Interspecific Hybridization and Evolutionary Relationships of *Cajanus cajan* (L.) Millspaugh and Four of the Wild Species

P. Balarama Swamy Yadav and V. Padmaja*

Department of Botany, Andhra University, Visakhapatnam-530 003, India

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Summary Genetic upgradation of the cultivated species, *Cajanus cajan* (L.) Millspaugh the protein rich pulse crop of tropics was sought through interspecific hybridization involving wild species of the genus as donors for the desirable genes. F₁ hybrids obtained between *C. cajan* × *C. cajanifolius* and *C. sericeus* × *C. cajan* were characterized. On the other hand, *C. cajan* × *C. platycarpus* and *C. cajan* × *C. scarabaeoides* crosses yielded hybrid pods containing shrivelled, non-viable seed. Based on the data, crossability relationships and phylogenetic affinities among *C. cajan* and the 4 wild species were elucidated.

Key words *Cajanus cajan*, Wild species, Reciprocal crosses, Interspecific hybridization, Phylogeny.

The genus *Cajanus* DC. of Fabaceae comprise of 31 wild species (including *Atylosia*, *Rhynchosia*, *Dunbaria* etc.) (van der Maesen 1990) distributed in different climatic conditions and exhibiting wide range of phenological differences. In spite of substantial genetic improvement in the yield and other agronomical traits in the Pigeonpea cultivars, further advancement is yet to be discerned with respect to resistance to pod borer, Fusarium wilt, Phytophthora blight and abiotic stresses such as salinity, waterlogging, frost and drought. There is an ample scope for genetic upgradation of cultivated species, *Cajanus cajan* through introgression of desirable genes from wild species. *Cajanus platycarpus* possess a number of beneficial traits such as resistance to Phytophthora blight, high protein content, early flowering, low flower drop and photo-insensitivity. *C. scarabaeoides* is an important wild species showing resistance to the pod borer, *Heliothis armigera*. On the other hand, *C. sericeus* possesses the characteristic feature of high pod set in addition to high protein content. *C. cajanifolius* largely resembles the cultivated species and possesses the desirable traits such as hardiness, drought resistance, salinity tolerance and resistance to pod borer.

Success rate of interspecific hybridization in the genus is meagre due to low rate of crossability, hybrid inviability and sterility besides high flower drop, non-synchronous flowering of the cultivated and wild species and cross-incompatibility. F₁ hybrids were reported between *C. cajan* and *Atylosia cajanifolia* Haines (Tripathi *et al.* 1984, Kumar *et al.* 1985, Mohanty and Patnaik 1989) and *C. cajan* and *A. lineata* (Kumar and Thombre 1958, Reddy and De 1983, Kumar *et al.* 1990). The present study primarily aims at obtaining interspecific hybrids between *C. cajan* and 4 wild species for subsequent use in genetic improvement programme besides elucidating crossability relationships and phylogenetic affinities among the species.

Materials and methods

Cajanus cajan (L.) Millspaugh cv. ICPL 93115 and the 4 wild species viz., *C. cajanifolius*, *C.*

* Corresponding author, e-mail: VPadmaja4@rediffmail.com

platycarpus, *C. scarabaeoides* and *C. sericeus*, selected for the hybridization programme were obtained from Genetic Resources Unit, ICRISAT, Hyderabad, India.

In view of variations in the time of flowering of these species, staggered sowings were made, facilitating synchronous flowering period for cultivated as well as wild species. *C. platycarpus* is the only photoinsensitive species and flowers in about 40 d. Batch-wise sowings of *C. platycarpus* were made at intervals of 2 weeks for the availability of flowers continuously during the hybridization programme. Emasculations were made 1 d before opening of the flower buds between 6 to 8 A.M. and 4 to 6 P.M. After emasculation, the flower buds were covered by specially made light weight butter paper bags. Pollinations were carried out on the next day by dusting the pollen of desirable male parent directly on the stigmas of the emasculated flower buds. 100 ppm of Gibberellic acid mixed with 40 ppm of α -Naphthalene acetic acid and 10 ppm of Kinetin was sprayed on the pedicels of emasculated flower buds in order to arrest abscission and minimize flower fall. Pollinations were repeated twice or thrice for ensuring the availability of viable pollen to the receptive stigmas. Crosses were repeated in 2 successive seasons for estimating percentage of crossability based on the formula used by Pundir and Singh (1985).

