

Plant Genetic Resources

Interspecific hybridization between *Cajanus cajan* (L.) Millsp. and *C. lanceolatus* (WV Fitzg) van der Maesen

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Short Communication

Interspecific hybridization between *Cajanus cajan* (L.) Millsp. and

***C. lanceolatus* (WV Fitzg) van der Maesen**

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Abstract

The cultivated pigeon pea has a narrow genetic base. Wild relatives play an important role in the efforts to broaden its genetic base. In this report, we report a successful wide-cross between the cultivated pigeon pea and *Cajanus lanceolatus*, a wild relative from the secondary gene pool, native to Australia, with desirable traits such as frost and drought resistance. A range of F₁ progeny were obtained and the resultant F₁ hybrid plants set mature pods and seeds. Hybrids had intermediate morphology sharing traits of both the parents. All the F₁ hybrids flowered

profusely. Some of the hybrids were completely male sterile and some were partially fertile with the pollen fertility ranging from 35-50 %. Meiotic analysis of the fertile F₁ hybrids revealed high degree of meiotic chromosome pairing between the two parental genomes. Meiotic analysis of the sterile F₁ hybrids revealed that the break-down in microsporogenesis was at the post meiotic stage after the formation of tetrads. Fertile plants formed regular bivalents with normal disjunction except for occasional asynchrony at meiotic II division.

Key words: Bruchid, *Cajanus lanceolatus*, gene pools, meiosis, pollen fertility, pigeonpea, sterility.

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is a multipurpose grain legume grown by the resource poor farmers in the semi-arid tropics and sub-tropics. The crop has narrow genetic diversity and is susceptible to a range of diseases and pests such as pod borer [*Helicoverpa armigera* (Hub.)], pod fly [*Melanagromyza obtusa* (Malloch)] and bruchids [*Callosobruchus chinensis* (F.)]. High levels of resistance to many of these pests and diseases are low to moderate in the cultivated

germplasm (Sharma, 2005), but the wild relatives of pigeonpea have shown high levels of resistance to many of the constraints (Green *et al.*, 2006; Sharma *et al.*, 2009). The utilization of wild species from the secondary gene pool are important as they are closely related, leading to normal chromosome recombination. This helps in the transfer of useful genes/traits to cultivated pigeonpea (Mallikarjuna *et al.*, 2011). *Cajanus lanceolatus*, a native of northern Australia, is a wild relative from secondary gene pool. Until now *C. lanceolatus* had not been successfully crossed as the previous work (Sateesh Kumar, 1985) reported F₁ hybrids which died in the vegetative stage. The present paper reports successful crosses between pigeonpea and *C. lanceolatus*.

Experimental

Materials and methods

Cajanus lanceolatus (ICP 15639) and *C. cajan* (ICPL 85010) plants were grown and maintained in a glasshouse. Crosses were made using *C. cajan* as the female parent and *C. lanceolatus* as the pollen donor. Pollinations were carried out soon after emasculations in the morning before 10am. Out of 86 pollinations, 20 pods were obtained. Pods were harvested 40-45 days after

pollinations. For cytological analysis of meiocytes, immature flower buds from F₁ hybrids were fixed in Carnoy's II solution (acetic acid 1: chloroform 3: and ethanol 6) for 24 hr at 4⁰C and then transferred to Carnoy's I solution (acetic acid 1: ethanol 3). Meiocytes were squashed and stained in 4% acetocarmine and well spread meiotic preparations were taken for analysis and photographed.

Results and discussion

Pod formation was 23% when *C. cajan* was crossed with *C. lanceolatus*. More than half of the seeds were normal with the exception of few semi-shrunken seeds (34%). Of the 35 morphologically normal seeds, 14 germinated to produce hybrid plants under *in vivo* germination conditions. The plants initially grew slowly, but later normal growth was observed.

Morphologically the hybrid plants had excessive growth compared to both the parents. F₁ hybrids were screened for morphological traits such as plant height, branching pattern, flower size and shape, pod shape and size, and seed color. Hybrids were tall, measuring 325cm (P₁₀-F₁) to 380cm (P₁₃-F₁) in height, resembling the male parent *C. lanceolatus* (height 285cm) compared to a height of 185cm of the *C. cajan* female parent (Fig. 1a). All the hybrids flowered at 98 to 160 days from the date of germination. Sateesh Kumar, 1985 reported that F₁ hybrids died in the vegetative stage. It is possible that the authors of this study failed to notice that hybrids inherited the long duration trait of the male parent, and did not maintain the hybrid plants till they reached the flowering stage. Alternatively, it is possible that the genotype of the female cultivars used in their study, in combination with *C. lanceolatus*, were not genetically successful.

