Euphytica **121:** 93–98, 2001.

Production and characterization of hybrids between *Cajanus cajan* × *C. reticulatus* var. *grandifolius*

L.J. Reddy, N. Kameswara Rao & K.B. Saxena
ICRISAT, Patancheru, Andhra Pradesh – 502 324, India

Received 24 December 1999; accepted 25 October 2000

Key words: crossabilty, cytology, interspecific hybridization, morphology, pigeonpea

Summary

Cajanus reticulatus var. *grandifolius*, endemic to Australia and a wild relative of the cultivated species, *C. cajan,* was successfully crossed with the latter as the female parent. The major wild species characters such as persistent stipules, long pod hairs, pod shattering, brown seeds with grey speckles, and presence of seed strophiole were dominant in the hybrid. For growth and branching habit, and leaflet, flower, pod, and seed size, the hybrid was intermediate between the parents. The meiotic cells of the hybrid were found to have quadrivalents, trivalents, univalents, and showed reduced chromosome pairing as revealed by the increased number of rod bivalents per cell at metaphase-I, and stickiness and precocious movement of chromosomes to poles in the second division. In comparison to the parents, the hybrid had fewer pods and seeds. However, these anomalies in the interspecific hybrid are not significant enough to preclude the gene transfer from *C. reticulatus* to the cultivated species through a sexual route.

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is a major legume crop grown for its multiple uses (dietary protein, firewood, fodder, building materials and medicine) in Asia, southern and eastern Africa, Latin America, and the Caribbean. Although a wide diversity occurs among pigeonpea land races, some of the economically most important traits such as resistance to pod borer (*Helicoverpa armigera)* are not available in the cultivated genepool, and hence they need to be identified in the related wild species for utilization in crop improvement. Wild *Cajanus* species of Indian origin have been extensively utilized in pigeonpea improvement (Saxena et al., 1998; Reddy et al., 1997; Ariyanayagam et al., 1995; Reddy, 1990; Saxena et al., 1990; Reddy & Faris, 1981). However, there is little information on the crossability and usefulness of the wild relatives of Australian origin.

C. reticulatus var. *grandifolius* (= *Atylosia grandifolia*) is a wild relative of pigeonpea endemic to Australia. It has been reported to be resistant to the pod borer (Dodia et al., 1996), as well as being fire tolerant (Akinola et al., 1975). This Australian species was successfully crossed with pigeonpea at ICRISAT center, Patancheru. This paper describes the morphology and cytology of this interspecific hybrid.

Materials and methods

The parents used in the study are a short-duration improved pigeonpea cultivar, Pant A 2 and a wild relative of Australian origin, *C. reticulatus* (Dryander) F.v. Muell. Var. *grandifolius* (F.v. Muell) van der Maesen [*Atylosia grandifolia* (F.v.Muell.) Benth]. The hybrid was produced by using the pigeonpea cultivar (var. Pant A 2) as the female parent (P1) and *C. reticulatus* as a male parent (P 2). For morphological studies, 10 plants each of P1 and P2, and five hybrid plants were used. Pod setting was recorded on about 180 flowers tagged on the main stem and primary branches of all the available plants of parents and hybrid. Seed setting was estimated from about 100 pods collected from all the plants. The smooth and plump seeds without wrinkles were considered as normal and

Table 1. Morphological characters of *Cajanus cajan* (cv. Pant A2), *C. reticulatus* var. *grandifolius*, and their hybrid

Character	*C. cajan* (cv. Pant A 2)	*C. reticulatus* var. *grandifolius* [1]	F_1 hybrid
Growth habit	Erect shrub	Semi-spreading shrub	Intermediate
Plant type	Indeterminate	Indeterminate	Indeterminate
Branching	Narrow-angled	Wide-angled	Intermediate
Leaflets:			
Shape	Lanceolate	Elliptic-rhomboid	Intermediate
Length (cm)	4.70 (± 1.52)[2]	8.5 (± 1.75)	6.58 cm (± 0.73)
Breadth (cm)	1.90 (+ 0.34)	5.21 (± 0.17)	3.15 (± 0.13)
Venation	Palmately reticulate	Palmately reticulate	Palmately reticulate
Petiole length (cm)	2.8 (± 0.41)	5.38 (± 0.37)	4.33 (± 0.26)
Stipules	Fugacious	Persistent	Persistent
Duration of flowering	Sep-Jan	Feb-Mar	Dec-April
Days from sowing to the first flush of flowers	79 (± 4.2)	Not recorded[3]	149 (± 3.7)
Standard petal (L× B) (cm)	1.51 × 1.26	2.62 × 1.78	2.15 × 1.65
Petals	Deciduous	Mostly persistent	Deciduous
Pod length	5.2 (± 0.23)	1.8 ± 0.08	3.9 (± 0.07)
Pod hairs	Short	Long	Long
Pod beak	Prominent	Minute	Intermediate
Pod shattering	Non-shattering	Shattering	Shattering
Locules per pod	3.8 (± 0.03)	3.0 (± 0.02)	2.3 (± 0.07)
Seed color	Brown	Brown with grey speckles	Brown with grey speckles
Strophiole	Absent	Present	Present
100-seed mass (g)	7.4 (± 1.21)	2.6 (± 0.34)	4.7 (± 0.43)
Seed protein (%)	24	33	27

