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Review Article

Progress in the utilization of *Cajanus platycarpus* (Benth.) Maesen in pigeonpea improvement

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With 1 figure and 4 tables

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Abstract

Cultivated pigeonpea (*Cajanus cajan* L. Millsp.) has a narrow genetic base; hence, utilization of wild relatives in the crossing programme would broaden its genetic base and introduce useful traits. *Cajanus platycarpus (*Benth.) Maesen, an annual wild relative from the tertiary gene pool, was successfully crossed with the cultigen, utilizing hormone-aided pollinations, embryo rescue and tissue culture techniques, and backcrossed using cultigen as the recurrent parent. Advance generation progeny showed a range of useful traits such as resistance to phytophthora blight, pod borer, bruchid and podfly resistance. Variation was also observed for plant type, growth habit and seed colour. A new source of cytoplasmic male sterility was identified in one of the progeny lines. Molecular analysis of the progeny after four backcrosses showed the presence of genomic segments from *C. platycarpus* accompanied by the presence of recombinant DNA sequences signifying recombination between the parental genomes.

Key words: bruchids — *Cajanus platycarpus* — Diversity Array Technology — embryo rescue — *Helicoverpa armigera* — pigeonpea — tertiary gene pool

Genetic variation is the most important tool for creating new recombinant genotypes which may lead to the development of popular varieties. Plant improvement scientists invariably search for new variability from different sources. Of these, the first and foremost choice is primary gene pool (following the classification of germplasm by Harlan and de Wet 1971) mainly because of ease in hybridization and selection. There are many examples in pigeonpea (*Cajanus cajan* (L.) Millsp.), where the primary gene pool species have become popular cultivars and basic sources of disease resistance (Saxena 2008). Alternatively, if the desired traits are not available in the primary gene pool, then breeders scan its wild relatives generally grouped in secondary (crossable with cultivated) and tertiary (non-crossable by conventional hybridization techniques) gene pools.

In pigeonpea, the secondary gene pool consisting of compatible species has been effectively utilized to breed for certain specific traits such as high-protein, cytoplasmic male sterility (CMS) and disease resistance traits (Reddy et al. 1997, Mallikarjuna and Saxena 2005). The use of tertiary gene pool in traditional plant breeding is uncommon and the same is true with pigeonpea. This paper reviews the successful utilization of *Cajanus platycarpus*, an important member of tertiary gene pool, in broadening the genetic base and introgression of some useful traits.

There are 20 wild species in the tertiary gene pool of pigeonpea. Of these, only of pigeonpea *C. platycarpus*, but with the same chromosome number as that of cultivated pigeonpea (2n = 22), is now amenable to interspecific hybridization and gene transfer (Mallikarjuna and Moss 1995, Mallikarjuna et al. 2006). *Cajanus platycarpus* is a species of interest to pigeonpea improvement scientists because it has various traits of interest which can be used for genetic improvement of pigeonpea. These include extra-early flowering and maturity, photoperiod insensitivity, prolific flowering and podding, high harvest index, annuality and rapid seedling growth, and resistances to biotic stresses such as pod borer (Sujana et al. 2008), wilt, phytophthora blight (Ariyanayagam and Spence 1978, Pundir and Singh 1987, Dundas 1990), nematodes (Sharma 1995), sterility mosaic (Lava Kumar et al. 2005) and salinity (Subbarao 1988). The present paper gives a summary of diversity between the accessions of *C. platycarpus*, a brief of the methods to overcome incompatibility and development of backcross progeny. A range of morphological and disease resistance traits were observed in the progeny lines such as pod borer (*Helicoverpa armigera*), bruchid (*Callosobruchus maculatus* F.), pod fly (*Melanagromyza obtusa* Malloch) and phytophthora blight (*Phytophthora drechsleri* Tucker f. sp. *Cajani*) resistance. The presence of *C. platycarpus* genome in advance generation progeny lines (BC_4) was quantified utilizing Diversity Array Technology (DArT) markers.

Materials and Methods

F_1 hybrid plants (*C. platycarpus* × *C. cajan*) were obtained by rescuing aborting hybrid embryos in vitro (Mallikarjuna 1998). Embryo rescue and tissue culture techniques were as described by Mallikarjuna and Moss (1995). F_1 hybrids were backcrossed to cultivated parent *C. cajan*. All the BC_1 (2n = 22) embryos aborted. To obtain BC_1 plants, aborting embryos were rescued using the techniques developed to save F_1 hybrids. BC_1 plants set mature seeds. Mature seeds were germinated to obtain BC_2 generation onwards.

