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RESEARCH ARTICLE

Phenotypic diversity in *Cajanus* species and identification of promising sources for agronomic traits and seed protein content

**H. D. Upadhyaya · K. N. Reddy · Sube Singh ·
C. L. L. Gowda**

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Abstract A total of 198 accessions representing 18 species of the genus *Cajanus*, assembled at the ICRISAT genebank, was characterized for 27 morpho-agronomic traits at ICRISAT farm, Patancheru, India. Newman–Keuls test of significance for mean values indicated significant differences among the species for one or more traits under study. Mean diversity for all traits was maximum in *C. scarabaeoides* ($H' = 0.590 \pm 0.010$). First three principal components (PCs) captured 84.3 % of total variation among all species. Cluster analysis resulted in three clusters. *C. albicans* and *C. mollis* formed Cluster 1; *C. cajanifolius*, *C. crassus* and *C. platycarpus* formed Cluster 2 and *C. acutifolius*, *C. scarabaeoides*, *C. lineatus* and *C. sericeus* formed Cluster 3. *C. platycarpus* for extra early flowering (34–40 days); *C. scarabaeoides* for early flowering (51–118 days); *C. albicans* for broad pods; *C. mollis*, *C. albicans*, *C. cinereus* for more seeds per pod (>6) and *C. crassus*, *C. cajanifolius*, *C. mollis*, *C. platycarpus* and *C. albicans* for high seed protein (>30 %) were found as promising sources. Long duration perennial species such as *C. crassus*, *C. mollis* and *C. albicans* are good sources for forage. Five accessions (ICP 15661, ICP 15664, ICP 15666, ICP 15668 and ICP 15671) of *C. platycarpus*, two accessions (ICP 15653 and ICP 15658) of *C. mollis* and one accession each of *C. acutifolius* (ICP 15611), *C. albicans* (ICP 15620), *C. cajanifolius* (ICP 15632), *C. crassus* (ICP 15768), *C. lineatus* (ICP 15646), *C. scarabaeoides* (ICP 15922) and *C. sericeus* (ICP 15760), found as promising for multiple trait combinations are useful in pigeonpea improvement programs.

Keywords *Cajanus* species · Characterization · Diversity · Genepool · Germplasm · Wild relatives

Introduction

Pigeonpea (*Cajanus cajan* (L.) Millspaugh) is the sixth most important legume crop in the world with seed protein up to 31 %. The crop originated in India, has several special traits and finds a unique place in the farming systems adopted by the farmers in a large number of developing countries. Besides its cultivation to produce dry grains, pigeonpea is grown to culture lac-producing insects, to reduce soil erosion on sloping lands and its green seeds are used as vegetables, leaves as fodder and stems as fuel. Pigeonpea enriches soil through fixing atmospheric nitrogen by Rhizobium bacteria present in the roots. Considerable progress in pigeonpea improvement has been made by using variability within the cultivated species and

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H. D. Upadhyaya (✉) · K. N. Reddy · S. Singh ·
C. L. L. Gowda
International Crops Research Institute for the Semi-Arid
Tropics (ICRISAT), Genetic Resources,
Patancheru 502324, Andhra Pradesh, India
e-mail: H.Upadhyaya@CGIAR.ORG

globally, pigeonpea is grown on 4.5 million ha with a production of 3.5 million metric tons and productivity of 863 kg ha^{-1} (FAO 2009). However, the crop still suffer from production constraints such as pod borer (*Helicoverpa armigera* Hübner) and podfly (*Melanagromyza chalcosoma* Spencer); the diseases sterility mosaic disease (SMD), wilt (*Fusarium udum* Butler) and phytophthora blight (*Phytophthora drechsleri* Tucker); and abiotic stresses such as salinity and water logging conditions.

Wild species have evolved to survive natural constraints such as droughts, floods, extreme heat, cold, pests and diseases that cause much damage to related crops. Wild species also possess genes for useful agronomic traits (Mallikarjuna et al. 2011a; Upadhyaya 2006; Upadhyaya et al. 2011). Crop wild relatives (CWR) are important components of agroecosystems as they constitute potential sources for genes for future breeding programs. Genetic base of cultivated genepools of many food crops are narrow. Discovery and incorporation of genes from wild species provide a means to sustain crop improvement. Pigeonpea breeders have a rich source of variability in the form of wild relatives, particularly in the genus *Cajanus*, which can play a major role in the introgression of genes for pest and disease resistance, useful agronomic traits, nutritional traits, and cytoplasmic male sterility systems (CMS) (Bohra et al. 2010; Rao et al. 2003). *C. albicans* was found as a source for sterility mosaic disease (SMD), pod borer and podfly resistance, high protein content, drought tolerance and high pod set; *C. acutifolius* for pod borer and podfly resistance and salinity tolerance; *C. lineatus* for SMD and podfly resistance, high protein content, salinity and drought tolerance; *C. sericeus* for SMD, phytophthora blight, pod borer and podfly resistance, high protein content, salinity and drought tolerance and high fruit set; *C. volubilis* (*C. crassus*) for SMD and high protein content; *C. mollis* for high seed protein content, *C. reticulatus* for pod borer resistance; *C. platycarpus* for phytophthora blight and pod borer resistance, salinity tolerance, early flowering, photoperiod insensitivity and high pod set are the important sources for useful genes (Bohra et al. 2010; Dodia et al. 1996; Mallikarjuna et al. 2006; Rao et al. 2003; Remanandan 1981; Saxena et al. 2002; Sharma et al. 2003; Srivastava et al. 2006).

The genebank at ICRISAT, Patancheru, India conserves 13,632 accessions of pigeonpea from 74 countries. This is the single largest collection of pigeonpea germplasm assembled in the world. The germplasm collection includes 8,215 landraces, 4,795 breeding lines, 67 improved cultivars and 555 wild accessions belonging to 66 species of six genera. The pigeonpea wild relatives' collection at ICRISAT genebank has not been characterized and evaluated systematically. Main reasons could be low seed quantity, lack of resources, difficulties in phenology and growth habit and lower priority than the cultivated species. However, limited evaluation of different species by researchers across the world indicated that the wild genepool of pigeonpea, particularly the secondary genepool is a promising source for various biotic and abiotic stresses (Bohra et al. 2010; Mallikarjuna et al. 2011a). Most of the species belonging to the secondary genepool and some species of the tertiary genepool such as *C. platycarpus*, crossable with cultivated pigeonpea either by conventional breeding or by using biotechnology tools, possess genes for useful agronomic traits and play an important role in pigeonpea improvement (Mallikarjuna et al. 2011a; Reddy et al. 2000). Therefore, the present study aims at systematic characterization of *Cajanus* species germplasm assembled at ICRISAT genebank for morpho-agronomic characters to assess the diversity within and between different species to enhance their utilization in pigeonpea improvement programs globally.