[1] Recorded from plants maintained in the botanical garden as perennials.
[2] Figures in parentheses refer to standard errors.
[3] Not recorded as *C. reticulatus* was maintained as a perennial.

the shrunken seeds with wrinkles as immature. For cytological study in the hybrid, flower buds were collected periodically and 86 pollen mother cells (PMCs) examined. For comparative cytological study of parents and hybrid, flower buds developed on the same day were used and 30 PMCs examined. The collected flower buds were fixed in propionic acid and ethanol mixture (1: 3), with the acid component saturated with ferric acetate. The flower buds were kept overnight in the fixative, and the anthers were stained in 1% propiono carmine. The microphotographs were taken at uniform magnification of × 2100 from the temporary preparations. Pollen viability was determined from 20 flowers collected for each of the parents and hybrid, and by staining the pollen grains by propiono carmine.

Results and discussion

Crossability

From the 450 pollinations, only nine mature pods were obtained. Ninety two percent of flowers dropped within four days after pollination and 6% of the developing young pods either dropped or contained only aborted ovules. From the mature pods seven fully developed and 11 immature shriveled seeds were obtained which produced only five fully developed F_1 plants.

Morphology

A comparative account of the morphological features of the parents and the hybrid is given in Table 1. The hybrid was closer to its male parent in its gross morphology expressing several dominant characters of

Table 2. Chromosome associations at diakinesis in the F_1 hybrid of *C. cajan* × *C. reticulatus* var. *grandifolius* (Total cells scored: 86)

Chromosome associations				No. of cells
IV	III	II	I	
4	–	3	–	1 (1.2)[1]
3	–	5	–	4 (4.7)
2	–	7	–	11(12.8)
1	–	9	–	17(19.7)
1	–	8	2	5 (5.8)
1	1	7	1	1 (1.2)
–	2	7	2	1 (1.2)
–	1	9	1	1 (1.2)
–	–	11	–	36 (41.9)
–	–	10	2	9 (10.4)
–				

[1] Figures in parentheses are% of cells for each association.

C. reticulatus i.e. persistent stipules, long pod hairs, shattering nature of the mature pods, brown seeds with grey speckles, and presence of strophiole. These observations confirmed the hybrid nature of plants. Branching habit, leaf shape and size, petiole length, size of the standard petals, pod length and its beak (Figure 1), 100-seed mass, and seed protein content were intermediate in the hybrid, compared to its parents.

Pollen fertility

Pollen fertility in the hybrid ranged from 26 to 58% (mean, 42.6%). In pigeonpea cultivar (Pant A-2) it ranged from 93 to 97% (mean, 95.7%), and in *C. reticulatus* from 90 to 95% (mean, 92.2%).

Pod setting

Pod setting on the hybrid plants ranged from 17 to 29% (mean, 1.3%), compared with 52 to 67% (mean, 61.0%) in pigeonpea and 38 to 47% (mean, 40.4%) in *C. reticulatus*.

