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Wide hybridization between *Cajanus cajan* and *Cajanus scareboides*

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Abstract
Interspecific hybridization between seven *Canajus cajan* genotypes *viz*., ICPL 88039, ICPL 11253, ICPL 11256, ICPL 11265, ICPL 11333, ICPL 11300, and ICPL 11306 with the wild species *C. scareboides* were attempted during *kharif* 2018. Successful pod set was observed in direct crosses involving *C. cajan* as ovule parent and *C. scareboides* as pollen parent while the reciprocal crosses were failed to set pods. The only surviving F_1 of ICPL 88039 × *C. scareboides*, ICPL 11253 × *C. scareboides*, ICPL 11306 × *C. scareboides* and ICPL 11300 × *C. scareboides* were vigorous in growth and exhibited variation in leaf, flower, pollen fertility, plant habit, pod colour and seed morphology with predominance of *C. scareboides* plant type. Seed size variation was observed in all crosses. With respect to test weight, cross ICPL 88039 × *C. scareboides* have 4.80 g seed weight while its parents have 2.33 g and 10.10 g, male and female respectively. The partial homology coupled with seed set suggested the possibility of incorporating traits of economic importance such as pest resistance from *C. scareboides* into *C. cajan*.

Keywords: Wide hybridization, *C. cajan*, *C. cajanifolius*, crossability.

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) [24], of which *Cajanus cajan* is the only cultivated species. It is grown for its protein-rich seeds since ancient days (De 1974) [3]. This crop is endowed with a rich germplasm base. According to van der Maesen (1990) [25], the gene pool of pigeon pea 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.

Narrow genetic base of cultivated pigeonpea and repeated use of a few elite breeding lines (Kumar *et al*., 2004) [9] in breeding programs are the major factors hindering its genetic improvement. Further, various biotic and abiotic stresses cause huge yield losses in pigeonpea worldwide and high levels of resistance/tolerance are not available in cultivated genepool.

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 (Patel *et al*. 2020) [13-14]. 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 the 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 and diversifying cytoplasmic male sterility (Sharma and Shadakshari 2021 and Sharma *et al*. 2022) [21, 20]. Since *C. cajan* is the only cultivated species (Reddy 1981) [17], it is reasonable to seek additional variation in the wild and related species of the 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 with *C. cajan* (Pundir and Singh 1985a, Dundas *et al*. 1989, Kumar *et al*. 1990, Mallikarjuna and Moss 1995) [15, 4, 10].

Attempts to transfer high protein content to the cultivated type from *C. albicans*, *C. sericeus*, and *C. scarabaeoides* have been reported (ICRISAT 1987) [5]. 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) [23] and Alternaria leaf spot disease (Singh *et al*. 1984) [22], with a protein content of more than 30% (Singh and Jambunathan 1980). However the crossability of Cajanus wild species 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) [18]. This study aimed to investigate the crossability of *C. scarabaeoides* and discuss the morphological performance of the F_1 and F_2.

Materials and Methods

Seeds of the wild species *C. scarabaeoides* (2n=22) and seven cultivars of *C. cajan* (2n=22) *viz*., (ICPL 88039, ICPL 11253, ICPL 11256, ICPL 11265, ICPL 11333, ICPL 11300 and ICPL 11306) were sown in a UV black net house at the pulses Research Station, S. D. Agricultural University, Sardarkrushinagar, Gujarat, India. Crosses were made in direct directions. The crossability was determined as a percentage of the pod set and F_1 was obtained for seven crosses to raise F_2 generations.