Materials and methods

The pigeonpea wild genepool assembled at ICRISAT genebank includes 213 accessions representing 19 species of genus *Cajanus* including *C. acutifolius* (F.v.Muell.) Maesen, *C. albicans* (Wight et Arn.) Maesen, *C. cajanifolius* (Haines) Maesen, *C. cinereus* (F.v.Muell.) F.v.Muell., *C. confertiflorus* F.v.Muell., *C. crassus* (Prain ex King) Maesen, *C. elongatus* (Benth.) Maesen, *C. goensis* Dalzell, *C. lanceolatus* (W.V.Fitzg.) Maesen, *C. latisepalus* (Reyn. et Pedl.) Maesen, *C. lineatus* (W. et A.) Maesen, *C. marmoratus* (R. Br. ex Benth.) F.v.Muell., *C. mollis* (Benth.) Maesen, *C. platycarpus* (Benth.) Maesen, *C. reticulatus* (Dryander) F.v.Muell., var. *reticulatus*, var. *grandifolius* (F.v.Muell.) Maesen, *C. rugosus* (Wight et Arn.) Maesen, *C. scarabaeoides* (L.) Thouars, *C. sericeus* (Benth. ex Baker) Maesen and *C. trinervius*

(DC.) Maesen. The other related genera assembled include *Rhynchosia* (33 species, 303 accessions), *Flemingia* (8 species, 18 accessions), *Eriosema* (3 species and 7 accessions), *Dunbaria* (2 species, 12 accessions) and *Paracalyx* (1 species, 2 accessions). A total of 198 accessions belonging to 18 species of genus *Cajanus* from nine countries were characterized and evaluated at ICRISAT farm, Patancheru (17.53°N, 78.27°E, 545 m.a.s.l, and 600 km away from the sea), India. Because of low seed quantity available in the genebank, one accession of *C. marmoratus*, two accessions each of *C. mollis* and *C. scarabaeoides*; 3 accessions of *C. reticulatus* and 4 accessions of *C. rugosus* and all accessions (3) of *C. trinervious* were not sown and excluded from the study. Seeds of all species were scarified before sowing by giving a small cut on seed coat to overcome the problem of hard seed coat-induced dormancy. The accessions were sown in alfisol-Patancheru Soil Series (Udic Rhodustolf) precision field on 30 June 2007. Mature pods of different species were harvested depending on their maturity during 2007–2009. Accessions were grown in a single row of 8-m length with a row to row distance of 100–300 cm and plant to plant distance of 50–100 cm within the row depending upon the growth habit of the species. The soil received a basal dose of 20 kg N and 40 kg P_2O_5 ha^{-1}. Standard agronomic practices were followed to raise a good crop. The crop was protected from weeds, pests and diseases to the research standards. The crop was irrigated as and when necessary. Depending upon the growth habit of some of the species, plants were given support using bamboo pegs and iron frames to overcome the problem of damage to the plants and pods due to soil contamination.

Observations were recorded on 18 qualitative traits (life cycle, growth habit, stem color, leaf color, leaflet shape, leaf hairiness, flowering pattern, base flower color, flower streak color, flower streak pattern, pod color, pod shape, pod hairiness, seed color pattern, primary seed color, secondary seed color, seed shape and seed strophiole) and 9 quantitative traits (leaflet length, leaflet width, days to 50 % flowering and days to maturity (first 5 pods), pod length, pod width, seeds per pod, 100 seed weight and seed protein content following the Descriptors for Pigeonpea (IBPGR and ICRISAT 1993). Pattern of growth habit in whole plot was recorded as growth habit. Color of the stem was recorded at the time of days to 50 % flowering on plot basis. Leaflet hairiness on lower surface was scored on a 1–4 scale (1 = short and 4 = long). The main color of standard petals was recorded as the base color. Color of streaks on dorsal side of the vexillum (flag) was recorded as flower streak color. Pattern of streaks on the dorsal side of the flag (standard petal) was recorded as flower streak pattern. Pattern of flowering habit in whole plot was recorded as flowering pattern. Main color of pods was recorded as pod color at seed filling stage. Pod hairiness was scored on 1–6 scale (1 = short hairs and 6 = long hairs). The color pattern on the seed coat was recorded after sun drying. Base color of seed coat was recorded as primary seed color. Days to 50 % flowering, days to maturity (first five pods) and 100 seed weight was recorded on plot basis. Leaflet length and width was recorded on 10 fully expanded leaflets picked from three plants. Pod length, pod width and seeds per pod were recorded on 10 pods harvested from three representative plants. A random well cleaned seed sample from each plot was used to estimate seed protein content by Kjeldal method with sulphuric acid-selenium digestion, at the ICRISAT's crop quality unit (Sahrawat et al. 2002).

To assess the diversity within and between species, 184 accessions of 9 wild *Cajanus* species (*C. acutifolius*, *C. albicans*, *C. cajanifolius*, *C. crassus*, *C. lineatus*, *C. mollis*, *C. platycarpus*, *C. scarabaeoides* and *C. sericeus)* having more than three accessions and sufficient plant stand in the field were considered for data analysis. In all other species, observations were recorded on single plants. Agronomic data were analyzed using residual maximum likelihood (REML) procedure in Genstat 14 release (http://www.vsni.co.uk) to partition genotypic variance into between species and between accessions within species. The respective standard errors were estimated and used to determine the significance of variance components. Mean, range and variances were calculated for 9 quantitative characters of each species and for the entire collection. The mean values of different traits were compared using the Newman–Keuls procedure (Newman 1939; Keuls 1952). Homogeneity of phenotypic variances was tested by Levene's test (Levene 1960). Shannon and Weaver (1949) diversity index (H') was used to measure and compare the phenotypic diversity for all 9 traits of all species. Phenotypic correlations were estimated among all quantitative characters of each species as well as over all accessions of all species and tested for their significance (Snedecor and Cochran 1980). Mean observations of traits were standardized by subtracting the mean value of the character from each observation and subsequently dividing by its standard deviation. This resulted in standardized values for each trait with average 0 and standardized deviation of 1 or less. The standardized values were used to perform principal component analysis (PCA) on Genstat 14 release. Cluster analysis (Ward 1963) was performed using scores of first three principal components (PCs) to group the species. Most diverse accessions were identified based on genetic distances between accessions of each species. In total, 14 best accessions of different *Cajanus* species possessing multiple trait combinations were identified as promising. Using Gower's dissimilarity matrix (Gower 1985), these accessions were clustered to study the grouping of species and genepool classification.

Results

Geographical distribution

The *Cajanus* wild species genepool assembled at ICRISAT genebank includes 213 accessions representing 19 species from nine countries (Table 1). India was the major source for *Cajanus* germplasm accounting for 126 accessions of 12 species followed by Australia with 31 accessions of 9 species and Sri Lanka with 29 accessions of four species. India was the predominant source for germplasm of *C. scarabaeoides*, *C. platycarpus*, *C. albicans* and *C. lineatus* representing 10 or more accessions each. Among the species, *C. scarabaeoides* (102 accessions), *C. albicans* (20 accessions), *C. platycarpus* (17 accessions) and *C. acutifolius* (12 accessions) are the major collections of *Cajanus* species at the ICRISAT genebank.

Diversity in the collection

Qualitative traits

Frequency distribution of different qualitative traits of *Cajanus* species are given in Table 9.

Life cycle All accessions of *C. cajanifolius*, *C. lanceolatus*, *C. marmoratus*, *C. platycarpus*, *C. scarabaeoides* and *C. cericeus*, which completed life cycle in a year were grouped as annuals and all other species completing their life cycle in more than 1 year were recorded as perennials. There were six annuals and 12 perennial species in the collection.