To generate tetraploid progeny, apical buds of F_1 hybrids (2n = 22) were treated with an aqueous solution of 0.05% colchicines with 10% Tween-20 using a soaked cotton swab placed on the apical buds. The treatment was given for 3 days, later washed with water and allowed

the auxiliary buds alone to grow. Hybrids were selfed to obtain F_2 tetraploid (2n = 44) progeny.

Immature flower buds were fixed in Carnoys II mixture (alcohol/acetic acid/chloroform; 6 : 3 : 1) for 48 h and transferred to Carnoys I (alcohol/acetic acid; 3 : 1). Buds were squashed in 2% aceto carmine and meiotic analyses were made on suitable preparations.

Screening for phytophthora blight disease caused by virulent stain of the fungus *Phytophthora drechsleri* Tuckvker f. sp. *Cajani* called the P_3 isolate (Reddy et al. 1996) was carried out by isolating Phytophthora fungi growing on pigeonpea plant. Twelve- to fifteen-day-old seedlings were sprayed with inoculum. Susceptible seedlings were killed with 15 days of inoculation, whereas resistant seedlings remained healthy. Details of the screening procedure are as given by Mallikarjuna et al. (2005).

Field screening for *Helicoverpa armigera* (pod borers), *Melanagromyza obtusa* (pod fly) and *C. maculatus* (bruchids) was carried out by growing the plants under unprotected field conditions for three consecutive years. Cultivated pigeonpea which is susceptible to all the three diseases was grown along with the test material. Adult bruchids were initially collected from the pigeonpea field and initial rearing was maintained for two generations on a bruchid susceptible variety. Bruchids were further screened in the laboratory under a Percival incubator with 24º ± 2°C with 70% RH and 14 : 10 (L : D). In a 13 × 11 cm cylindrical transparent box, 20 seeds each of the three accessions of *C. platycarpus* and their derivatives along with a susceptible check were screened. Four beetles in each box were placed for 48 h and removed subsequently. Observations on no. of eggs laid and no. of eggs hatched were recorded under a binocular microscope. Data were recorded on the number of adults emerged and percentage seed damage along with other parameters.

Total DNA was extracted from fresh leaf tissues of individual plants by CTAB method. PCR amplification of microsatellite loci using 14 fluorescent-dye-labelled primer pairs was carried out in 15 µl volume. The reaction mixture contained 10 mM Tris–HCl, 50 mM KCl, 10 ng of genomic DNA, 2–4 mM $MgCl_2$, 300–400 µM of dNTP and 1 unit of *Taq* DNA polymerase. Amplified products were pooled as per multiplex plan and separated on an ABI 3700 fragment analyser. The results were evaluated using the software package Genotyper 3.7 (Applied Biosystem, Foster City, CA, USA). Analysis was performed using data generated by 14 Simple sequence repeat (SSR) markers. Genetic polymorphism was measured in terms of number of alleles per locus, expected and observed heterozygosity, average genetic distance between accessions (Dg) and the polymorphic information content (PIC) using Power marker V3 (Liu and Muse 2005). Genetic distance is a measure of the dissimilarity of genetic material between different species or individuals of the same species. Depending upon the difference and correcting the values of genetic distances for known rates of evolution, genetic distance is used as a tool to construct cluster diagrams. Genetic diversity analysis was carried out by using the program DARwin version (Perrier and Jacquemoud-Collet 2006).

Diversity Array Technology was performed essentially as reported by Wenzl et al. (2004). A genomic representation was generated from a mixture of genomic DNA from pigeonpea genotypes including the parental lines of mapping populations available at ICRISAT and few wild species including *C. platycarpus*, using the *Pst*I/*Aci*I-based complexity reduction method. The DArT array consisting of 7680 clones was used to genotype the backcross progeny lines of *C. platycarpus* × *C. cajan* and their parents.

Results

Diversity among *Cajanus platycarpus* accessions

Cajanus platycarpus accessions were used in molecular diversity study using SSR markers, differences were observed between the accessions and cultivated pigeonpea cultivars, and the genetic diversity indices varied from 0.17 to 0.50 among the accessions showing their individuality but at the same time showing partial relatedness (Table 1). The diversity indices varied from 0.67 to 0.94 between *C. platycarpus* accessions and cultivated pigeonpea showing greater diversity between the two groups (R. Varshney, and N. Mallikarjuna, unpublished results) and corroborating their placement in the tertiary gene pool of pigeonpea.