Results and Discussion

Crossability and hybrid survival successful pod set was obtained in direct crosses involving *C. cajan* as ovule parent and *C. scarabaeoides* as pollen parent. From 535 *C. cajan* buds pollinated with *C. scarabaeoides* pollen, F_1 plants were obtained and survived until maturity. The percentage of pod set in the *C. cajan* x *C. scarabaeoides* cross ranged from 0.9 to 6.5%. (Table 1). This finding confirms earlier reports of Dundas *et al*. (1989) [4] and Kumar *et al*. (1990) [8]. However, Pundir and Singh (1985a) [15] obtained successful crosses between *C. cajan* and *C. cajanifolius* using the latter as afemale 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) [16].
Though only seven *C. cajan* genotypes were used in the present investigation, a marked difference between them was observed in their crossability with *C. scarabaeoides*. These results 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 (Bozorgipour and Snape 1990) [1]. This explains the wide range of variation amongst *C. cajan* genotypes concerning 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, all F_1 hybrids survived and grew to maturity.

Morphology of parents and F_1 hybrid

A detailed comparative account of the morphological features of *C. cajan* cv. ICPL 88039, *C. scarabaeoides* and their F_1 hybrid revealed that the parents differed in some of the morphological characters, especially in pod color and the presence of strophile (Table 2). ICPL 88039 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, dwarf, profusely branched, and abundantly flowered (Figure 1). For most of the flower, pod, and seed characters, the hybrid showed an intermediate nature with predominant characters of *C. scarabaeoides* (Figures 2 and 3). Some traits such as ovate leaf shape, green petiole, yellow standard petal, glabrous pods, color of the mature pod, presence of strophile, and prominent beak in the pod were properties of *C. scarabaeoides*. 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 the shape of the first leaflet pair, petiole length, petiole colour size of the standard petal, pod length, and a 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 conform with those reported by Mohanty and Patnaik (1989) [11] for most characters and Reddy *et al*. (1980) [19] regarding the dominanat relationship concerning the traits of 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 pod fly and Alternaria leaf spot disease under field conditions although no artificial screening was done.

Morphology of F_2

Darwin (1877) [2] opined that natural gene flow across related and unrelated individuals through natural cross-pollination helps in the evolutionary processes through enhanced adaptation. In a crop like pigeonpea where natural cross-pollination is a common event, the insect-aided gene flow via pollen transfer from one individual/population to another is inevitable. Kassa *et al*. (2012) [6] while analyzing genetic patterns of domestication of pigeonpea and its wild relatives reported the occurrence of a substantial gene flow from wild species to cultivated types due to cross-pollination; and resulted in natural genetic admixtures and alteration of allele contents in the primary gene pool. Also, sometimes rare alleles are not available in the primary gene pool such as trichomes and disease resistance, etc., and may pass from wild species to the cultivated types. Result in enhanced genetic variability in F_2 generation which could be used by pigeonpea breeders. seven parents of *C. cajan* (2n=22) *viz*., (ICPL 88039, ICPL 11253, ICPL 11256, ICPL 11265, ICPL 11333, ICPL 11300, and ICPL 11306) showed significant variation for all morphological traits F_2 individuals under study also showed segregation for all morphological traits. The F_2 population revealed plant habit varied from tall to dwarf. Tall with erect plant habit was observed in 12 F_2 individuals, while 45 individuals were recorded medium tall with semi spreading and 35 individuals recorded dwarf with spreading type of plant habit. Green stem colour was found in 5 F_2 individuals and purple stem colour was observed in 81 individuals. Pod color with dark purple was recorded in 40, green in 23 and 4 individuals were recorded (Green with brown streak pod color). Obovate leaf shape was observed in 2 F_2 individuals, whereas, 51 individuals were recorded oblong and 34 individuals observed lanceolate leaf shape. Yellow flower color was recorded in 49 plants, 1 with red and 39 individuals with yellow with red streak flower color. Pod pubescence and Pod surface stickiness were recorded in 67 individuals. Those individuals, which are not bearing pod, may be due to self-incompatibility (Table 3). This helps to understand the varying degrees of phenotypic values.

The result of this investigation indicated that a certain degree of homology is maintained between *C. scarabaeoides* and *C. cajan* chromosomes, which is further supported by their ready crossability and the production of fertile hybrid. The easy crossability indicates close relations of pigeonpea with *C. scarabaeoides*, which is considered a progenitor of *C. cajan* (Pundir and Singh 1985a) [15]. 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 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. scarabaeoides* to *C. cajan*. The different traits can be effectively utilized in the selection program and aimed towards improvement in pigeon pea.