Growth habit Five growth habits (climber, creeper, erect, spreading and semi-spreading) were observed in the collection. *C. albicans*, *C. crassus* and *C. goensis* were found to be climbers. *C. elongatus*, *C. marmoratus*, *C. mollis*, *C. platycarpus*, *C. rugosus* and *C. scarabaeoides* were creepers. One accession of *C. reticulatus* and all accessions of *C. sericeus* showed erect growth habit. *C. acutifolius*, *C. confertiflorus*, *C. lineatus* and four accessions of *C. reticulatus* had spreading growth habit. All accessions of *C. cajanifolius*, *C. cinereus*, *C. lanceolatus* and *C. latisepalus* showed semi-spreading growth habit.

Stem color Six stem colors (brown, dark brown, green, purple, green + purple and purple + green) were observed in the collection. Within *C. acutifolius*, two accessions had brown stems and 10 accessions had green stems. The plants of the *C. confertiflorus* accession produced a mixture of green and purple stems. In *C. platycarpus*, six accessions produced green stems, 9 accessions green and purple stems and one accessions purple and green stems. In *C. reticulatus*, one accession produced brown and one accession produced dark brown and two accessions produced green stems. In *C. scarabaeoides*, 58 accessions were with a mixture of green and purple stem plants and 42 accessions with purple and green stem plants. Plants of all other species produced green color stems. One accession of *C. cajanifolius* produced purple stems.

Leaf color Three leaf colors (dark green, green and light green) were observed in the collection. All accessions of *C. albicans*, *C. crassus*, *C. lineatus*, 16 accessions of *C. platycarpus* and one accession of *C. reticulatus* produced dark green leaves. One accession of *C. acutifolius* and one accession of *C. latisepalus* produced light green leaves. Plants of all accessions of other species possessed green leaves.

Leaflet shape All accessions of all species produced trifoliate leaves. Six leaflet shapes were found in the collection of *Cajanus*. All accessions of *C. mollis*

Table 1 Geographical origin of *Cajanus* species germplasm assembled, characterized and evaluated at ICRISAT genebank, Patancheru, India

Species	Australia	Fiji	UK	Indonesia	India	Sri Lanka	Myanmar	Philippines	Papua New Guinea	Unknown	Accessions characterized	Total accessions
C. acutifolius (F.v.Muell.) Maesen	12										12	12
C. albicans (Wight et Arn.) Maesen					14	6					20	20
C. cajanifolius (Haines) Maesen					5						5	5
C. cinereus (F.v.Muell.) F.v.Muell.	1										1	1
C. confertiflorus F.v.Muell.	1										1	1
C. crassus (Prain ex King) Maesen					8		2				10	10
C. elongatus (Benth.) Maesen					1						1	1
C. goensis Dalzell					1						1	1
C. lanceolatus (W.V.Fitzg.) Maesen	1										1	1
C. latisepalus (Reyn. et Pedl.) Maesen	1										1	1
C. lineatus (W. et A.) Maesen					10						10	10
C. marmoratus (R. Br. ex Benth.) F.v.Muell.	1										1	2
C. mollis (Benth.) Maesen					6						6	8
C. platycarpus (Benth.) Maesen					17						17	17
C. reticulatus (Dryander) F.v.Muell.												
var. *reticulatus*	2										2	3
var. *grandifolius* (F.v.Muell.) Maesen	1								2		3	5
C. rugosus (Wight et Arn.) Maesen						2					2	6
C. scarabaeoides (L.) Thouars	6	2	1	16	55	16	1	2		1	100	102
C. sericeus (Benth. ex Baker) Maesen	1				3						4	4
C. trinervius (DC.) Maesen					1	2					–	3
No. of species characterized	9	1	1	1	10	3	2	1		1	18	–
No. of accessions characterized	27	2	1	16	120	24	3	2	2	1	198	–
Total species	9	1	1	1	12	4	2	1	1	1	19	–
Total accessions	31	2	1	16	126	29	3	2	2	1	–	213

produced elliptic leaflets. All accessions of *C. acutifolius*, *C. cajanifolius*, *C. lanceolatus* and *C. lineatus* produced lanceolate leaflets. Leaflet shape was oblanceolate in *C. sericeus*; obovate in *C. albicans*, *C. confertiflorus*, *C. elongatus*, *C. rugosus* and *C. scarabaeoides*; ovate in *C. cinereus*, *C. goensis*, *C. latisepalus*, *C. platycarpus* and *C. reticulatus*, and rhomboidal in *C. crassus* and *C. marmoratus.*

Leaflet hairiness Leaflets of all accessions of all species were hairy. Except *C. lineatus*, leaves of all species scored 1–2 for leaf hairiness. Eight accessions of *C. lineatus* scored 3 and two accessions scored 4 for leaf hairiness.

Flowering pattern This was a monomorphic trait. All accessions of all species showed indeterminate flowering habit.

Base flower color This was a monomorphic trait and all the accessions of all species produced basically yellow flowers.

Flower streak color Four flower streak colors (no streaks, purple, red and red + no streaks) were observed in the collection. Red color streaks were predominant in *C. acutifolius*, *C. albicans*, *C. cajanifolius*, *C. cinereus*, *C. confertiflorus*, *C. elongatus*, *C. lanceolatus*, *C. latisepalus*. *C. reticulatus* and *C. scarabaeoides.* Flowers with no streak color were observed in one accession of *C. albicans*, nine accessions each of *C. crassus* and *C. lineatus*; all accessions of *C. goensis*, *C. marmoratus*, *C. mollis*, *C. platycarpus*, *C. sericeus* and *C. rugosus*; 12 accessions of *C. scarabaeoides* and one accession of *C. reticulatus.*

Flower streak pattern Five classes were observed for this trait. Dense streaks were found in one accession of *C. acutifolius*, 10 accessions of *C. albicans*, 3 accessions of *C. cajanifolius* and one accession each of *C. cinereus*, *C. lanceolatus* and *C. scarabaeoides*. Few streak pattern was observed in 51 accessions belonging to *C. acutifolius* (2), *C. albicans* (9), *C. confertiflorus* (1) *C. elongatus* (1), *C. lineatus* (1), *C. reticulatus* (1) and *C. scarabaeoides* (36). Medium streaks were observed in 63 accessions belonging to *C. acutifolius* (9), *C. cajanifolius* (2), *C. crassus* (1), *C. latisepalus* (1), *C. reticulatus* (1) and *C. scarabaeoides* (49). Two accessions of *C. scarabaeoides* were with a mixture of plants producing medium streaks and no streaks. As many as 63 accessions of 11 species produced no streaks.

Pod color Two classes (green and mixed) of pod color were found in the collection. Except 3 accessions of *C. cajanifolius*, one accession each of *C. marmoratus* and *C. platycarpus*, which produced mixed color pods, all accessions of all other species only produced green pods.

Pod shape Only two classes (cylindrical and flat) were observed for this trait. Except one accession each of *C. cajanifolius* and *C. elongatus* and four accessions of *C. sericeus*, which produced cylindrical pods, all accessions of other species produced flat pods.