Crossability studies: barriers to hybridization and methods to overcome them

Application of gibberellic acid (GA_3) to the base of pollinated pistils delayed the abortion of hybrid embryo from 3 to 6 days to 18 to 22 days. Even at 18–22 days, the hybrid embryos from cross-pollinations were slow in growth in comparison with embryos from self-pollinations. Embryos from cross-pollinations were not more than 1.0 mm in size, being immature and at the cotyledonary stage of development.

Embryo rescue

Immature aborting seeds with aborting embryos inside were cultured to produce hybrid plants (Fig. 1). Embryo rescue technique that was standardized for the cross *C. platycarpus* × *C. cajan* took about 6 months to obtain a F_1 hybrid plant. This plant, once established, grew vigorously into a short and bushy plant and male sterile but female fertile. The F_1 progeny did not set mature seeds when backcrossed to the cultivated parent and immature aborting BC_1 seeds were obtained. It was not possible to self the F_1 hybrid plants because of complete male sterility.

Table 1: Genetic diversity between *Cajanus platycarpus* accessions and *C. cajan* as revealed by SSR markers

@Darwin 5.0 – DIS
16

	61	62	63	65	66	68	69	70	71	72	**85010**	**87119**
61	0											
62	0.17	0										
63	0.31	0.28	0									
65	0.31	0.42	0.47	0								
66	0.42	0.42	0.50	0.47	0							
68	0.33	0.31	0.39	0.42	0.19	0						
69	0.22	0.22	0.28	0.42	0.25	0.17	0					
70	0.31	0.31	0.36	0.39	0.33	0.22	0.25	0				
71	0.22	0.17	0.28	0.42	0.25	0.14	0.06	0.19	0			
72	0.50	0.36	0.47	0.56	0.33	0.25	0.33	0.39	0.31	0		
ICPL85010	0.78	0.83	0.92	0.83	0.92	0.94	0.89	0.89	0.94	0.97	**0**	
ICPL87119	0.67	0.72	0.78	0.72	0.83	0.83	0.78	0.72	0.78	0.83	**0.61**	**0**

61–72 are *C. platycarpus* accessions, each number with ICPW prefix. 85010 and 87119 are pigeonpea cultivars with ICPL prefix.

