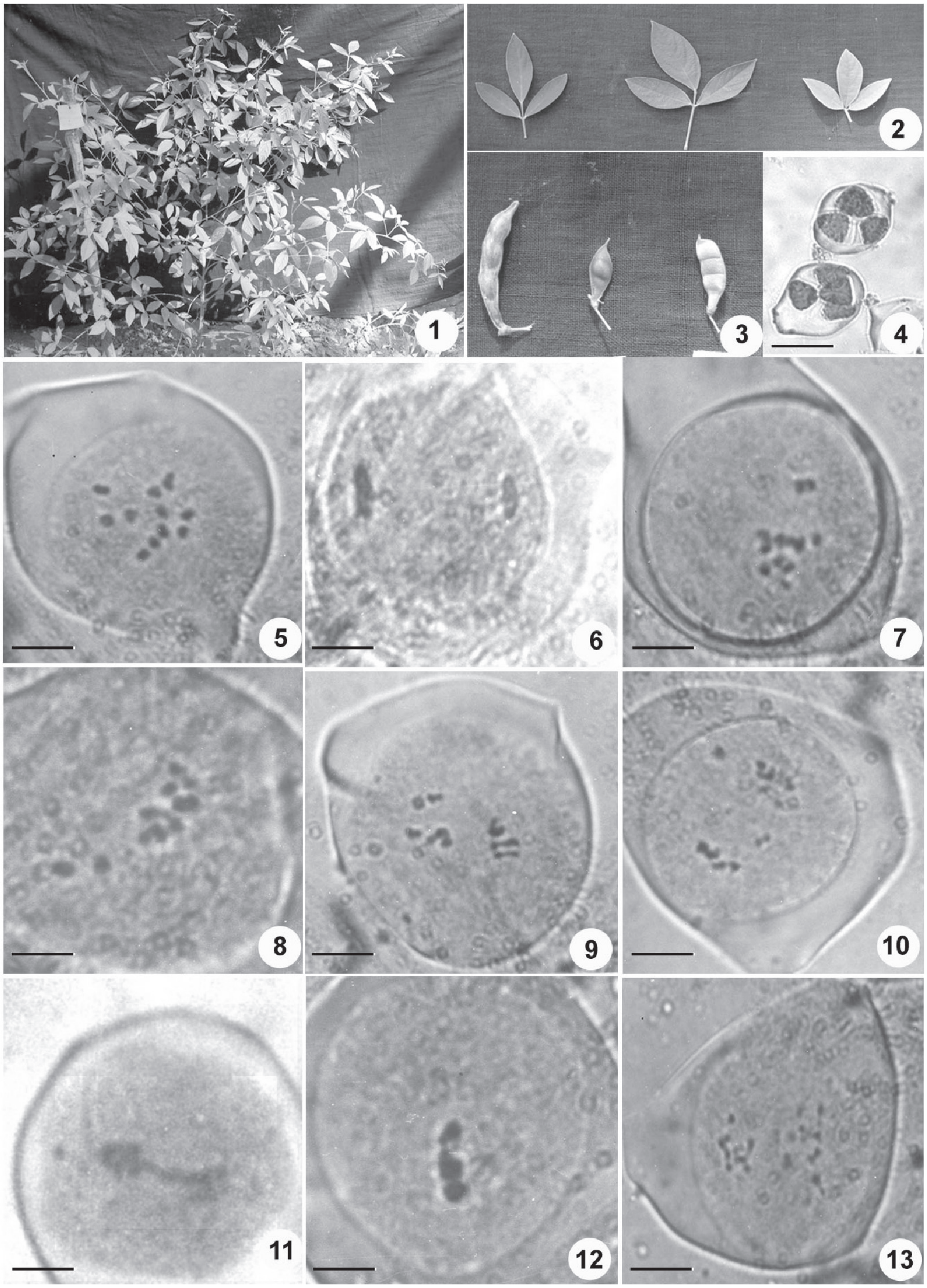
Table 2. Morphological comparison of *C. cajan*, *C. cajanifolius* and F_1 hybrid