Pod hairiness Pods of all accessions of *C. acutifolius*, *C. albicans*, *C. cajanifolius*, *C. cinereus*, *C. crassus*, *C. elongatus*, *C. mollis* and *C. rugosus* produced relatively short hairs with a score ranging from 1 to 3. On the other hand, *C. goensis*, *C. lanceolatus*, *C. latisepalus* and *C. lineatus*, *C. marmoratus*, *C. platycarpus*, *C. reticulatus*, *C. scarabaeoides* and *C. sericeus* produced longer hairs with a score ranging from 4 to 6.

Seed color pattern In the collection, three solitary (mottled, plain and speckled) and four mixed (mottled + plain, plain + mottled, plain + speckled and speckled + plain) seed color patterns were found. Mottled pattern was predominant in most of the species. *C. rugosus* and *C. goensis* produced plain and speckled seed color patterns, respectively.

Primary seed color Six primary seed colors were observed in the collection. Only one accession of *C. lineatus* produced brown seeds. The seed color of *C. rugosus* was dark purple. Primary seed colors of all other species were variants of gray ranging from light gray to dark gray. About 54 % of total accessions produced dark gray colored seeds.

Secondary seed color Six classes (black, dark gray, gray, gray brown, light gray and none) were observed for this trait. One accession each of *C. marmoratus* and *C. mollis* produced black seeds. Seeds of 39 accessions of *C. scarabaeoides* and 2 accessions of *C. rugosus* have no secondary color. Seeds of all other species produced variants of gray as secondary color. About 62 % of accessions of 15 species produced seeds having gray as secondary seed color.

Seed shape Three seed shapes (elongate, square, and oval) were found in the collection. Six accessions of *C. platycarpus* produced elongate seeds and one accession produced elongate and square seeds. Seeds of *C. goensis*, *C. latisepalus* and *C. marmoratus* were of oval shape. All accessions of other species produced square shape seeds.

Seed strophiole This was the important diagnostic trait to identify wild species. Seed strophiole was observed in seeds of all accessions of all species, in *C. cajan* it is very rare.

Quantitative traits

(REML) analysis Genotypic variance (σ^2g) was significant for all the traits in the entire set (184 accessions) and in all species except *C. sericeus* (4 accessions), *C. cajanifolius* (5 accessions), and *C. mollis* (8 accessions). The genotypic variances estimated for all traits of all species (with >8 accessions) and entire collection revealed significant differences among species (Table 2). Highly significant ($P < 0.0001$) Wald (1943) statistics revealed significant differences among the species.

Range and means Wide variation was observed in *C. acutifolius* for leaf width (1.6–9.5 mm) and days to maturity (130–290 days); in *C. albicans* for pod width (9.6–15.0 mm) and seeds per pod (4.8–7.2); in *C. crassus* for leaflet length (8.3–11.3 cm); in *C. mollis* for days to 50 % flowering (222–392 days) and in *C. platycarpus* for pod length (3.5–4.7 cm), 100-seed weight (3.8–6.9 g) and seed protein content (17.9–33.3 %) (Table 3). Minimum and maximum values for different traits indicated *C. crassus* for long and broad leaflets; *C. mollis* for long pods; *C. albicans* for late flowering (days to 50 % flowering = 460–502 days), maturity (510–560 days) and broad pods (up to 15 mm); *C. mollis* for more seeds per pod (8.6); *C. platycarpus* for large seeds (6.9 g 100 seeds^{-1}) and *C. crassus* for high seed protein content (33.8 %) are the important sources. On the other hand, days to 50 % flowering in *C. platycarpus* ranged from 34 to 40 days

Table 2 Genotypic variance (σ^2g) for different traits of *Cajanus* species evaluated at ICRISAT, Patancheru, India

Trait	Leaflet length (cm)	Leaflet width (cm)	Days to 50 % flowering	Days to maturity (first 5 % pods)	Pod length (cm)	Pod width (mm)	Seeds per pod	100 seed weight (g)	Seed protein content (%)
Entire collection	0.20**	0.50**	493.50**	1,083.00**	0.10**	0.40**	0.20**	0.20**	9.20**
C. acutifolius	0.20*	4.80*	2,129.00*	2,206.00*	0.01*	0.50*	0.30*	0.01*	2.80*
C. albicans	0.60**	0.60**	131.10**	7,200.00**	0.10**	1.20**	0.30**	0.10**	18.90**
C. cajanifolius	<0.0001	<0.0001	210.00	55.20	0.10	1.10	0.50	0.80	28.50
C. crassus	1.20*	0.90*	294.40*	73.30*	0.10*	0.10*	0.10*	0.10*	12.20*
C. lineatus	0.50*	0.70*	687.50*	387.80*	0.10*	0.20*	0.10*	0.01*	26.80*
C. mollis	0.20	0.20	6,527.00	1,888.00	0.10	0.20	0.10	<0.0001	22.30
C. platycarpus	0.10**	0.40**	3.30**	53.00**	0.10**	0.00**	0.20**	1.30**	27.20**
C. scarabaeoides	0.10**	0.10**	182.60**	148.40**	0.01**	0.30**	0.20**	0.10**	2.30**
C. sericeus	<0.0001	0.10	2.70	3.70	<0.0001	0.10	<0.0001	0.10	22.30
Wald statistic (species)	2,463.50	1,279.60	6,514.00	2,757.30	2,421.80	2,820.58	1,097.10	1,072.70	162.10
F probability	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

* Significant at $P = 0.05$; ** significant at $P = 0.01$

and maturity from 64 to 84 days) and it ranged from 51 to 118 days in *C. scarabaeoides*.

Newman–Keuls (Newman 1939; Keuls 1952) test of significance for mean values indicated significant differences among the species for one or more traits under study (Table 4). *C. crassus* produced long (9.7 cm) and broad leaflets (8.4 mm) and differed significantly from all other species for these traits. Whereas, *C. sericeus* produced short leaflets and *C. lineatus* produced narrow leaflets. *C. albicans* flowered and matured very late and differed significantly with all other species. In this respect, *C. platycarpus* was extra early for flowering (36 days) and maturity (74 days) and produced larger seeds (5.7 g 100 seeds^{-1}) and differed significantly from all other species for these traits. *C. scarabaeoides*, which is crossable with cultivated pigeonpea and possess resistant genes for several biotic and abiotic stresses, flowered early (70 days) and matured early too (126 days). *C. mollis* produced longest pods (4.6 cm) among wild species with more seeds per pod (8.3) and differed significantly from all other species for these traits. *C. albicans* produced broader pods than all other wild species. *C. crassus* had highest seed protein content (28.3 %) but did not differed significantly from *C. cajanifolius* (27.2 %), *C. sericeus* (26.7 %), *C. lineatus* (26.5 %), *C. albicans* (25.5 %), *C. mollis* (25.1 %) and *C. platycarpus* (23.8 %). Raw data of other species, which were not included in the analysis (few accessions) indicated longest leaflets in *C. reticulatus* (12.5 cm) and very late flowering (520 days) and maturity (555 days) in *C. goensis*.