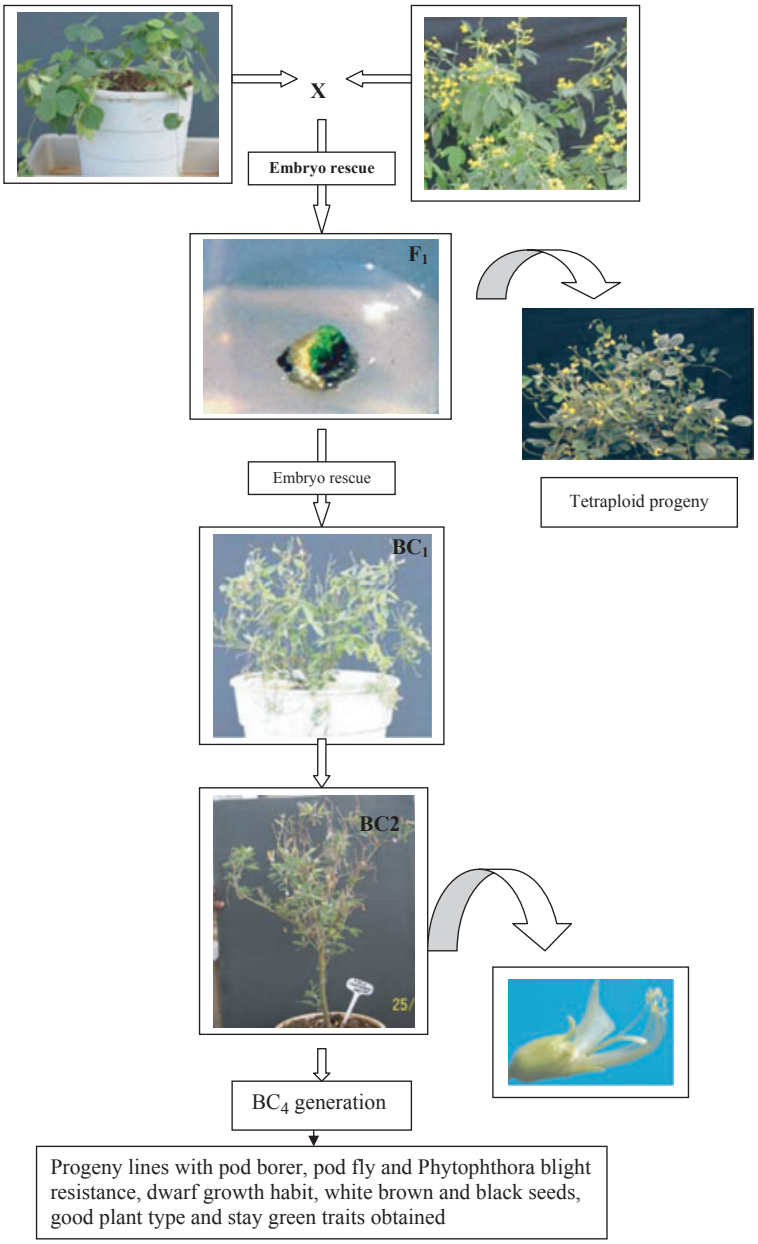

Fig. 1: Tapping useful genetic variation from *Cajanus platycarpus*

Backcross generation

F_1 hybrids were backcrossed using cultivated pigeonpea as the recurrent male parent. Application of GA_3 was mandatory to obtain well-developed pods and seeds from BC_1F_1 plants, but they failed to reach maturity (Fig. 1). The BC_1 embryos grew for 2–3 weeks with the application of GA_3. Without the application of GA_3, BC_1F_1 embryos also aborted by 3–6 DAP. Majority of the BC_1F_1 embryos were smaller than the F_1 embryos, but could be saved by the rescue of the aborting embryos on the ovule culture medium standardized for pigeonpea. It took nearly 4 months for the BC_1F_1 embryo to grow into a plant that could be transferred to soil. BC_1F_1 plants were large with semi-spreading growth habit and produced a large number of flowers. Mature seeds were obtained for the first time on BC_1 plants and they were black in colour. BC_2F_1 seeds germinated in soil producing BC_2 plants. Seeds in the BC_2F_1 plants varied from light brown to black. Variation for seed colour and size was observed for the first time in the progeny from the cross *C. platycarpus* × *C. cajan*.

Variability observed in the progeny

One progeny line derived from BC_2F_1 was called line A, and it was backcrossed to cultivated recurrent parent ICPL 85010 and BC_4F_1-A lines were developed. The progeny was selfed twice and screened for various traits of interest for pigeonpea improvement.

Days to flower

Days to first flower in the progeny lines varied from 49 to 120 days and 77 to 122 days for 50% flowering. Majority of the lines flowered between 60 and 75 days. In the parental lines, *C. platycarpus* flowered at 50 days and cultivar ICPL 85010 flowered at 83 days.

Dwarf growth habit

One line BC_4F_2 – line A 22 showed short and bushy growth habit, not seen in any of the other 21 lines grown in the field. The other lines had erect, semi-spreading growth habit. The plants of line A 22 also flowered early and set a few pods. The short and bushy growth habit continued in subsequent generations.

Stay green

Two BC_4F_4 lines stayed green when all other lines had completed their life cycles after setting pods. These two lines continued to stay green, produced new shoots, flowers and set seeds. Stay-green plants are being screened for third year in a row to test for stay-green trait.

High seed number and weight

One of the line namely BC_4F_4-A17 showed higher seed number than normally encountered in other progeny lines. Pods with five locules were also more frequent in this line which varied from 4 to 57%, with one plant showing 37% of the pods with five locules and produced a total of 294 pods and 950 seeds per plant. Usually, pods with five locules were rare in pigeonpea. Cultivated pigeonpea cultivar ICPL 87 produced a total of 67 pods in which six of the pods had five locules and produced a total of 208 seeds per plant. *Cajanus platycarpus* had a few pods with 4–5 locules, but the total number of pods per plant did not exceed 20–25. Compared with the control used in the study, all the progeny lines showed significantly more 100 seed weight, which varied from 8.8 to 11.00 g (Table 2).

Seed colour

In BC_4F_3 lines, variation for seed colour was observed, which were dark brown, light brown and white. *Cajanus platycarpus*, the maternal parent of the cross has black seeds and *C. cajan*, the pollen parent, had brown seeds. After generating the F_1 and BC_1 progeny through embryo rescue, it was not possible to self the progeny till BC_4 population was obtained. Plants in BC_4 progeny were selfed to produce BC_4F_2 population which had only brown seed colour. In BC_4F_3 population, variation for seed colour was observed.