Characters	*C. cajan* cv. ICPL 87	F_1 hybrid	*C. cajanifolius*
1. Shape of first pair of simple leaves	Lanceolate	Ovate lanceolate	Ovate
2. Leaf lets			
a) Shape	Lanceolate	Ovate lanceolate with oblique base	Ovate with oblique base
b) Length (cm)	5.90	6.82	2.92
c) Breadth (cm)	1.90	2.86	1.12
d) Venation	Palmately reticulate	Palmately reticulate	Palmately reticulate
3. Petiole length (cm)	2.02	3.70	2.28
4. Petiole color	Greenish white	Green	Green
5. Days to flower initiation	61	91	86
6. Size of the standard petal (cm)	1.60 × 1.40	1.50 × 1.40	1.30 × 1.10
7. Color of the standard petal	Yellow without streaks	Yellow with medium streaks	Light yellow with dense streaks
8. Pod length (cm)	5.18	2.40	2.80
9. Pod color (immature)	Mixed (green/purple)	Purple	Purple
10. Pod color (mature)	Straw	Straw	Straw
11. Hairs on mature pods	Present	Absent	Absent
12. No. of seeds per pod	3.20	2.31	4.13
13. Seed color	Reddish brown	Light gray	Light gray
14. Strophiole	Absent	Present	Present
15. Beak of the pod	Less prominent	More prominent	Prominent

small chromosome size and some of the chromosomal configurations are difficult to visualize. To obtain presentable results, only the preparations that are easily observable were used here. The results showed normal chromosome pairing in parents with regular bivalent (n=11) formation at diakinesis (Figure 5), normal orientation in metaphase I and equal separation of chromosomes in anaphase I (Figure 6) with no detectable chromosomal abnormalities. In contrast, pairing was not normal in the hybrid during diakinesis. About 50.6% of 150 PMCs analyzed exhibited the formation of both univalents and bivalents (Figure 7). The univalent, bivalent and quadrivalent configurations and their frequencies are shown in Table 3. The formation of univalents may be due to the absence of pairing between homologous chromosomes or might have occurred due to an early disjunction in partially homologous chromosomes.

During diakinesis and metaphase, heteromorphism was quite frequently observed in two bivalents (Figure 8). The occurrence of frequent heretomorphism in two bivalents indicated lacking homology in the chromosomes of the two species (Reddy and De 1983). At metaphase I, precocious separation of bivalents (Figure 9) was observed in most cells (98 out of 150 analyzed) followed by formation of laggards (22.7%) (Figure 10). Non-synchronization of genomes of two species at different meiosis stages was observed in 7.4% of the cells. This may be due to the failure of bivalents to congress because of repulsion of some of the chromosomes on a metaphase plate which led to reduced chromosome pairing in the interspecific hybrid. Similar meiotic behavior had been reported in plants such as Triticinae (Riley and Chapman 1957) and Brassica (Olsson and Hagberg 1955). Bridge formation was found in 4.6% of the cells (Figure 11). Besides these chromosomal abnormalities, chromosome stickiness at metaphase I was also noted in a few of the cells (Figure 12).

At anaphase I, the separation was normal (11/11) in about 74.7% of the PMCs studied while 22.7% showed unequal separation (10/2/10) due to laggards in the metaphase plate (Table 3; Figure 13). Unequal chromosome separation might be a physiological



Figures 1-13. Morphological characteristics and meiotic behavior in the hybrid *C. cajan* cv. ICPL 87 x *C. cajanifolius* and its parents. 1) F_1 plant. 2) Leaf morphology, left to right *C. cajan* cv. ICPL 87, F_1 hybrid, *C. cajanifolius*. 3) Pod characters, left to right *C. cajan* cv. ICPL 87, F_1 hybrid, *C. cajanifolius*. 4) Triads and tetrads in the F_1 hybrid. 5) *C. cajan* cv. ICPL 87, diakinesis (11_{II}) (2n=22). 6) *C. cajanifolius*, anaphase I (11-11 separation). 7) Hybrid, metaphase I with univalents and bivalents. 8) Hybrid, metaphase I with heteromorphism in two bivalents. 9) Hybrid, metaphase I with precocious movement of bivalents. 10) Hybrid, anaphase I presenting laggards. 11) Hybrid, anaphase I with a chromatin bridge. 12) Hybrid, metaphase I presenting chromosome stickiness. 13) Hybrid, anaphase I with unequal chromosome separation Scale bar:1 bar =10 µm