Variances The homogeneity of variances of the species was tested for all the nine quantitative traits by Levene's test (Levene 1960) (Table 4). The variances were heterogeneous ($P \leq 0.0001$) for leaflet length, days to 50 % flowering, pod length, 100 seed weight and seed protein content. Variance for leaflet width was significant only at 5 %.

Diversity The Shannon–Weaver diversity index (H') (Shannon and Weaver 1949) was calculated over all accessions of all species and for each species to compare phenotypic diversity among the species for nine quantitative traits (Table 5). A low H' indicates extremely unbalanced frequency classes for an individual trait and lack of genetic diversity in the collection or species. The diversity index values (H') were variable among traits. In the entire collection, diversity index (H') ranged from 0.313 ± 0.026 for days to 50 % flowering to 0.553 ± 0.026 for seed protein content. Mean diversity over all traits was maximum in *C. scarabaeoides* ($H' = 0.590 \pm 0.010$) and it was lowest in *C. sericeus* ($H' = 0.322 \pm 0.051$). Accessions of *C. scarabaeoides* were found to be highly diverse for leaflet length and width, days to maturity, pod width, 100 seed weight and seed protein content. Similarly, accessions of *C. albicans* were diverse for days to 50 % flowering and seeds per pod and those of *C. platycarpus* were highly diverse for pod length (Table 5).

Cluster analysis Principal component analysis was carried out using standardized data of nine quantitative traits. First three PCs captured 84.3 % of total variation. A hierarchical cluster analysis (Ward 1963) conducted on the scores of the first three PCs resulted in three clusters (Fig. 1). *C. albicans* and *C. mollis* formed Cluster 1; *C. cajanifolius*, *C. crassus* and *C. platycarpus* formed Cluster 2 and *C. acutifolius*, *C. scarabaeoides*, *C. lineatus* and *C. sericeus* formed Cluster 3.

Character associations Correlation coefficients were also calculated for different trait combinations in each species and over all accessions of all species. Skinner et al. (1999) suggested that correlation coefficients greater than 0.707 or less than −0.707 are biologically meaningful, so that at least 50 % of the variation in one trait is predicted by the other. The trait combinations showing meaningful correlations in different species and over all accessions of all species are given in Table 6. A total of 28 trait combinations showed correlations greater than 0.707 or less than −0.707. Twenty of these were positive and eight were negative correlations. Leaf length and leaf width showed strong correlation ($r = 0.825$–0.931) in *C. albicans*, *C. mollis* and across all tested species. Days to 50 % flowering and days to maturity had strong positive correlation in *C. acutifoilus*, *C. crassus*, *C. lineatus*, *C. platycarpus*, *C. scarabaeoides* and over all species. Pod length with pod width in *C. sericeus*, pod length with seeds per pod in *C. lineatus* and with 100-seed weight in *C. platycarpus* also showed significant positive correlation (Table 6). Correlations were positive between pod width and seeds per pod in

Table 3 Range of variation for different traits of *Cajanus* species evaluated at ICRISAT, Patancheru, India

Species	Leaflet length (cm)	Leaflet width (cm)	Days to 50 % flowering	Days to maturity (first 5 % pods)	Pod length (cm)	Pod width (mm)	Seeds per pod	100 seed weight (g)	Seed protein content (%)
Entire collection	2.3–11.3	1.1–9.6	34–502	64–560	1.2–5.0	1.3–15.0	2.0–8.6	1.1–6.9	16.3–33.8
C. acutifolius	4.1–5.5	1.6–9.5	60–220	130–290	2.0–2.5	6.6–9.0	2.8–4.4	2.1–2.4	16.3–20.7
C. albicans	4.4–6.8	3.1–5.8	460–502	510–560	3.4–4.4	9.6–15.0	4.8–7.2	1.7–2.8	20.1–32.5
C. cajanifolius	6.1–6.5	2.1–2.4	84–120	140–158	3.8–4.5	7.8–10.0	3.6–5.2	3.5–5.8	19.3–33.6
C. crassus	8.3–11.3	6.5–9.6	140–198	220–240	3.1–4.2	9.6–10.6	4.4–5.3	3.2–4.1	20.3–33.8
C. lineatus	3.3–5.7	1.1–4.1	160–220	220–270	1.5–2.4	8.0–9.4	2.0–2.8	1.8–2.3	18.2–30.2
C. mollis	6.0–7.0	3.9–5.1	222–392	310–420	4.2–5.0	7.0–8.2	7.9–8.6	1.6–2.1	21.7–33.4
C. platycarpus	5.7–7.1	5.1–6.9	34–40	64–84	3.5–4.7	1.3–1.6	4.0–6.0	3.8–6.9	17.9–33.3
C. scarabaeoides	2.3–3.8	1.4–2.4	51–118	102–168	1.8–2.7	5.6–8.4	3.6–5.8	1.1–2.9	17.8–27.1
C. sericeus	2.3–2.6	5.2–5.8	98–102	134–138	1.2–1.4	6.0.4–7	2.0–2.0	1.4–2.3	20.9–30.6

Table 4 Mean values[a] and variances for different traits of *Cajanus* species evaluated at ICRISAT, Patancheru, India

Species	Leaflet length (cm)	Leaflet width (cm)	Days to 50 % flowering	Days to maturity (first 5 % pods)	Pod length (cm)	Pod width (mm)	Seeds per pod	100 seed weight (g)	Seed protein content (%)
C. acutifolius	4.52 d	2.56 d	174.17 dc	246.67 c	2.25 d	7.90 d	3.39 e	2.26 d	18.56 b
C. albicans	5.28 c	4.29 c	483.40 a	516.50 a	3.77 c	11.36 a	6.14 b	2.34 d	25.48 a
C. cajanifolius	6.27 b	2.24 d	96.00 e	146.80 d	4.08 b	8.76 c	4.12 d	4.28 b	27.20 a
C. crassus	9.70 a	8.43 a	163.00 d	231.70 c	3.59 c	10.16 b	4.81 c	3.61 c	28.25 a
C. lineatus	4.27 d	1.77 d	190.20 c	239.00 c	1.97 e	8.60 c	2.27 f	2.07 d	26.50 a
C. mollis	6.49 b	4.42 c	286.17 b	346.00 b	4.56 a	7.68 d	8.26 a	1.93 d	25.08 a
C. platycarpus	6.35 b	6.07 b	36.24 g	74.41 e	4.12 b	1.43 f	4.98 c	5.72 a	23.81 a
C. scarabaeoides	3.09 e	1.90 d	70.37 f	126.33 d	2.23 d	6.96 e	4.88 c	1.81 d	20.31 b
C. sericeus	2.46 f	5.50 b	100.00 e	135.50 d	1.26 f	6.55 e	2.00 f	1.80 d	26.70 a
F value (variance)	11.16	17.17	17.13	1.14	4.87	1.89	1.20	20.36	10.15
P	<0.0001	0.0321	<0.0001	0.3406	<0.0001	0.0639	0.301	<0.0001	<0.0001
Species not considered for statistical analysis									
C. reticulatus	8.9	5.9	325	360	2.6	7.9	3.7	1.7	24.1
C. rugosus	5.9	4.4	452	360	2.6	7.9	3.7	1.6	24.1
C. cinereus	6.9	5.2	175	255	4.5	9.2	6.0	2.9	19.4
C. confertiflorus	7.7	6.4	360	–	–	–	–	2.0	–
C. elongatus	3.0	2.2	237	300	–	–	–	1.3	–
C. goensis	4.8	4.6	520	555	4.7	7.8	5.8	2.9	–
C. lanceolatus	10.5	3.6	135	198	3.6	8.4	4.8	4.1	16.9
C. latisepalus	7.6	4.0	160	260	2.9	9.2	3.2	2.4	–
C. marmoratus	3.2	4.0	85	134	2.8	12.8	3.6	6.9	16.8