Cytoplasmic nuclear male sterility

A selection was made in BC_2F_1 generation for open flower morphology and low pollen fertility, and it was designated as BC_2F_1 – line E (Fig. 1). In this material, two progeny were found to be totally male sterile. Its anthers were pale white and diminished in size and papery in appearance. In the other plants although some of the pollen grains were fertile, self-pollination and seed set were not obtained. BC_3F_1-E15, one of the male sterile progeny, was crossed with pigeonpea cultivars ICPL 85010, ICP 14444, ICPL 88014 and ICPW 69. Crosses with ICPL 85010 produced totally male sterile progeny with no fertile pollen and with no pod set. Hence, it was classified as a maintainer of this male sterility system. Crosses with ICP 14444 produced progeny with partial fertility (25–60%), while crosses with ICPL 88014 produced progeny with 40–65% fertility. Crosses with ICPW 69 (*C. platycarpus*) also produced progeny with 5–20% pollen fertility.

Insect resistance

Helicoverpa armigera

All the derivatives were screened for resistance to insects *H. armigera* (Hubner) also called pod borer, *Melanagromyza obtusa* (pod fly) and *Callosobruchus maculates* (bruchids) under unprotected field conditions. Damage in BC_4F_1-A derivatives ranged from 6.85 to 22.84%, with majority of the lines with

Table 2: Performance of progeny lines derived from *Cajanus platycarpus* for resistance to pod borer, pod fly, bruchid and seed weight and their significance with respect to check [ICPL 85010 (S)]

	Pod damage/plant (%)				
	Healthy pods per plant (no.)	*Helicoverpa armigera*	*Melanagromyza obtusa*	*Callosobruchus maculatus*	100 seed wt (g)
F_1BC_4A4 10-7-1	81.30	9.92*	14.55	1.03	10.30*
F_1BC_4A4 10-12-1	99.50	16.61*	12.05	2.12	9.82*
F_1BC_4A4 13-2-1	91.25*	10.15*	10.24	2.74	9.45*
F_1BC_4A4 13-5-1	79.28	12.59*	12.52	6.28*	9.52*
F_1BC_4A4 14-4-1	72.06	18.56*	9.47	1.98	9.70*
F_1BC_4A4 14-6-1	106.94	15.90*	10.64	1.01	9.93*
F_1BC_4A4 14-9-1	111.35	13.18*	14.95	3.50	8.65*
F_1BC_4A4 14-16-1	95.95	14.68*	14.52	1.55	9.22*
F_1BC_4A4 14-21-1	118.22*	9.71*	12.94	1.38	10.27*
F_1BC_4A4 14-18-1	74.33	10.26*	7.68	7.44*	8.57*
F_1BC_4A4 15-14-1	73.53	13.42*	3.73*	2.01	9.60*
F_1BC_4A4 17-1-1	50.16	9.43*	16.68	0.13*	8.82*
F_1BC_4A4 17-5-1	67.60	13.28*	14.61	0.00*	9.15*
F_1BC_4A4 17-8-1	73.11	11.42*	10.98	14.34*	11.02*
F_1BC_4A4 19-1-1	76.00	9.46*	7.74	7.69*	9.62*
F_1BC_4A4 19-8-1	99.70	14.19*	11.48	1.06	9.29*
F_1BC_4A4 19-12-1	77.95	7.23*	15.71	8.65*	9.42*
F_1BC_4A4 19-14-1	8.55*	15.25*	41.75*	0.00*	9.37*
F_1BC_4A4 19-20-1	97.00	22.85*	16.57	2.52	10.46*
F_1BC_4A4 20-5-1	68.39	10.55*	20.19*	0.00*	9.82*
F_1BC_4A4 20-10-1	34.18*	21.52*	21.65*	0.33*	9.98*
F_1BC_4A4 13-2-1	63.20	11.12*	15.84	0.04*	8.65*
F_1BC_4A4 13-5-1	70.55	6.85*	12.85	0.23*	9.11*
F_1BC_4A4 14-6-1	54.50	24.15*	10.80	0.47*	9.85*
ICPL85010(S) check	66.40	41.55	10.85	1.45	6.20

*Significantly different from check at $P < 0.05$.

< 15% damage. Damage because of *H. armigera* in the cultivated parent ICPL 85010 was 41.55%. All the treatments as indicated in the (Table 2) were found significant ($P < 0.05$) compared with the control.