$$\text{Crossability (\%)} = \frac{\text{No. of crossed pods}}{\text{No. of pollinated flowers}} \times 100$$

Comparison of parents and F_1 hybrid plants was made based on 7 morphological and 19 reproductive features (Table 2). F_2 generation was raised for ascertaining the inheritance pattern of contrasting characters of the 2 parents and for understanding their genetic basis.

Results

The pooled data of the hybridization programme of the 2 seasons involving *C. cajan* and 4 of the wild species indicated feasibility of crosses between *C. cajan* $\times$ *C. cajanifolius*, *C. cajan* $\times$ *C. platycarpus*, *C. cajan* $\times$ *C. scarabaeoides* and *C. cajan* $\times$ *C. sericeus* and their reciprocal crosses (Table 1). However, in the case of *C. cajan* and *C. cajanifolius* out of 530 crosses made only 3.20% showed pod formation out of which apparently normal seed set was found in only 0.94%.

The 4 wild species and the cultivated variety used for the interspecific hybridizations were grown in the same plot with 2' $\times$ 2' spacing between the rows and 3 feet in between the plants by allotting 2 rows for each species. *C. cajan* and *C. sericeus* were in the adjacent rows and were used for emasculation and dusting. Seed set could not be obtained from the reciprocal crosses involving these 2 species in 2 successive seasons. However, seeds collected from field grown *C. sericeus* plants when used for raising seedlings for the next generation showed interesting result. Three seedlings from among a total of about 200 showed morphologically distinct traits. The shape and size of the leaflets was intermediate between the obovate leaflets of *C. sericeus* and the lanceolate leaflets of *C. cajan*.

Crosses between *C. cajan* $\times$ *C. platycarpus* and their reciprocal cross showed pods containing small shrivelled seed at a very low frequency. More or less the same results were obtained in *C. cajan* $\times$ *C. scarabaeoides* and *C. cajan* $\times$ *C. sericeus* used as both male and female parents.

Characteristics of the hybrids

a) *C. cajan* $\times$ *C. cajanifolius* hybrid: Quantitative data relating to a total of 26 characters of the parents and F_1 hybrid showed significant differences with respect to size of the stipules, arrangement of the leaves, leaflet shape and size, branching pattern and plant height (Table 2). Most prominent feature was that of drooping nature of the leaflets after sunset in the hybrid which was associated with *C. cajanifolius* (Fig. 1). Expression of male characters in hybrid confirms hybrid nature of

Table 1. Particulars of hybridization between *Pigeonpea* and its four wild species

S. No.	Parents		No. of pollinated flowers	No. of pollinated flowers retained*				No. of pods set**		Crossability (%)	No. of seeds from F ₁ pods		No. of seeds germinated
	Female	Male		5th	10th	15th	20th	+	++		Filled	Shrivalled	
1.	<i>C. cajan</i>	<i>C. cajanifolius</i>	530	105	50	25	19	12 (2.26)	5 (0.94)	3.20	21	16	21
2.	<i>C. cajan</i>	<i>C. platycarpus</i>	650	50	18	10	4	3 (0.46)	1 (0.15)	0.61	—	2	—
3.	<i>C. cajan</i>	<i>C. scarabaeoides</i>	540	45	13	9	5	3 (0.56)	2 (0.37)	0.93	—	3	—
4.	<i>C. cajan</i>	<i>C. sericeus</i>	510	48	15	14	12	7 (1.37)	3 (0.59)	1.96	—	5	—
5.	<i>C. cajanifolius</i>	<i>C. cajan</i>	550	24	18	8	7	4 (0.73)	2 (0.36)	1.09	—	8	—
6.	<i>C. platycarpus</i>	<i>C. cajan</i>	515	29	21	18	10	4 (0.78)	3 (0.58)	1.36	—	12	—
7.	<i>C. scarabaeoides</i>	<i>C. cajan</i>	520	28	23	13	8	5 (0.96)	3 (0.58)	1.54	—	10	—
8.	<i>C. sericeus</i>	<i>C. cajan</i>	510	32	25	15	13	7 (1.37)	5 (0.98)	2.35	—	6	—

