Pre-breeding to expand primary genepool through introgression of genes from wild *Cajanus* species for pigeonpea improvement

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Abstract: Narrow genetic base coupled with low levels of resistance against important biotic/abiotic stresses in cultivated pigeonpea is the major constraint affecting its production and productivity globally. Wild *Cajanus* species are the reservoir of many important genes and can be utilized to improve the crop cultivars, enrich variability, and broaden the genetic base. Pre-breeding populations involving wild *Cajanus* species from the secondary and tertiary genepools as donors and pigeonpea cultivars as recipients have been developed. Considerable variability was observed in these populations for morpho-agronomic traits and for biotic stresses. Agronomically desirable and disease resistant introgression lines have been identified for use in pigeonpea improvement programs.

Key words: genepool, interspecific hybridization, pre-breeding, pigeonpea, wild *Cajanus*

Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is the sixth most important grain legume crop of rainfed tropics and subtropics. Though a short-lived perennial shrub, it is traditionally cultivated as an annual crop in Asia, Africa, Latin America, and the Caribbean regions. Pigeonpea has a wide adaptability to diverse climates and is cultivated in 82 countries on 6.23 million ha area with an annual production of 4.68 m t and productivity of 751 kg ha^{-1} (3). India is the major pigeonpea growing country in the world with 3.02 t of production from 4.65 million ha area (3).

In spite of large breeding efforts for pigeonpea improvement in India and elsewhere, there is a huge gap between the potential (2.5 t ha^{-1} - 3.0 t ha^{-1}) and actual yield (~0.8 t ha^{-1}). This is due to the damage caused by several biotic (wilt, sterility mosaic disease (SMD), phytophthora blight and pod borer (*Helicoverpa armigera* L.)) and abiotic (water logging, salinity and frost/cold) stresses. The use of working collection consisting mainly of a few and elite breeding lines coupled with intense selection for high yield has resulted in the recirculation of the same germplasm and hence narrow genetic base of the improved cultivars. The cultivation of a few and genetically similar cultivars has resulted in the vulnerability to pests and diseases. Therefore, efforts are immediately required towards broadening the genetic base of pigeonpea cultivars and developing improved plant types having high levels of resistance to various biotic and abiotic stresses.

Wild relatives for pigeonpea improvement

Crop wild relatives (CWR) have been used as a diverse pool of genetic resources for crop improvement, including insect and disease resistance (1, 4). Wild *Cajanus* species harbor high levels of genetic diversity to survive against various biotic/abiotic stresses, especially under changing climatic conditions and thus hold a great potential for pigeonpea improvement. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in Patancheru, India, has the global responsibility of collecting, conserving and distributing the pigeonpea germplasm comprising of landraces, obsolete varieties, breeding lines, modern cultivars, genetic stocks, mutants and wild *Cajanus* species. The ICRISAT genebank holds 13,216 accessions of cultivated pigeonpea and 555 accessions of wild species in the genus *Cajanus* from 60 countries. Based on the crossability with cultivated pigeonpea, the cultivated and wild *Cajanus* species are divided into three genepools (Fig. 1).

In wild *Cajanus* species, new and diverse sources of resistance/tolerance to various biotic stresses, such as alternaria blight (12), phyto-phthora blight (6, 8, 10), sterility mosaic disease (7), pod borer (2, 13, 18), cyst nematode (15), and abiotic stresses, such as salinity (16, 17), as well as accessions with agronomically desirable traits, such as early flowering, high seed number per pod, high seed protein content (19) and photoperiod insensitivity (9) have been identified (Table 1) for use in pigeonpea improvement.

Pre-breeding for broadening the genetic base

Although high levels of resistance is available in wild *Cajanus* species, these are not being utilized adequately in pigeonpea breeding programs. The major limitation is due to the linkage drag and different incompatibility barriers between cultivated and wild species. Under such situations, pre-breeding provides a unique opportunity to expand primary genepool by exploiting genetic variability present in wild species and cultivated germplasm, and will ensure continuous supply of new and useful genetic variability into the breeding pipelines to develop new cultivars having high levels of resistance and broad genetic base.