Seed setting

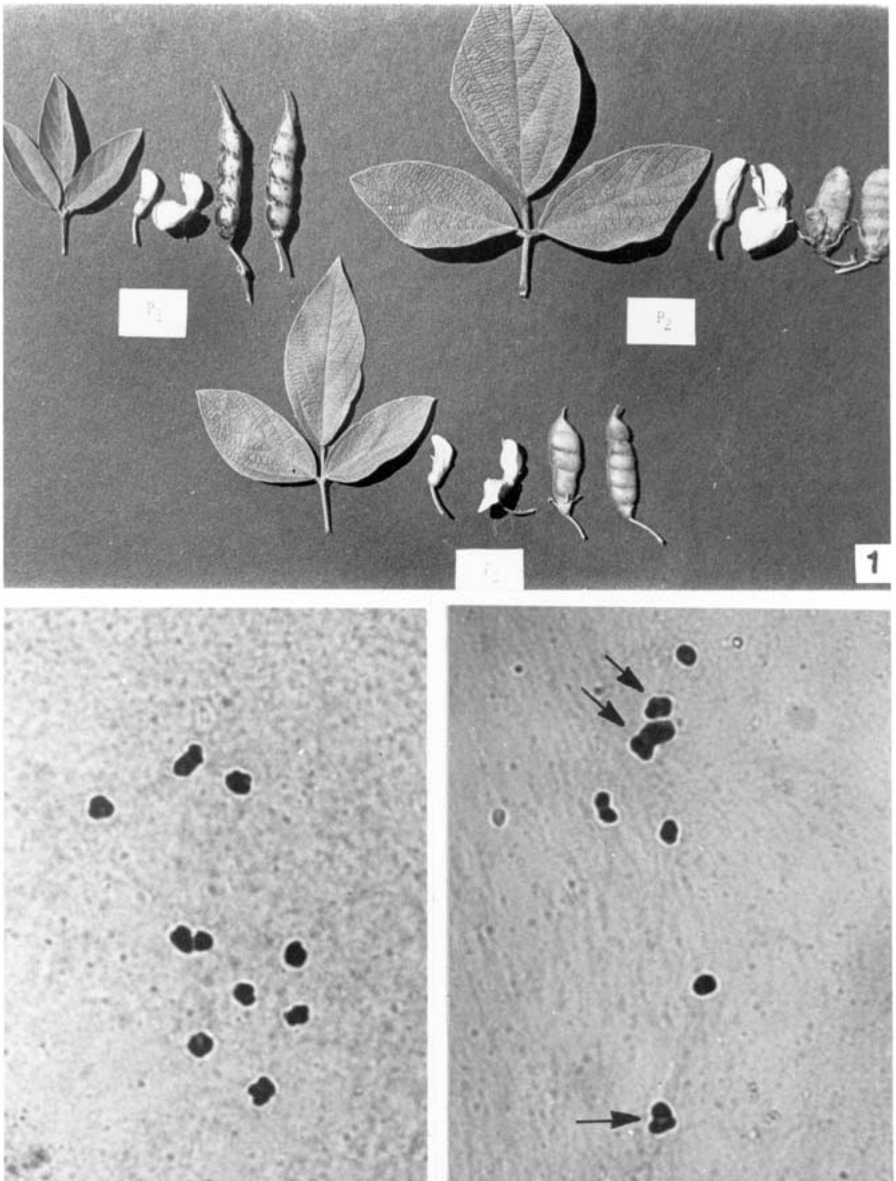
In the pigeonpea parent 85.3% of the pod locules were filled with seed (of which 5.9% immature) and in the *C. reticulatus* parent 76.7% of the pod locules contained seed (of which 15.2% immature). In the hybrid 57.1% of the locules were filled with seed, of which 22.3% were immature.

Cytology

Both the parents exhibited normal chromosome pairing at pachytene stage and during diakinesis and metaphase-I. Eleven bivalents were always observed with predominance of the ring bivalents and rare occurrence of rod bivalents. Anaphase-I showed regular separation of chromosomes with equal number of chromosomes to each pole. The meiocytes in the hybrid at pachytene stage showed different degrees of synapsis. While, some chromosomes showed almost normal pairing, most of them showed both interstitial and terminal unpaired segments. In the hybrid various chromosome associations were noticed at diakinesis and metaphase-I (Figures 2 to 4); Only 41.9% of cells showed normal 11 bivalents. Quadrivalents, trivalents, and univalents were observed in 45.4%, 3.5%, and 12.8% of cells, respectively. The various combinations of quadrivalents, trivalents, bivalents and univalents in each cell and their frequencies are given in Table 2. Of the different combinations, cells with one quadrivalent and nine bivalents were more frequent. A comparison of different types of bivalents occuring at diakinesis and number of chaismata per cell in the parents and the hybrid is given Table 3. In both the parents, ring bivalents were prevalent, whereas rod bivalents were predominant in the hybrid. While, no higher chromosome associations were noticed in the parents, such associations occurred in the hybrid frequently. The chiasmata per cell were lower in the hybrid, compared to those of the parents.

In the hybrid, two bivalents exhibited frequent heteromorphism (Figure 5). At anaphase-I, unequal distribution of the chromosomes to the poles in 6.9% cells and laggards in 10.3% of cells were observed. In the second division, occasionally stickiness and precocious movement of chromosomes to poles during metaphase-II (Figure 6) and presence of micronuclei at telophase-II were noticed.