Meiotic analysis of pollen mother cells of the F₁ hybrids exhibited regular formation of eleven bivalents which were predominantly rings (Fig.1b). It is clear from Table 1 that the number of bivalents ranged from 11 in anther from the fertile plant to 7 in the sterile F₁ plant (Fig.1c). Univalents were also found in many cells and the average number of univalents per cell varied from 1-5 in the sterile F₁ plant. Meanwhile, trivalents and tetravalents were appeared at a lower frequency, ranging from 0-2. Normal bivalent formation in the majority of the pollen mother cells is an indication that there is good recombination between the parental genomes. Meiotic anaphase I showed 50-70% of pollen mother cells with normal disjunction and remaining 30-50% with abnormal disjunction of chromosomes (Fig.1e). At the tetrad stage 100% normal tetrads were observed in all hybrids except in P₇ in which 6% of tetrads contained micronuclei. Pollen fertility was found to vary between 35-50% in fertile hybrids (Fig.1f). In some of the F₁ hybrids (P₁, P₄, P₇, P₁₀ and P₁₂) total male sterility was observed with all the anthers having

100% sterile pollen grains, a result of unseparated tetrads (Fig. 1g). An important observation made was that male sterility was a post meiotic process. Development of tetrads was normal, but none of them formed pollen grains. Instead, they grouped together and the tetrads did not separate into individual pollen grains. Such sources may be useful in the development of cytoplasmic male sterile systems in pigeonpea, as such a phenomenon was observed in the A₇ CMS system derived from *C. platycarpus* (Mallikarjuna et al., 2012). Pigeonpea crossed with different wild *Cajanus* species has given rise to different cytoplasmic male sterile systems (Saxena et al., 2010; Mallikarjuna et al., 2011). Hence it is worth exploring if a CMS system can be developed from this cross, as complete male sterility was observed in the F₁ hybrids.

The cross between pigeonpea and *C. lanceolatus* generated two categories of progenies. The first one is the fertile progeny with good recombination between the parental genomes leading to fertile plants, good material for broadening the genetic base of pigeonpea and to look for traits of interest. The second progeny category is the CMS lines, F₁ hybrids with 100% male sterility which can be used to develop another CMS source, distinct from the currently available A₅ CMS system (Mallikarjuna and Saxena, 2005), which was developed from the cross between cv. ICPL 85010 and *C. acutifolius*, and developed on cultivated pigeonpea cytoplasm. Since CMS is developed as a result of interaction between cytoplasmic genome of the female parent and nuclear genome of the pollen parent (Saxena et al., 2010). It is envisaged that gametic recombination between cv. ICPL 85010 and *C. lanceolatus* may have given rise fertile and sterile hybrid plants.

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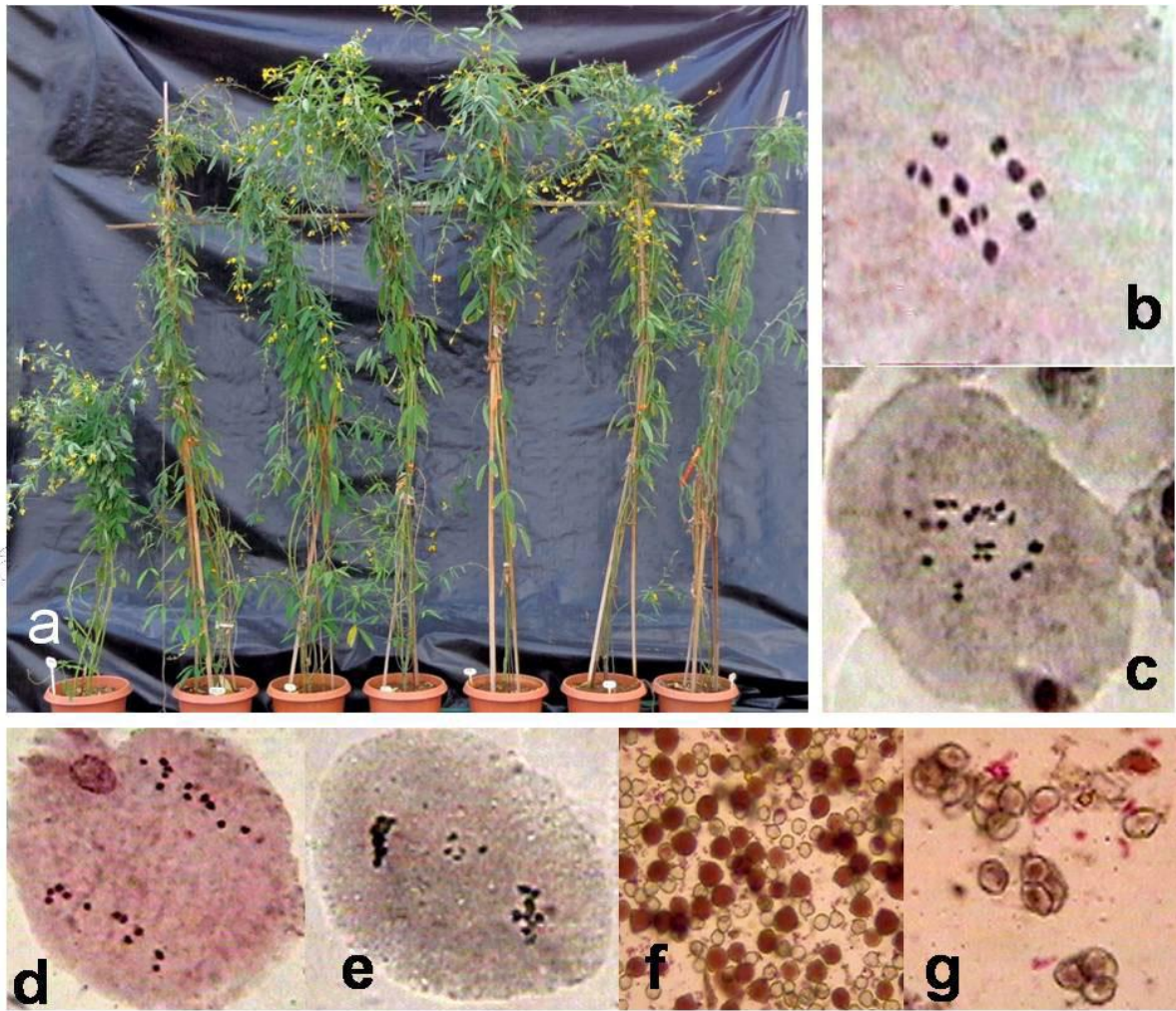