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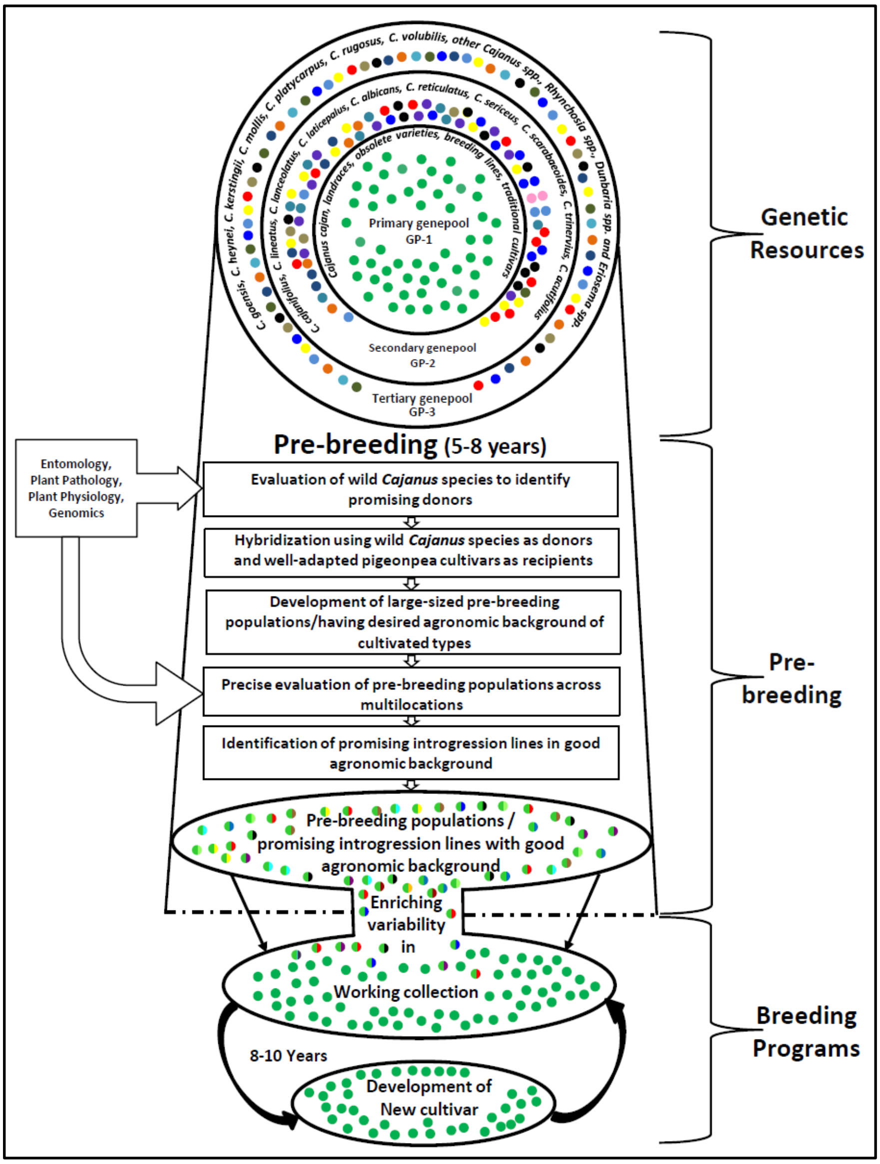

Figure 1. Pre-breeding using wild *Cajanus* species and pigeonpea cultivars for broadening the genetic base for pigeonpea improvement

Pre-breeding is comprised of three major activities: i) Identification of desirable traits and/or genes from unadapted germplasm such as exotic landraces/wild species (donors); ii) Population development following hybridization for transferring these traits into well-adapted genetic backgrounds; and iii) Evaluation of pre-breeding populations to identify desirable ILs for ready use by the breeders in breeding programs (14). Overall, pre-breeding is a time consuming and resource demanding affair (Fig. 1).

At ICRISAT, pre-breeding activities are being undertaken by utilizing the wild *Cajanus* species from secondary and tertiary genepool for pigeonepa improvement. One major achievement of using wild *Cajanus* species is the development of cytoplasmic nuclear male sterility systems (CMS). These CMS systems have been developed with cytoplasm derived from different wild *Cajanus* species (11).