[a] Means were tested by Newman–Keuls test and means followed by different letters are significantly different at $P = 0.05$

C. cajanifolius and *C. crassus* and between seeds per pod and seed protein content in *C. acutifolius*. Days to 50 % flowering had highest positive correlation with 100-seed weight ($r = 0.982$) in *C. sericeus*. Days to 50 % flowering had shown significant negative correlation with pod length and 100-seed weight in *C.*

Table 5 Shannon–Weaver diversity indices (H') for important agronomic traits of different *Cajanus* species, evaluated at ICRISAT, Patancheru, India

Species	Leaflet length (cm)	Leaflet width (cm)	Days to 50 % flowering	Days to maturity (first 5 % pods)	Pod length (cm)	Pod width (mm)	Seeds per pod	100 seed weight (g)	Seed protein content (%)	Mean	SE
Entire collection	0.479	0.421	0.313	0.374	0.531	0.481	0.525	0.425	0.553	0.456	0.0264
C. acutifolius	0.364	0.125	0.537	0.521	0.477	0.537	0.244	0.439	0.468	0.412	0.0478
C. albicans	0.427	0.566	0.580	0.086	0.532	0.434	0.597	0.506	0.471	0.467	0.0518
C. cajanifolius	0.413	0.458	0.217	0.458	0.413	0.413	0.413	0.217	0.413	0.379	0.0313
C. crassus	0.473	0.530	0.507	0.473	0.473	0.473	0.473	0.556	0.473	0.492	0.0105
C. lineatus	0.518	0.278	0.447	0.447	0.530	0.507	0.507	0.458	0.415	0.456	0.0259
C. mollis	0.439	0.439	0.276	0.217	0.458	0.458	0.458	0.377	0.217	0.371	0.0350
C. platycarpus	0.516	0.588	0.474	0.476	0.573	0.524	0.523	0.368	0.416	0.495	0.0236
C. scarabaeoides	0.615	0.635	0.554	0.612	0.555	0.591	0.551	0.609	0.587	0.590	0.0102
C. sericeus	0.452	0.301	0.452	0.452	0.244	0.244	0.000	0.452	0.301	0.322	0.0505

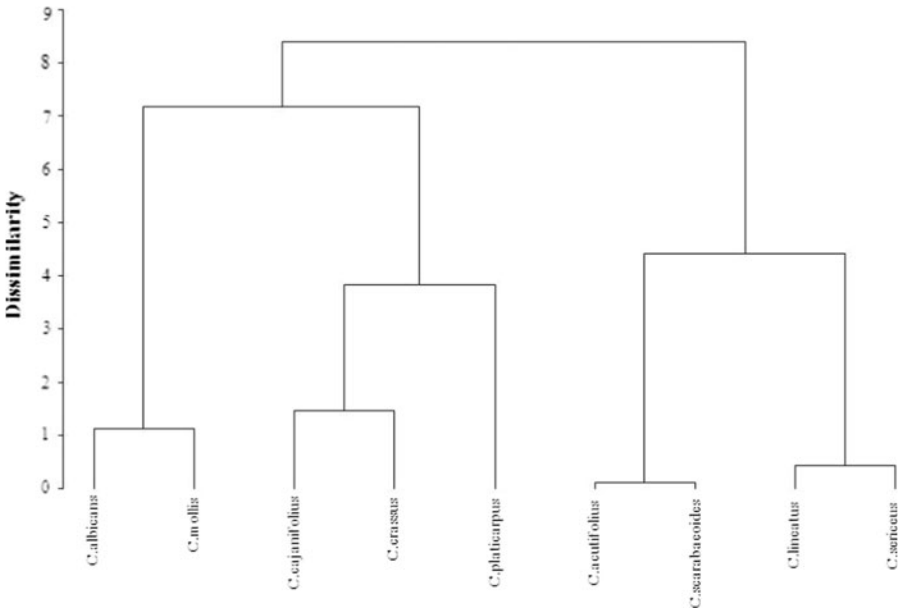

Fig. 1 Dendrogram showing the clusters of *Cajanus* species based on first three principal components (84.3 % variation)

platycarpus and with seeds per pod in *C. acutifolius*. Days to maturity correlated negatively with pod width in *C. mollis*; with pod width and 100 seed weight in *C. platycarpus* and with seeds per pod in *C. acutifolius*. Pod width had significant negative association with seeds per pod in *C. mollis*. A maximum of (seven) meaningful correlations were found in *C. platycarpus* including three positive and four negative correlations.

Promising sources identified

Five accessions of *C. platycarpus*, two accessions of *C. mollis* and one accession each of *C. acutifolius*, *C. albicans*, *C. cajanifolius*, *C. crassus*, *C. lineatus*, *C. scarabaeoides* and *C. sericeus* were found as promising for multiple trait combinations (Table 7). These 14 accessions were clustered using Gower's

Table 6 Important correlation coefficients ($r \geq 0.707$ and <0.707) for different traits of *Cajanus* species, evaluated at ICRISAT, Patancheru, India

Trait combination	Species	*r* value
Leaflet length and width	All species	0.825
	C. albicans	0.931
	C. mollis	0.876
Leaflet length and pod length	All species	0.746
Leaflet width and days to maturity	*C. cajanifolius*	0.938
	C. platycarpus	0.745
Days to 50 % flowering and days to maturity	All species	0.963
	C. acutifolius	0.893
	C. crassus	0.733
	C. lineatus	0.909
	C. platycarpus	0.827
	C. scarabaeoides	0.865
Days to 50 % flowering and pod length	*C. platycarpus*	−0.761
Days to 50 % flowering and seeds per pod	*C. acutifolius*	−0.819
Days to 50 % flowering and 100-seed weight	*C. platycarpus*	−0.840
	C. sericeus	0.982
Days to maturity and pod width	All species	0.729
	C. mollis	−0.891
	C. platycarpus	−0.728
Days to maturity and seeds per pod	*C. acutifolius*	−0.762
Days to maturity and 100-seed weight	*C. platycarpus*	−0.838
Pod length and pod width	*C. sericeus*	0.974
Pod length and seeds per pod	*C. lineatus*	0.829
Pod length and 100-seed weight	*C. platycarpus*	0.882
Pod width and seeds per pod	*C. cajanifolius*	0.891
	C. crassus	0.765
	C. mollis	−0.858
Seeds per pod and seed protein content	*C. acutifolius*	0.793