Table 1: Crossability relationship between *C. cajan* and C. scereboides

Cross combinations	No. of buds pollinated	No. of pods set	Percentage pod set	Number of seed sown	Number of surviving hybrid plants
116	47	3	6.5	10	3
117	65	1	1.5	3	1
119	53	1	1.9	2	1
120	105	2	1.9	5	2
121	67	1	1.5	2	1
125	109	1	0.9	2	2
126	89	2	2.5	4	2

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

Characters	C. scereboides	F_1 hybrid	*C. cajan* cv. ICPL 88039
1. Shape of first pair of simple leaves	ovate	Ovate lanceolate	lanceolate
2. Leaf lets			
a) Shape	ovate	Ovate lanceolate	lanceolate
b) Length (cm)	2.9	4.2	5.7
c) Breadth (cm)	1.2	2.7	1.8
d) Venation	Palmately reticulate	Palmately reticulate	Palmately reticulate
3. Petiole length (cm)	2.4	1.9	2.0
4. Petiole color	Green	Dark green	Light green
5. Days to flower initiation			
6. Size of the standard petal (cm	1.20 × 1.00	1.40 × 1.20	1.70 × 1.40
7. Color of the standard petal	Yellow	Yellow	Yellow
8. Pod length (cm)	2.5	4.2	7.2
9. Pod color (immature)	Green	Green with black strip	Strip pod
10. Pod color (mature)	Brownish	Brownish	Light brown strip
11. Hairs on mature pods	No	No	No
12. No. of seeds per pod	4.17	4.60	4.20
13. Seed color	Brownish black	Dark brown	Reddish brown
14. Strophiole	Present	Present	Absent
15. Beak of the pod	Prominent	Prominent	Less prominent

Table 3: Morphological Variants in F_2 generation

Cross combinations	Total No. of plants	Plant habit			Stem colour		Pod colour			Leaf shape		
		Tall with erect habit	Medium tall semi spreading	Dwarf with spreading	Green	Puple	Dark purple	Green	Green with brown streak	Obovate	oblong	Lanceolate
116	37	06	30	1	6	31	31	-	04	1	35	1
117	2	0	0	02	0	02	02	-	-			
119	3	01	0	02	1	02	Non podding					03
120	27	0	05	22	0	27	05	21	0	0	5	21
121	5	0	0	05	0	05	01	01	0	0	4	1
125	8	5	0	03	2	6	01	01	0			8
126	10	0	10	0	2	8	Non podding			1	7	
Total	92	12	45	35	5	81	40	23	04	02	51	34

Table: 3 cont….

Cross combinations	Total No. of plants	Flower colour			Pod pubescence		Pod surface stickiness	
		Yellow	Red	Yellow with red streak	Absent	Present	Absent	Present
116	37	34	0	03	00	35	00	35
117	2	2	0	0	00	02	00	02
119	3	01	01		Non podding		Non podding	
120	27	0	0	27	00	26	00	26
121	5	0	0	05	00	02	00	02
125	8	05		03	00	02	00	02
126	10	07		01	Non podding		Non podding	
Total	92	49	1	39	00	67	00	67

Fig 1: F_1 ICPL 88039 x *C. Scaraboides*

Conclusion

This study confirms successful hybridization between *C. cajan* and *C. scarabaeoides*, with F1 hybrids exhibiting intermediate traits from both parents. Crossability varied among *C. cajan* genotypes, emphasizing the role of genotype in hybrid success. The F1 hybrids grew to maturity, displaying distinct morphological features, and the F2 generation showed significant variation, suggesting potential for genetic improvement. The study also highlights the genetic relationship between *C. cajan* and *C. scarabaeoides*, supporting the transfer of valuable traits from wild relatives to cultivated pigeonpea. These findings contribute to advancing pigeonpea breeding for improved traits and resilience.

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