Besides this, pre-breeding activities involving wild *Cajanus* accessions as donors and popular pigeonpea varieties as recipients are in progress to develop new genepools with high frequency of useful genes, wider adaptability, and a broad genetic base for resistance/tolerance to important biotic and abiotic stresses, as well as for agronomic and nutrition-related traits. Further, to minimize the linkage drag associated with utilizing wild species in crop improvement programs, we are focusing on the development of advanced backcross populations. Using secondary genepool species, *C. cajanifolius* (Haines) Maesen (ICPW 29), *C. acutifolius* (F. Muell.) Maesen (ICPW 12 and ICPW 004), *C. scarabaeoides* (L.) Thouars (ICPW 281) and tertiary genepool species, *C. platycarpus* (Benth.) Maesen (ICPW 68) having useful traits such as tolerance to salinity and pod borer resistance (5, 16, 18), and two pigeonpea cultivars (ICPL 87119 and ICPL 85010), advanced backcross populations have been generated from five interspecific crosses, ICPL 87119 × ICPW 29, ICPL 87119 × ICPW 12, ICPL 87119 × ICPW 281, ICPL 85010 × ICPW 004 and ICPW 68 × ICPL 85010, at ICRISAT, Patancheru, India. ICPL 87119 (Asha) is a medium-duration variety and ICPL 85010 is an early-duration pigeonpea variety, both cultivated in India. Evaluating these populations for agronomic traits revealed considerable variability for days to flowering, growth habit as well as for pod and seed traits. Promising introgression lines (ILs) having high numbers of pods per plant and seeds per pod and 100-seed weight (> 20.0 g) have been identified from the population derived from a ICPL 85010 × ICPW 004 cross (Fig. 2). Evaluation of two populations derived from ICPL 85010 × ICPW 004 and ICPW 68 × ICPL 85010 crosses for sterility mosaic disease and wilt identified 15 ILs derived from *C. acutifolius* and two ILs from *C. platycarpus* having combined resistance against SMD and wilt. Besides this, one population derived from ICPL 87119 × ICPW 12 was also screened against phytophthora blight under controlled environmental conditions and the resistant ILs have been selected for confirming the resistance. Overall, pre-breeding activities have led to the development and identification of agronomically desirable and disease resistant ILs for further use in pigeonpea improvement programs.

Recently, the efforts have been initiated to introgress pod borer resistance from wild *Cajanus* species into pigeonpea cultivars. The evaluation of wild *Cajanus* species has

Table 1. Wild *Cajanus* species identified as promising donors for important biotic/abiotic stresses and agronomic traits

Species	Traits
	Secondary genepool
C. acutifolius	Pod borer
C. albicans	Alternaria blight, sterility mosaic disease, pod borer, salinity, high seed number per pod (> 6) and high seed protein (> 30%)
C. cajanifolius	Alternaria blight, and high seed protein (> 30%)
C. lineatus	Alternaria blight
C. reticulatus	Pod borer
C. scarabaeoides	Alternaria blight, streility mosaic disease, pod borer, cyst nematode, salinity, early flowering (between 51 and 118 days) and high seed protein
C. sericeus	Alternaria blight, phytophthora blight, pod borer, and salinity
	Tertiary genepool
C. cinereus	High seed number per pod (> 6)
C. crassus	High seed protein (> 30%)
C. mollis	High seed number per pod (> 6) and high seed protein (> 30%)
C. platycarpus	Alternaria blight, phytophthora blight, salinity, photoperiod insensitivity, super-early flowering (between 34 - 40 days) and high seed protein (> 30%)
C. volubilis	Alternaria blight
Flemingia spp.	Cyst nematode
Rhynchosia aurea	Pod borer and cyst nematode
R. bracteata	Pod borer
R. rothii	Cyst nematode
R. densiflora	Cyst nematode