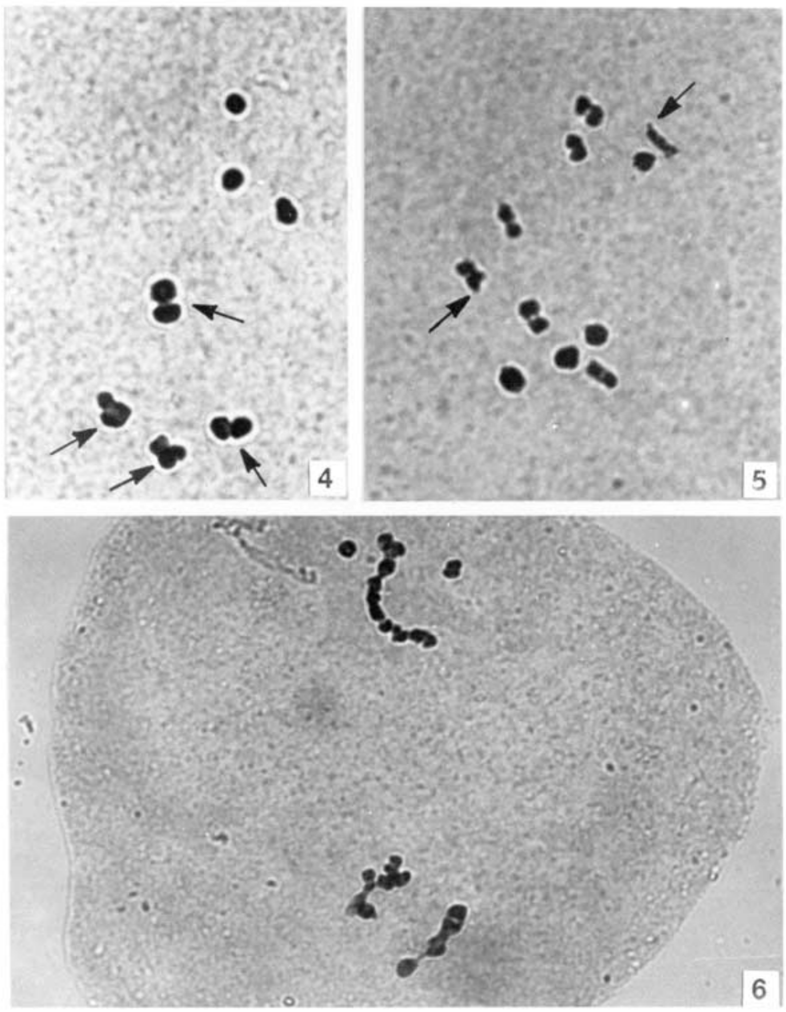
Information on crossability, fertility in F_1s, and the possibility of gene transfer from the related species is essential to decide the course of utilization of alien species in crop improvement programs. Although such information is available on some of the wild *Cajanus* species of Indian origin, it is not available for wild *Cajanus* species of Australian origin. *C. reticulatus*, a species endemic to Australia, has been recently reported (Dodia et al., 1996) to possess antibiosis against the pod borer, the most important constraint in pigeonpea production, for which resistance is not available in the cultivated genepool. In the present study, *C. re-*



Figures 1–3. Figure 1. Leaves, flowers, and pods of *C. cajan* (left), *C. reticulatus* var. *grandifolius,* (right) and their hybrid (bottom). Meiocytes of hybrid (× 2100); *Figure 2.* A cell showing 2 univalents (arrows) and 10 bivalents. *Figure 3.* A cell with 3 quadrivalents (arrows) and 5 bivalents.

Table 3. Frequency of ring and rod bivalents and chiasmata per cell at metaphase-1 of parents and hybrid of *C. cajan* (cv. Pant A-2) × *C. reticulatus* var. *grandifolius* (No. of cells scored: 30)

Description	*C. cajan*		*C. reticulatus*		F_1 Hybrid	
Description	Range	Mean	Range	Mean	Range	Mean
Higher associations	Nil	Nil	Nil	Nil	0–3	0.8
Ring bivalents	9–11	9.8	8–11	9.1	3–10	6.9
Rod bivalents	0–2	0.6	0.3	0.8	1–8	2.9
Univalents	Nil	Nil	Nil	Nil	0–2	0.4
Chiasmata	18–22	21.3	17–22	20.9	14–22	18.3



Figures 4–6. Meiocytes of hybrid (× 2100); *Figure 4.* A cell showing 4 quadrivalents (arrows) and 3 bivalents. *Figure 5.* A cell showing 2 heteromorphic bivalents (arrows). *Figure 6.* A cell in second division showing stickiness of chromosomes and precocious movement of chromosomes to poles.

ticulatus was successfully crossed with the cultivated pigeonpea and fertile hybrids were produced. In the hybrid, the dominant nature of the wild species traits such as persistent stipules, seed color, and presence of seed strophiole was evident. The extent of pollen viability, pod setting, and seed formation in the hybrid was relatively less compared to those in the parents.