* In days after pollination, + = pods with shrivalled, nonviable seeds, ++ = pods with apparently normal seeds, ** Numbers in parenthesis indicate per cent values.

Table 2. Characteristics of F_1 hybrid between *C. cajan* (♀) and *C. cajanifolius* (♂)

S. No.	Character	<i>C. cajan</i>	<i>C. cajanifolius</i>	F_1 hybrid
1.	Petiole length (cm)	4.1 ± 0.06	4.4 ± 0.19	4.2 ± 0.05
2.	Stipule size (mm)	4.1 ± 0.11	2.1 ± 0.02	3.0 ± 0.13
3.	Arrangement of leaves	Directed towards the stem	Directed away from the stem	Slightly directed towards the stem
4.	Leaflet (middle)			
	Shape	Lanceolate	Elliptic	Elliptic
	Size (L/B) (cm)	5.2/2.1	5.5/2.3	5.1/1.8
5.	Branching	Erect	Spreading	Semi-spreading
6.	Plant height (cm)	175.1 ± 1.57	120.0 ± 0.99	150.0 ± 2.11
7.	Drooping of leaflets	Absent	Present	Semi-drooping
8.	Days for the first flush of flowering	147.4 ± 1.33	173.2 ± 0.32	135.1 ± 1.56
9.	Dorsal side colour of the standard petal	Red	Yellow with purplish black streaks	Intermediate
10.	Standard petal size (L/B) (cm)	1.35 ± 0.34	1.21 ± 0.04	1.29 ± 0.04
11.	Longevity of the fully opened flower (h)	20 ± 0.47	21 ± 0.33	18 ± 0.40
12.	Pollen fertility (%)	96.0 ± 0.26	95.1 ± 0.38	83.0 ± 0.63
13.	Pod colour (immature)	Green with purplish black streaks	Green with two purplish margins	Green with two purplish margins
14.	Pod length (cm)	4.7 ± 0.6	3.9 ± 0.89	4.3 ± 0.06
15.	Beak of the pod	Prominent	Minute	Intermediate
16.	No. of seeds per pod	5.3 ± 0.15	4.1 ± 0.23	4.2 ± 0.20
17.	Pod setting (%)	65.3 ± 0.47	60.9 ± 0.95	45.9 ± 0.80
18.	Nature of mature pods	Non-shattering	Shattering	Semi shattering
19.	Seed coat colour	Reddish brown	Black	Grey
20.	Mottles on seed-coat	Absent	Present	Present
21.	Seed strophiole	Absent	Present	Present
22.	No. of chambers per pod	5.7 ± 0.15	4.3 ± 0.21	4.5 ± 0.17
23.	Seed setting (%)	96.0 ± 0.21	94.6 ± 0.37	79.4 ± 1.27
24.	100 seed weight (g)	10.01 ± 0.16	5.08 ± 0.29	7.02 ± 0.07
25.	Seed germination (%)	94.8 ± 0.27	91.0 ± 0.32	53.2 ± 0.41
26.	Plant survival (%)	89.2 ± 0.41	86.8 ± 0.47	72.2 ± 0.47

± Values indicate standard error.

the plants under study. Early flowering was observed in the F_1 hybrids (135 d after sowing) compared to 147 and 173 d for the female and male parents respectively. Several floral and pod characters like size of the standard petal, longevity of the fully opened flowers, pollen fertility, pod length, number of chambers per pod, number of seeds per pod, seed set percentage, seed size (Fig. 2), 100 seed weight, percentage of germination as well as survival of the hybrids were intermediate between the 2 parental values.