Bruchids

Resistance to storage pest bruchid *C. maculatus* F. *C. chinensis* (L.) is very important in pigeonpea and is lacking in cultivated species (Lateef and Reed 1990). With delayed harvest, bruchid menace is commonly observed. Progeny lines derived from *C. platycarpus* showed 0–7.44% damage compared with 1,45% damage in the control lines. Some of the lines with no bruchid damage also had significantly lower *H. armigera* damage (Table 2). Three accessions of *C. platycarpus* were screened for bruchid resistance. On all the three accessions, 82–91% of the eggs failed to hatch. Although 44% of the eggs failed to hatch on cultivar ICPL 85010, the number of non-viable eggs on the wild species was more than double. The minimum seed damage was recorded on *C. platycarpus* accession ICPW 66 (14%), while the damage was moderate to medium in other *C. platycarpus* derivatives compared with the susceptible check 85010 (75% damage). Many interspecific derivative lines derived from *C. platycarpus* were found to have low damage because of bruchids in the present study. In advance generation interspecific derivative lines, the number of eggs that failed to hatch varied from 32.78 to 92%. Although some eggs hatched, the days required for the emergence of the adult were more than in the cultivars. In most of the wild accessions as well as in the advance lines, the number of days for the adult emergence was higher. Later emergence produced smaller and weaker adults (Table 3).

Podfly resistance

Low to moderate resistance was observed for the podfly in the BC_4F_1-A derivatives, which ranged between 4 and 22%, with majority of derivatives having moderate damage between 10 and 16%, with a single derivative BC_4F_1-A 15-14-1 showing a low damage of 3.73%. The line also showed low damage to *H. armigera* (13.42; Table 2). Earlier reports on podfly damage have revealed a mean damage of over 20% in north India and above 11% damage in south India.

Phytophthora blight

Phytophthora blight is an important disease of short duration pigeonpea where the atmospheric moisture content is high. The disease is caused by *Phytophthora drechsleri* tucker var. *cajani* Pal, Grewal and Sarbhoy. There are three isolates of Phytophthora blight (P_1, P_2 and P_3) (Gupta et al. 1997). Resistances sources for P_1 and P_2 isolates are available which are not as virulent as the P_3 race and no source is resistant to the P_3 race. *Cajanus platycarpus* accessions ICPW 61 and ICPW 66 are the only known sources of resistance to phytophthora blight P_3 race of pigeonpea (Reddy et al. 1996). Fifty-four F_2 seedlings were screened for P_3 isolate of Phytophthora blight disease. Of these, 14 plants showed resistance to the disease, whereas the rest succumbed to the disease. The resistant plants were subjected to the disease during the seedling stage, before flowering stage and at the flowering stage, and in all the tests, the resistant plants did not show any disease symptoms. Resistance to Phytophthora blight was identified as monogenic and recessive (Mallikarjuna et al. 2005). Fourteen advance generation diploid hybrid lines were subjected to the disease and one of the lines showed < 1% disease (N. Mallikarjuna, and L. Kaur, unpublished). Progeny were screened for 3 years and segregation for Phytophthora blight disease resistance has been observed.

Tetraploid generation

In order to avoid embryo rescue for the second time, F_1 embryos were colchicine treated while still in culture. Percentage conversion of diploid hybrids to tetraploids was 2.5%. A large number of F_1 diploid hybrid embryos were treated to obtain F_1 tetraploid plants (Fig. 1). The tetraploids had robust vegetative growth but with spreading growth habit. Leaves and flowers were larger than the diploid F_1 plant and set a large number of mature seeds. F_2 and F_3 progeny had spreading growth habit with large leaves and set a large number of seeds. It was not possible to backcross tetraploid F_1 or F_2 plants with cultivated pigeonpea, as the embryos aborted because of ploidy differences between the hybrid (4×) and cultivated (2×). It was observed that all the tetraploids had high levels of resistance to phytophthora blight disease at seedling as well as at mature growth stages, a trait transferred from *C. platycarpus*.

Cytogenetical studies

Cytological analysis of the F_1 hybrid ($2n = 22$) showed variation in meiotic chromosome configuration with a mean of six univalents (ranging from 5.2 to 6.7) and eight bivalents (7.4–8.4) per cell. Trivalents were rarely observed (Mallikarjuna and Moss 1995). Homology between eight chromosomes of pigeonpea and *C. platycarpus* showed that more than half of

Table 3: Bruchid response on three accessions of *Cajanus platycarpus* and their derivatives

1	2	3	4	5	6	7	8
ICPW 64	92	82	10	20	0	53.47 ± 3.88	45–58
ICPW 66	55	50	5	14	0	48.77 ± 0.58	48–49
ICPW 68	129	110	21[1]	30	2	43.53 ± 1.46	39–45
BC4A4-10-7-1	66	54	10	30	2	48.23 ± 0.73	47–49
BC4A4-10-7-2	60	29	29	50	2	44.40 ± 1.00	42–46
BC4A4-10-7-4	71	45	28[1]	40	1	46.13 ± 1.03	42–47
BC4A4-10-7-7	70	64	4	15	2	44.25 ± 1.26	43–46
BC4A4-10-7-19	79	73	3	10	3	43.05 ± 0.71	41–44
BC4A4-10-7-20	61	20	40	50	1	41.88 ± 0.66	40–43
ICPL 85010 check	158	70	90[1]	75	1	34.95 ± 1.39	33–38