Genetic recombination in the hybrids is best visualized during meiosis at metaphase-I by chiasmata that hold the separating chromosomes momentarily together and represent the cross over points. The ring bivalents result when chiasmata occur on both the arms, while rod bivalents result when chiasmata occur only on one arm. The *C. cajan* × *C. reticulatus* hybrid in the present study showed both ring and rod bivalents, but rod bivalents were more frequent compared to those of both the parents indicating some restriction of recombination in the hybrid. Similar observations on the nature of bivalents were made in the hybrids involving pigeonpea and wild relatives of Indian origin (Reddy & De, 1983; Pundir & Singh, 1985). The occurrence of multivalents in the hybrid indicates the presence of duplicate segments on the chromosomes or heterozygous translocations. Due to these cytological abnormalities during meiosis, the fertility in the F_1 hybrid was lower resulting in the decreased pod set and increased immature seeds, compared to those in the parents. In the hybrid, there appeared to be no parity between the relatively higher percent of pollen fertility observed from pollen stainability and higher frequency of quadrivalents observed at diakinesis. The higher frequency of quadrivalents observed in the present material could be due to reasons other than gross chromosomal abnormalities. It is noteworthy, that Reddy (1984) reported in interspecific hybrids of pigeonpea and three wild *Cajanus* species, a phenomenon known as 'star formations' at pachytene due to fusion of two to four bivalents at centromeric region and subsequent formation of secondary associations at diakinesis. These associations may mimic quadrivalent formations.

Considering that *C. reticulatus* could be crossed successfully with pigeonpea and the F_1 during meiosis showed sufficient homology between the parental chromosomes, with adequate pollen fertility, pod and seed setting, it is concluded that this species can be utilized for pigeonpea improvement through gene transfer by sexual means.

References

Akinola, J.O., P.C. Whiteman & E.S. Wallis, 1975. The Agronomy of Pigeonpea (*Cajanus cajan*). Commonwealth Bureau of Pastures and Field crops Review Series 1. pp. 57.

Ariyanayagam, R.P., A. Nageswara Rao & P.P. Zaveri, 1995. Cytoplasmic-genic male sterility in interspecific matings of *Cajanus*. Crop Science 35: 981–985.

Dodia, D.A., A.J. Patel, I.S. Patel, F.K. Dhulia & S.B.S. Tikka, 1996. Antibiotic effect of pigeonpea wild relatives on *Helicoverpa armigera*. Int Chickpea & Pigeonpea Newsl 3: 100–101.

Pundir, R.P.S. & R.B. Singh 1985. Cytogenetics of F_1 hybrids between *Cajanus* and *Atylosia* species and its phylogenetic implications. Theor Appl Genet 71: 216–220.

Reddy, L. J., 1984. Fusion of centromeres and star formations at pachytene of *Cajanus* × *Atylosia* hybrids. Heredity 53: 435–439.

Reddy. L.J., 1990. Pigeonpea: Morphology. In: Y.L. Nene, S.D. Hall & V.K. Sheila (Eds.), Wallingford, Oxen, U.K. C.A.B. International: 47–87.

Reddy, L.J. & D.G. Faris, 1981. A cytoplasmic genetic male sterile in pigeonpea. Int Pigeonpea Newsl 1: 16.

Reddy, L.J. & D.N. De, 1983. Cytomorphological studies in *Cajanus cajan* × *Atylosia lineata*. Ind J Genet & Plant Breed 43: 96–103.

Reddy, L.J., K.B. Saxena, K.C. Jain, U. Singh, J. M. Green, D. Sharma, D.G. Faris, A.N. Rao, R.V. Kumar & Y.L. Nene, 1997. Registration of high protein elite germplasm ICPL 87162. Crop Sci 37: 94.

Saxena, K.B., L.J. Reddy, L. Singh, R.V. Kumar & D.G. Faris 1998. Registration of ICPL 87154, a partially cleistogamous pigeonpea germplasm with low natural out-crossing. Crop Sci 38: 556.