dissimilarity matrix (Gower 1985) to study the grouping of accessions belonging to different genepools. Accessions of *C. acutifolius*, *C. cajanifolius*, *C. scarabaeoides*, *C. albicans*, *C. crassus* and *C. lineatus* formed Cluster 1; accessions of *C. mollis* and *C. sericeus* formed Cluster 2 and all accessions of *C. platycarpus* formed Cluster 3 (Fig. 2). In Cluster 1, only *C. crassus* belongs to the tertiary genepool and other species belong to the secondary genepool. On the other hand, Cluster 2 consists of species belonging to secondary (*C. sericeus*) and tertiary (*C. mollis*) genepool. Results indicated deviation of *C. platycarpus* from all other species for traits under study. ICP 15768 (*C. crassus*); ICP 15632 (*C. cajanifolius*); ICP 15653 (*C. mollis*); ICP 15666, ICP 15668, ICP 15671 and ICP 15661 (*C. platycarpus*); ICP 15620 (*C. albicans*) and ICP 15760 (*C. sericeus*) have produced more than 30 % seed protein content (Table 7). One accession of *C. platycarpus* (ICP 15666) flowered in 39 days, produced more than five seeds per pod and had 33.3 % seed protein. Four accessions of *C. platycarpus* (ICP 15661, ICP 15666, ICP 15668 and ICP 15671) were extra early flowering (34–37 days) and maturity (64–84 days) and also produced seeds having more than 30 % protein. Accessions of *C. mollis* (ICP 15653 and ICP 15658) produced more than 7 seeds per pod are the promising sources for higher number of seeds per pod. Perennial species such as *C. albicans*, *C. crassus* and *C. mollis*, which produced large leaves and have high seed protein (>30 %) are good sources for forage also.

Most diverse and least diverse pairs of accessions were identified in each species for utilization in crop improvement programs (Table 8). ICP 15655 and ICP 15653 of *C. mollis* were found as the most diverse accessions in the collection with maximum distance (0.671) followed by ICP 15612 and ICP 15610 of *C. acutifolius* (0.555). ICP 15708 and ICP 15697 of *C. scarabaeoides* were found as the most similar with zero distance.

Discussion

Genebanks are able to conserve only a fraction of the total variability that exists in the wild relatives of the crops (Jarvis et al. 2008). The revised genus *Cajanus* consists of 32 species including the cultivated species *Cajanus cajan*. Of the 31 wild species, 13 are endemic to Australia, eight to Indian subcontinent and Myanmar, one to West Africa and rest of the species occur in more than one country (van der Maesen 1990). Though Eastern Africa is considered as the secondary center of diversity for pigeonpea, none of the East African countries were represented in the collection of *Cajanus* germplasm. The summary of *Cajanus* germplasm at ICRISAT genebank indicates wide

Table 7 Promising accessions of *Cajanus* species identified for multiple trait combinations

Species	Gene pool[a]	ICP no.	Leaflet length (cm)	Leaflet width (cm)	Days to 50 % flowering	Days to maturity (first 5 % pods)	Pod length (cm)	Pod width (mm)	Seeds per pod	100 seed weight (g)	Seed protein content (%)
C. acutifolius	GP2	15611	4.1	1.7	140	220	2.5	9.0	4.0	2.4	20.6
C. albicans	GP2	15620	6.6	5.8	500	560	4.4	15.0	6.2	2.5	32.5
C. cajanifolius	GP2	15632	6.2	2.3	84	148	3.8	9.8	4.4	3.5	33.6
C. crassus	GP3	15768	8.3	6.5	168	240	3.5	10.0	4.7	3.8	33.8
C. lineatus	GP2	15646	4.3	1.5	160	220	2.1	9.4	2.4	2.0	30.2
C. mollis	GP3	15653	6.0	4.1	238	310	4.6	8.0	7.9	2.0	33.4
C. mollis	GP3	15658	6.8	4.3	222	340	5.0	7.4	8.6	2.0	21.7
C. platycarpus	GP3	15661	6.0	5.5	35	70	4.7	1.5	5.0	6.9	30.1
C. platycarpus	GP3	15664	6.6	5.9	34	64	4.7	1.5	5.1	6.3	17.9
C. platycarpus	GP3	15666	6.7	6.4	39	82	3.7	1.3	5.0	4.1	33.3
C. platycarpus	GP3	15668	7.1	6.5	35	73	4.3	1.4	5.4	5.9	31.3
C. platycarpus	GP3	15671	6.6	6.6	37	84	3.7	1.3	6.0	4.1	31.3
C. scarabaeoides	GP2	15922	3.5	1.8	62	102	2.7	8.0	4.8	2.9	22.3
C. sericeus	GP2	15760	2.6	5.2	100	134	1.2	6.4	2.0	1.7	30.6

[a] *GP2* secondary genepool and *GP3* tertiary genepool

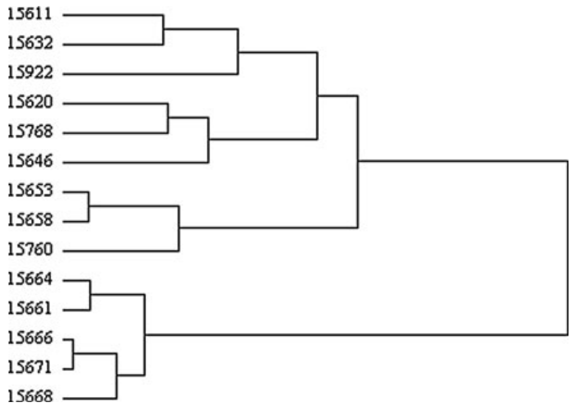

Fig. 2 Dendrogram of promising accessions belonging to different species based on Gower's dissimilarity matrix

geographical and taxonomical gaps in the collection. Hence, there is a need for critical assessment of existing *Cajanus* collection to identify geographical, taxonomical and diversity gaps and possibilities for exploration to fill these gaps before valuable material is lost forever due to rapid urbanization, shifting cultivation, forest fires, climate change, natural calamities, overgrazing, etc. (Upadhyaya and Gowda 2009). *C. scarabaeoides*, which has relatively more accessions (100) in the collection, was highly diverse for many traits emphasizing further exploration for all other species under study in priority areas for additional diversity.

Characterization refers to recording of distinctly identifiable, heritable characteristics, while preliminary evaluation involves recording of a limited number of important agronomic traits. At ICRISAT genebank, germplasm accessions were characterized and evaluated simultaneously. The main reason for characterization is to use wild species germplasm as a source for higher levels of resistance to biotic and abiotic stresses and for additional variability in agronomic traits. Characterization of wild species reveals the true potential of species in crop improvement and adaptation to the changing climate. But, characterization of wild relatives' germplasm is more problematic than characterization of cultivated germplasm. There may be several species of a crop with greater population differences on geographic scale, high genetic variability especially for flowering and maturity (both inter- and intra-species), range of breeding systems and perenniality of species (Lenne and David 1991). Lack of information on adaptation behavior of different species, variation in dormancy, germination, seed production, shattering are the other problems in characterization and evaluation of wild species. Probably because of the above mentioned reasons, only a

Table 8 Most and least diverse accessions (ICP nos.) of different *Cajanus* species

Species	Most diverse accessions	Distance	Least diverse accessions	Distance
C. acutifolius	15612 and 15610	0.555	15609 and 15607	0.017
C. albicans	15620 and 15901	0.507	15927 and 15925	0.013
C. cajanifolius	15630 and 15629	0.458	15873 and 15632	0.159
C. crassus	15776 and 15772	0.400	15770 and 15767	0.053
C. lineatus	15648 and 15645	0.461	15650 and 15649	0.091
C. mollis	15655 and 15653	0.671	15657 and 15654	0.055
C. platycarpus	15667 and 15664	0.501	15673 and 15665	0.010
C. scarabaeoides	15922 and 15913	0.361	15708 and 15697	0.000
C. sericeus	15762 and 15761	0.542	15763 and 15760	0.154

small proportion of total wild relatives germplasm conserved in many genebanks including that of *Cajanus* collection at ICRISAT genebank has been characterized for morpho-agronomic traits (Jarvis et al. 2008).