1: Identity; 2: No. eggs laid; 3: Eggs failed to hatch; 4: Adults emerged; 5: Seed damage (%); 6: Adults dead inside the seed 7: Average days for emergence; 8: Adult emergence (Min and Max days).
[1]In few accessions, more than single adult emerged from a single seed.

the genome of pigeonpea has regions in common with that of *C. platycarpus*. In the BC_1 plants, using cultivated pigeonpea as the recurrent parent, 1–4 univalents were observed in spite of increasing the genome contribution of cultivated pigeonpea. In the BC_2 plants, the number of univalents were less (1–2), with greater homology between chromosomes in the BC_3 plants, univalents was not observed (Mallikarjuna et al. 2006). It can be concluded from theoretical calculations (Mallikarjuna 2007) that 93.75% of the cultivated genome was essential to bring about the proportion of homologous regions in the progeny to form only bivalents.

Molecular analysis of advance generation (BC_4) progeny

Diversity Array Technology, a genome-wide marker technology, was used to genotype the parents and advance generation hybrids after four backcross. A total of 1225 markers were found polymorphic among the parents and the progeny. The results of the study showed that apart from DNA stretches from the female and male parent, there was some novel DNA polymorphism observed in the progeny not seen in both the parental species. It was interesting to observe that as per theoretical calculations, there should be 3.12% of *C. platycarpus* genome after four backcrosses with cultivated parent *C. cajan* (Mallikarjuna 2007). Diversity Array Technology analysis showed the presence of *C. platycarpus* genome ranging from 2.0 to 4.8%. The presence of non-parental DNA sequences presumably because of recombination ranged from 2.6 to 10.4% (Table 4).

Discussion

A total of 13 accessions of *C. platycarpus* were used to study diversity between accessions. Differences were observed among accessions for days to flowering and maturity, seeds per pod, 100 seed weight, grain yield and seed protein content. There were differences between accessions of *C. platycarpus* with respect to crossability with pigeonpea (N. Mallikarjuna, unpublished). Molecular analysis confirmed the diversity between the accessions of *C. platycarpus* and different accessions can be used to broaden the genetic base of cultivated pigeonpea.

The first-known attempt to cross pigeonpea with *C. platycarpus* was by Ariyanayagam and Spence (1978), which was followed by James (1978), who did not succeed in producing true hybrids. Kumar (1985) and Dundas (1990) reported embryo abortion in the cross *C. cajan* × *C. platycarpus* within 6 days of pollination. Pundir and Singh (1987) also reported embryo abortion in the crosses involving *C. platycarpus*. Fluorescence and light microscopy showed the barriers to hybridization to be postzygotic, accompanied by minor prezygotic barriers (Mallikarjuna and Moss (1995). This was overcome by the application of gibberellic acid to postpone the abortion of hybrid embryo so that a more developed embryo was obtained. A more developed embryo is amenable to embryo rescue techniques standardized by Mallikarjuna (1998). As a result, it was possible to obtain advance generation hybrids utilizing *C. platycarpus*.

Wide crosses with distantly related species give rise to novel variation generally not seen in both the parents used in the crossing programme (Hoisington et al. 1999). Many novel traits were noticed when the cross was advanced to BC_4F_1 generation. In the BC_2F_1 plants, the flower colour varied from yellow- to orange-coloured petals. Pollen fertility varied from 27 to 46%. Some plants had open flowers unlike that observed in pigeonpea or *C. platycarpus*, the parents of the cross (Cherian et al. 2006). Open flowers of pigeonpea are likely to play an important role in development hybrid breeding programme, as this trait will facilitate cross-pollination. There are already many sources of CMS (A_1, A_2, A_3, A_4, A_5 and A_6) reported (Saxena et al. 2010). The source reported with the cytoplasm from *C. platycarpus* will be an additional source and will be helpful in the diversification of the cytoplasmic base.

In the BC_4F_1-A plants, seed was black in colour. In BC_4F_2-A plants, seed was light brown in colour. Variation for seed colour was observed in BC_4F_3-A progeny. There was segregation for seed colour, and dark brown-, light brown- and white-coloured seeds were obtained. White-coloured seeds did not segregate for colour in subsequent generations. Based on published literature, it is known that white seed coat colour is a recessive trait (Rekhi 1966, Patil 1970, Singh and Pandey 1974) and one or two recessive genes control the expression of white seed colour (Shaw 1936, Patil 1970, Deokar et al. 1972).