b) *C. sericeus* × *C. cajan* hybrid: The female and male parents i.e. *C. sericeus* and *C. cajan* were morphologically distinct with respect to leaflet size, shape, texture and arrangement of leaves, stipule shape and size, size of the flower and drooping of leaflets after sunset (Table 3). The F_1 hybrid stood intermediate between the male and female parents with respect to some traits while in other cases they resembled the male parent, confirming hybrid nature (Fig. 4). Arrangement of leaves formed the diagnostic feature for this hybrid apart from other reproductive features. In *C. cajan* parent, the angle between the petiole and the stem was about 45° giving the overall appearance of the leaves being directed towards the growing apex. On the other hand, in case of *C. sericeus* the corresponding angle was about 70° giving appearance of being directed away from the stem while in the F_1 , it displayed intermediate nature (Fig. 3). Regarding the drooping of leaflets

Figs. 1–4. 1) Seedlings of a) *C. cajan*, b) F_1 hybrid, c) *C. cajanifolius* showing difference in drooping nature of leaflets after sunset. 2) Seeds of a) *C. cajan*, b) F_1 hybrid, c) *C. cajanifolius*. 3) Twigs of a) *C. sericeus*, b) F_1 hybrid, c) *C. cajan*. 4) Seeds of a) *C. sericeus*, b) F_1 hybrid, c) *C. cajan*.

after sunset, in *C. sericeus* and *C. cajan* parents are drooping and non-drooping respectively, while the F_1 hybrid displayed semi-drooping nature. *C. sericeus* was characterized by smaller flowers compared to *C. cajan*. In the F_1 hybrid, size of the standard petal was intermediate (1.23 cm) between the 2 parents (1.10, 1.35 cm) and resembled the male parent with respect to the red coloured prominent vein colouration. The size and shape of the pad as well as the colour of seed coat were intermediate in the hybrid (Fig. 4).

Discussion

Successful hybrids could be obtained between *C. cajan* and *C. cajanifolius* and between *C. sericeus* and *C. cajan*. In the remaining crosses, hybrid inviability was indicated by the formation of pods containing abortive embryos at different developmental stages. Relatively high pod set (3.20%) of *C. cajan* × *C. cajanifolius* reveal close affinities between these 2 species. On the other hand, non-realization of successful hybrid in the reciprocal cross, *C. cajanifolius* × *C. cajan*, in 550 flowers attempted might be due to low crossability with wild species as female parent.

Out of 650 pollinations attempted between *C. cajan* and *C. platycarpus*, only 1 pod was obtained carrying shrivelled seeds indicating successful germination of pollen of *C. platycarpus* on the stigma of *C. cajan*. However, the probable mechanism that retards the development embryo from the first division of zygote upto the final differentiation termed as hybrid inviability might account for non-realization of viable seed. Stebbins (1958) listed out the causes of hybrid inviability as due to disharmony in the genomes of the 2 parental species, due to disharmonious interaction between the genome of one species and the cytoplasm of the other, and due to inhibition of development of hybrid embryo by the endosperm nourishing the former. Mallikarjuna and Moss (1995) reported pollen-stigma compatibility in crosses between *C. cajan* and *C. platycarpus* and succeeded in rescuing the hybrid embryos by *in vitro* method. The crosses between *C. cajan* and *C. platycarpus* and between *C. cajan* and *C. scarabaeoides* though compatible as indicated by pod development with abortive embryos, furnish evidence for the involvement of post-zygotic factors, which could be overcome by following *in vitro* method of embryo rescue and culture (Balarama Swamy Yadav 1999).