Table 3. Chromosomal behavior at different stages of meiosis and sporad count in the F_1 hybrid *C. cajan* cv. ICPL 87 x *C. cajanifolius*

Chromosome associations	PMCs observed	
	No. of cells	Percentage
Diakinesis		
Univalents (I)	14	9.4
Bivalents (II)	39	26.0
I + II	76	50.6
Quadrivalents (IV)	21	14.0
Total	150	100.0
Metaphase I		
Precocious separation of bivalents	98	65.3
Bridges	7	4.6
Laggards	34	22.7
Non-synchronization of genomes at different stages	11	7.4
Total	150	100.0
Anaphase I		
11/11 distribution	112	74.7
10/12 distribution	4	2.6
10/2/10 distribution	34	22.7
Total	150	100.0
Sporad count		
Dyads	78	53.1
Triads	25	17.0
Tetrads	44	29.9
Total	147	100.0

phenomenon caused by disturbances in the cytochemical balance reaction (Rao and Lakshmi 1980). The lagging chromosomes are probably the univalents because their frequencies are comparable with each other. Though regular 11-11 separation is seen in most of the cells at anaphase I, a certain frequency of bridge formation was encountered which may be due to cryptic structural alterations as a consequence of crossing over with paracentric inversion (Mohanty and Patnaik 1989). When the frequency of bridges is near only 5%, this may be result of chromosome anomalies too difficult to explain, rather than an influence of structural hybridity (Singh and Roy 1986).

Studies on sporad formation revealed the failure of one of the meiotic divisions and conspicuous formation of triads in the F_1 hybrid (Figure 4). Frequencies of PMCs containing less than 4 microspores are presented in Table 3. The formation of triads in the F_1 hybrid confirmed the fact that, unlike in the parents, abnormal meiotic events occurred in the hybrid. The triad formation could also have resulted from a lack of second division in a dyad cell (Stuckey and Banfield 1946).

The pollen fertility of F_1 hybrid was found to be low (44.4%). This might have been caused by the meiotic irregularities since pollen fertility has been found to be directly related to the chromosome associations (Stebbins 1966).

The result of this investigation indicated that a certain degree of homology is maintained between *C. cajanifolius* and *C. cajan* chromosomes, which is further supported by their ready crossability and the production of fertile hybrid. The easy crossability and pairing behavior of chromosomes indicate close relations of pigeonpea with *C. cajanifolius,* which is considered a progenitor of *C. cajan* (Pundir and Singh 1985a). The present study broadened the understanding of cytogenetic affinities between cultivated and wild species of *Cajanus* which may be explored for transfer of desirable traits from the wild relatives to the cultivated plant. The partial homology in parental genomes combined with successful interspecific hybridization may be useful in transferring economically useful traits from *C. cajanifolius* to *C. cajan*.

Hibridação interespecífica entre *Cajanus cajan* e *Cajanus cajanifolius*

RESUMO – *A hibridação interespecífica entre cinco genótipos de* Canajus cajan - *ICPL 87, CORG 9302, CORG 5, TAT 93-47 e AS 46 – e a espécie silvestre* C. cajanifolius *foi tentada. Sucesso na frutificação foi observado nos cruzamentos diretos envolvendo* C. cajan *como genitor feminino e* C. cajanifolius *como genitor masculino, enquanto os cruzamentos recíprocos falharam. O único híbrido* F_1 *sobrevivente de ICPL 87 ×* C. cajanifolius *se mostrou crescimento vigoroso e exibiu morfologia de folhas e flores intermediária, com predomínio do tipo de planta de* C. cajanifolius. *Pareamento cromossômico no híbrido* F_1 *foi bastante irregular, com perda de pareamento, univalentes, quadrivalentes e outras anormalidades, separação precoce e não sincronização dos genomas. A fertilidade do pólen do híbrido foi baixa, com ocorrência de tríades. A homologia parcial e a produção de sementes sugerem a possibilidade de incorporar caracteres de importância como resistência a pragas de* C. cajanifolius *em* C. cajan.

Palavras-chave: Hibridação interespecífica, *C. cajan*, *C. cajanifolius*, cruzamento, citologia.

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