Days to flower is an important trait in short duration pigeonpea. They can complete their life cycle faster and therefore can fit well in various cropping systems. One of the traits of *C. platycarpus* is early flowering. Some of the derivatives showed early flowering trait, which was earlier than the cultivated parent used in the crossing programme. Earliness in pigeonpea is controlled by more than one dominant gene and is expressed in a quantitative manner

Table 4: Proportion of *Cajanus platycarpus* genome after four backcrosses with *C. cajan* (in the cross *Cajanus platycarpus* × *C. cajan*) as explained by DArT genotyping based on 1225 polymorphic markers

Genotypes	No. of non-parental alleles	% of non-parental alleles	No. of female parent alleles	% of female parent alleles
1. F_1BC_4A4-5-4-12-9	63	5.1	52	4.2
2. F_1BC_4A4-10-3-2-18	30	2.4	44	3.6
3. F_1BC_4A4-10-12-1-8	61	5.0	65	5.3
4. F_1BC_4A4-14-21-1-9	64	5.2	59	4.8
5. F_1BC_4A4-17-5-1-10	40	3.3	37	3.0
6. F_1BC_4A4-17-8-16-10	115	9.4	43	3.5
7. F_1BC_4A4-17-8-19-10	44	3.6	39	3.2
9. F_1BC_4A4-19-12-1-10	62	5.1	51	4.2
10. F_1BC_4A4-19-12-17-9	29	2.4	47	3.8
11. F_1BC_4A4-21-1-10-17	127	10.4	65	5.3
21. F_1BC_4A4-8-11-1-3	55	4.5	34	2.8
22. F_1BC_4A4-8-11-1-3	32	2.6	24	2.0

DArT, Diversity Array Technology.

(Saxena and Sharma 1990). Bushy growth habit is not a favourable trait for pigeonpea as it attracts *H. armigera* and the line with dwarf bushy growth habit showed 40% damage because of *H. armigera*. In contrast, all the other lines were tall with semi-spreading secondary and tertiary branches. It is reported in pigeonpea that plant height is a complex and quantitative trait (Byth et al. 1981). Stay-green trait is important in drought situations. Pigeonpea has inherent drought tolerance by growing and setting some seeds in marginal areas with scanty rainfall. Hence, additional stay-green trait maybe an added advantage as pigeonpea is normally grown by resource poor farmers in areas with scanty rainfall.

Cytoplasmic male sterility observed in two BC_2F_1-E lines with open flowers is a desirable trait in developing male sterility system. Open flowers encourage cross-pollination, and hence, it is important in a largely self-pollinated crop such as pigeonpea. Open flowers will allow the bees to cross-pollinate and thus aid in the exploiting heterosis in pigeonpea. Although there are six diverse CMS cytoplasms reported for pigeonpea, the reported sources are either from the primary or from the secondary gene pool. The CMS source developed with *C. platycarpus* cytoplasm is very diverse from other sources as *C. platycarpus* is a species from the tertiary gene pool of pigeonpea.

The tetraploid progeny will not be of use to develop pigeonpea lines with desirable traits because of ploidy incompatibility between diploid and tetraploid progeny. Because the tetraploids had extensive vegetative growth, trailing growth habit and mature seed set, they can be of use as forage cover providing useful leguminous proteins to grazing animals.

Evaluation for insect resistance data showed that there is good scope to transfer multiple insect resistances from *C. platycarpus*. A few lines with low pod borer, pod fly and bruchid damage were observed. More lately, lines with fusarium wilt and sterility mosaic disease (Patancheru isolate) were also observed (data not included in the present report). All the above-mentioned insects cause economic losses in pigeonpea, and lines with multiple resistances are much desired as farmers cannot afford to protect pigeonpea to multiple insects spraying an array of chemicals as chemicals are expensive, bad for the environment and to the farming community.

Disease and insect resistance traits as well as other morphological traits observed in the progeny lines were a result of crossing *C. platycarpus* with *C. cajan*. After four backcrosses and as per theoretical calculations, there should be 3.12% of the *C. Platycarpus* genome in the progeny lines (Mallikarjuna 2007). Molecular analysis using genome-wide DArT marker showed that there was 2–5% *C. platycarpus* genome, which tallies with the theoretical calculations. This suggests that the traits present in the lines are indeed derived from *C. platycarpus*.

To conclude, much desired variation has been created for pigeonpea utilizing *C. platycarpus*, a tertiary gene pool species. The effort to broaden the genetic base and introduce useful traits has been achieved.

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