One of the important problems restricting gene exchange between Mendelian populations is

Table 3. Characteristics of F₁ hybrid between *C. sericeus* (♀) and *C. cajan* (♂)

S. No.	Character	<i>C. sericeus</i>	<i>C. cajan</i>	F ₁ hybrid
1.	Shape of the cotyledonary leaves	Ovate	Lanceolate	Ovate
2.	Petiole length (cm)	1.5±0.08	4.1±0.06	2.5±0.07
3.	Stipule shape	Filiform	Lanceolate	Sub filliform
4.	Arrangement of the leaves	Directed away from the stem	Directed towards the stem	Slightly directed towards the stem
5.	Leaflet (middle)			
	Shape	Oblanceolate	Lanceolate	Ovate-lanceolate
	Size (L/B) (cm)	1.5/0.7	5.2/2.1	2.8/0.7
	Surface	White hairy	Thinly silky	White silky
	Tip	Mucronate	Acuminate	Acute
6.	Plant height (cm)	95±0.63	175.1±1.57	120±1.13
7.	Drooping of leaflets	Present	Absent	Semi-drooping
8.	Days for the first flush of flowering	172±0.70	147.4±1.33	140±1.84
9.	Dorsal side colour of the standard petal	Yellow with few veins	Red without veins	Yellow with dense veins
10.	Standard petal size (cm)	1.1±0.03	1.35±0.34	1.23±0.06
11.	Longevity of the fully opened flower (h)	18±0.42	20±0.47	17±0.33
12.	Pollen fertility (%)	95±0.37	96±0.26	81±0.58
13.	Pod colour (immature)	Green without purplish streaks	Green with purplish streaks	Green without purplish streaks
14.	Hairs on dried pods	Present	Absent	Present
15.	Pod length (cm)	1.15±0.04	4.71±0.6	2.31±0.08
16.	Beak of the pod	Minute	Prominent	Intermediate
17.	No. of seeds per pod	1.3±0.48	5.3±0.15	1.9±0.23
18.	Pod setting (%)	72±0.47	65.3±0.47	65±0.07
19.	Nature of mature pods	Shattering	Non-shattering	Semi-shattering
20.	Seed coat colour	Light brown	Reddish brown	Grey
21.	Mottles on seed-coat	Present	Absent	Present
22.	Seed strophiole	Present	Absent	Present
23.	No. of chambers per pod	1.4±0.16	5.7±0.15	2.1±0.18
24.	Seed setting (%)	92±0.47	96±0.21	89±0.73
25.	100 seed weight (g)	3.08±0.06	10.01±0.16	5.72±0.07
26.	Seed germination (%)	89±0.50	94.8±0.27	70±1.07
27.	Plant survival (%)	87±0.59	89.2±0.41	81±0.50

± Values indicate standard error.

that of isolating mechanisms. When 2 such groups which have developed isolation barriers exist side by side, they exploit their environment in different ways, stimulating divergent evolution. Therefore, the development of reproductive isolation is one of the important steps in evolution (Stebbins 1958). Self pollination itself is one of the inbuilt isolating mechanisms. Pigeonpea though predominantly self pollinated, permitted out-crossing to certain extent (Ariyanayagam 1976). Two such natural hybrids between *C. cajan*×*A. lineata* was reported by Deodikar and Thakar (1956). The natural hybrid between *C. sericeus* and *C. cajan* of the present study indicated that the 2 species though allopatric, did not show complete reproductive isolation, suggesting the affinity between the 2 species concerned, which can also be substantiated from 81% and 65% of pollen and ovule fertilities respectively in the F₁ hybrid (Table 3). Successful crosses between these 2 species were also reported by Kumar *et al.* (1966), Reddy *et al.* (1981), Kumar *et al.* (1985).

Natural fertile hybrids between *C. seiceus* and *C. cajan* revealed divergent evolutionary pattern in differentiation of these 2 species from a common ancestor and that the crossability barriers are

probably not fully operative and hence could permit cross pollination between them. The cultivated species *C. cajan* appear to have evolved from the wild species, especially *C. sericeus* and *C. cajanifolius*. The present study is in accordance with the concept of polyphyletic origin of *C. cajan*, as a result of continuous hybridization with more than 1 wild species.

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