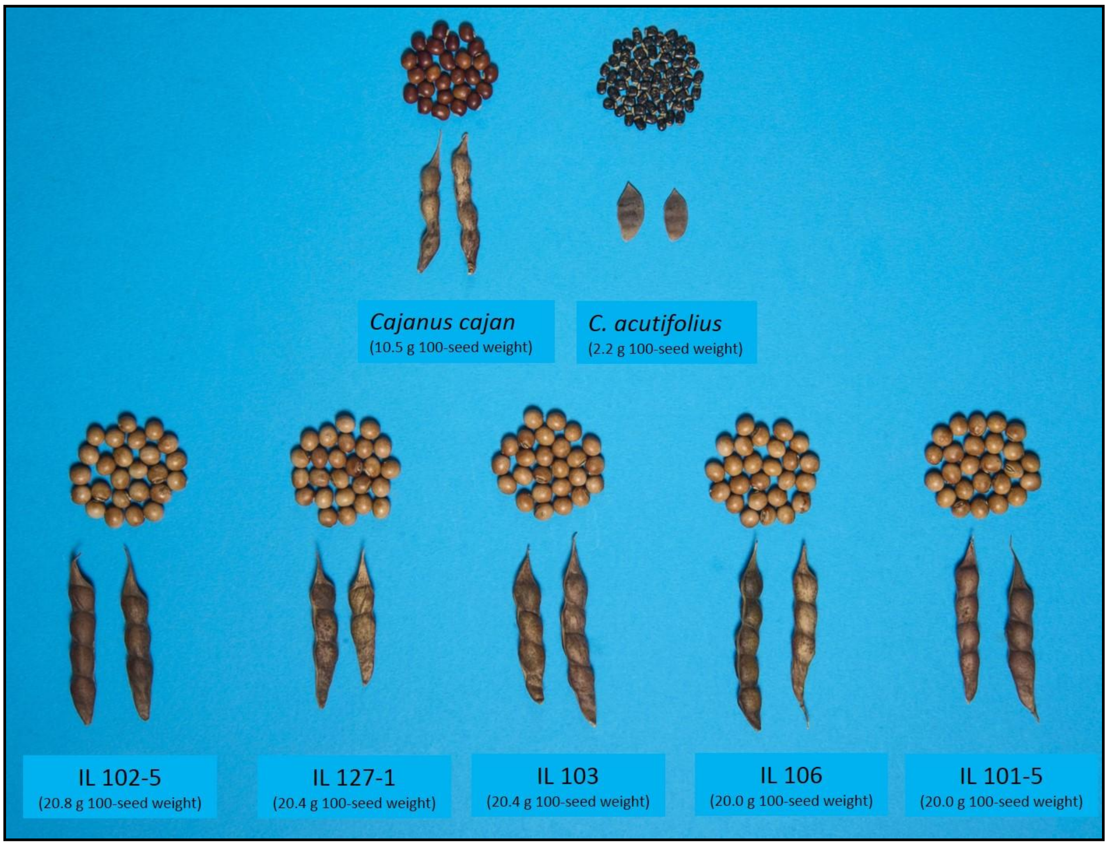

Figure 2. Introgression lines (ILs) derived from *Cajanus acutifolius* having variations for pod traits and high 100-seed weight

identified accessions/species with a diverse combination of morphological and biochemical components associated with expression of resistance to pod borer. Wild *Cajanus* accessions having high levels of antixenosis for oviposition (*C. acutifolius*, and *C. sericeus*), high expressions of antibiosis (*C. acutifolius* (Benth. ex Baker) Maesen) high density of type 'C' and 'D' trichomes (*C. scarabaeoides*, and *C. sericeus*) and low amounts of sugars and high amounts of tannins and polyphenols (13) were used in the crossing program with an objective to combine these components into common genetic background. Using four wild species, *C. acutifolius*, *C. sericeus*, *C. scarabaeoides*, and *C. albicans* (Wight & Arn.) Maesen and two pigeonpea cultivars, ICPL 87119, and ICP 8863, eight interspecific F_1 crosses were generated. Four F_1 crosses generated using a common cultivar were further crossed amongst themselves to generate two 4-way F_1 crosses in order to combine different components of pod borer resistance from different species into a common genetic background. These crosses are being advanced further to develop ILs with high levels of resistance against pod borer.

Conclusion

Wild species harbor many useful genes and can be utilized effectively to create enormous genetic variability following wide hybridization. At ICRISAT, utilization of wild *Cajanus* species has contributed significantly through the development of CMS systems for pigeonpea improvement. Further, development of pre-breeding populations using pigeonpea cultivars as recipients and wild *Cajanus* species as donors has resulted in enriching the variability for different traits in the primary genepool. Introgression lines (ILs) having good agronomic performance and high levels of resistance against important biotic stresses have been identified, which can be utilized in breeding programs to develop new cultivars with a broad genetic base. Efforts are underway to combine different components of pod borer resistance from different species into common genetic background following complex interspecific crosses and would pave a way to develop ILs having high levels of pod borer resistance. Overall, these pre-breeding activities have led to the genetic enhancement by increasing the extent of useful diversity in the primary genepool for further use by the breeders.

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