Fig.1. Morphological observations and Meiotic analysis of the F₁ hybrids from the cross *C. cajan* (ICPL 85010) X *C. lanceolatus* (ICP 15639).

Captions for Figure.1: (a) Comparison of hybrids (middle) with cultivar (female-left) and wild (male-right) parents. (b) Metaphase I (fertile plant P₆-F₁) showing two rod and nine ring bivalents. (c) Metaphase I (sterile plant P₇-F₁) showing four univalents and nine bivalents. (d) Anaphase I (fertile plant F₁ P₆) with normal disjunction of chromosomes. (e) Anaphase I (sterile plant F₁ P₇) showing five laggards. (f) Fertile and sterile pollen in fertile anther. (g) Unseparated and empty pollen grains in sterile anther.

Table 1. Meiotic studies of hybrids derived from the cross *C. cajan* (ICPL 85010) X *C. lanceolatus* (ICP 15639)

Hybrid Plant No.	Metaphase I				Anaphase I(%)		Pollen Fertility (%)
	Univalents	Bivalents	Trivalents	Tetrapolyploids	*N.D.	A.D.	
P-1	1.4 (1-4)	9.5 (8-11)	0.2 (0-1)	0 (0)	50	50	Sterile
P-2	0.7 (1-4)	10.2 (7-11)	0.1 (0-1)	0.15 (1-2)	65	35	37.8
P-3	1.15 (1-4)	10.1 (8-11)	0.15 (0-1)	0.05 (1-1)	45	55	24.3
P-4	1.05 (1-5)	10.1 (7-11)	0.25 (0-1)	0 (0)	60	40	Sterile
P-5	0.7 (1-4)	10.25 (8-11)	0.2 (0-1)	0.05 (0-1)	65	35	40.5
P-6	0.35 (1-4)	10.2 (7-11)	0.2 (0-1)	0 (0)	65	35	40.7
P-7	1 (1-4)	10.05 (8-11)	0.1 (0-1)	0.15 (1-2)	25	75	Sterile
P-8	0.35 (1-3)	10.2 (8-11)	0.05 (0-1)	0.3 (1-2)	65	35	56
P-9	0.8 (1-4)	10.3 (9-11)	0.2 (0-1)	0 (0)	55	45	36
P-10	1.35 (1-4)	9.75 (7-11)	0.25 (0-1)	0.1 (0-1)	20	80	Sterile
P-11	0.6 (1-2)	10.2 (7-11)	0.2 (0-1)	0.1 (0-1)	50	50	45
P-12	1.1 (1-4)	9.25 (8-11)	0.3 (0-1)	0.15 (0-1)	35	65	Sterile
P-13	0.65 (1-4)	9.9 (7-11)	0.15 (0-1)	0.3 (0-2)	50	50	50
P-14	0.75 (1-4)	9.15 (7-11)	0.15 (0-1)	0.35 (0-2)	45	55	48

*N.D-Normal distribution, A.D-Abnormal distribution at Anaphase I