Characterization and evaluation of different species by various researchers indicated that the wild pigeonpea genepool, particularly the genus *Cajanus* is a promising source for various biotic and abiotic stresses in addition to possessing some important agronomic traits (Mallikarjuna et al. 2011a; Reddy et al. 2000; Sharma et al. 1993; Sharma 2006; Srivastava et al. 2006). Most of the *Cajanus* species are palatable to cattle and goats, and many wild species of pigeonpea are useful in rearing lac insects (van der Maesen 1990). Therefore, the need to use wild *Cajanus* species, particularly from secondary genepool is well recognized. The results of the present study revealed that *C. albicans*, *C. crassus*, and *C. mollis* produced large leaves and high biomass for a longer period are important as animal feed. *C. platycarpus* and *C. scarabaeoides* were extra early and early flowering (ICRISAT 1978; Mallikarjuna et al. 2006; Upadhyaya 2006); *C. albicans*, *C. crassus* and *C. marmoratus* had broad pods; *C. albicans*, *C. cajanifolius*, *C. cinereus*, *C. crassus*, *C. goensis*, *C. mollis*, *C. platycarpus* and *C. scarabaeoides* had high seed number per pod (van der Maesen 1986), hence these are the species important for agronomic traits. Upadhyaya (2006) reported high pod set (74 % compared to about 20 % in pigeonpea) in *C. scarabaeoides*. Therefore, all *Cajanus* species may be evaluated for this important trait. The highest seed protein content in the entire collection of cultivated pigeonpea was 30.8 % (in ICP 15135). Evaluation of wild relatives' germplasm for seed protein content in the present study revealed that some accessions of *C. crassus* (33.8 %), *C. cajanifolius* (33.6 %), *C. mollis* (33.4 %), *C. platycarpus* (33.3 %) and *C. albicans* (32.5 %) as the best sources for higher levels of seed protein content (Reddy et al. 1997; Reddy et al. 2000; Saxena et al. 2002), but seeds are smaller.

The *Cajanus* species *C. lineatus*, *C. platycarpus*, *C. scarabaeoides*, *C. sericeus*, *C. reticulatus*, *C. goensis*, *C. lanceolatus*, *C. latisepalus* and *C. marmoratus*, which produced longer pod hairs with a score from 4 to 6 in the present study, may be useful in developing insect resistant pigeonpea cultivars. Rao et al. (2003) reported pod borer and podfly resistance in *C. acutifolius*, *C. albicans*, *C. platycarpus*, *C. scarabaeoides*, *C. sericeus* and *C. reticulatus*. Shanower et al. (1997) studied the survival, growth and fecundity of *H. armigera* on pods of *C. cajan* and two wild species and reported that larval survival on *C. scarabaeoides* was 21 % compared to 57 % on *C. platycarpus* and 78 % on *C. cajan*. The dense covering of non-glandular trichomes on *C. scarabaeoides* acts as physical barrier and prevents small larvae reaching the surface of the pods.

Clustering of different species in this study was in agreement with studies of several researchers. Parani et al. (2000) reported a close genomic relationship between *C. albicans* and *C. mollis*, which formed Cluster 1 in the present study. Pundir and Singh (1985a) reported almost similar gross morphology and close association of *C. lineatus* and *C. sericeus*, which formed Cluster 3 in the present study. Nadimpalli et al. (1994) using RFLP also reported close relationship between these species. Pundir and Singh (1985a) reported close morphology between *C. platycarpus*

Table 9 Frequency distribution for different qualitative traits of various *Cajanus* species characterized at ICRISAT, Patancheru, India

Character	Level	*C. acutifolius*	*C. albicans*	*C. cajanifolius*	*C. cinereus*	*C. confertiflorus*	*C. crassus*	*C. elongatus*	*C. goensis*	*C. lanceolatus*
Life cycle	Annual			5						1
	Perennial	12	20		1	1	10	1	1	
Growth habit	Climber		20				10		1	
	Creeper							1		
	Erect									
	Spreading	12				1				
	Semi-Spreading			5	1					1
Stem color	Brown	2								
	Dark brown									
	Green	10	20	4	1		10	1	1	1
	Purple			1						
	Green + purple					1				
	Purple + green									
Leaf color	Dark green		20				10			
	Green	11		5	1	1		1	1	1
	Light green	1								
Leaflet shape	Elliptic									
	Lanceolate	12		5						1
	Oblanceolate									
	Obovate		20			1		1		
	Ovate				1				1	
	Rhomboidal						10			
Leaf hairiness	1	12	20	3			10			1
	2			2	1	1		1	1	
	3									
	4									
Flowering pattern	NDT	12	20	5	1	1	10	1	1	1
Flower base color	Yellow	12	20	5	1	1	10	1	1	1
Flower streak color	No streaks		1				9		1	
	Purple			2						
	Red	12	19	3	1	1	1	1		1
	Red + no streaks									

Table 9 continued

Character	Level	*C. acutifolius*	*C. albicans*	*C. cajanifolius*	*C. cinereus*	*C. confertiflorus*	*C. crassus*	*C. elongatus*	*C. goensis*	*C. lanceolatus*
Flower streak pattern	Dense streaks	1	10	3	1					1
	Few streaks	2	9			1		1		
	Medium streaks	9		2			1			
	Medium streaks + no streaks									
	No streaks		1				9		1	
Pod color	Green	12	20	2	1	1	10	1	1	1
	Mixed			3						
Pod shape	Cylindrical			1				1		
	Flat	12	20	4	1	1	10		1	1
Pod hairiness	1		20	2			10			
	2	12		3	1			1		
	3									
	4									
	5									1
	6								1	
Seed color pattern	Mottled	12	20	1	1	1	10	1		1
	Mottled + plain									
	Plain									
	Plain + mottled			1						
	Plain + Speckled									
	Speckled			3					1	
	Speckled + Plain									
Primary seed color	Brown									
	Dark gray	5	10	5			2	1	1	
	Gray	5	5				6			
	Gray brown	2	5		1	1	2			1
	Light gray									
	Dark purple									

Table 9 continued

Character	Level	*C. acutifolius*	*C. albicans*	*C. cajanifolius*	*C. cinereus*	*C. confertiflorus*	*C. crassus*	*C. elongatus*	*C. goensis*	*C. lanceolatus*
Secondary seed color	Black									
	Dark Gray		3	1						
	Gray	9	13	4	1	1	4	1	1	1
	Gray brown		2							
	Light gray	3	2				6			
	None									
Seed shape	Elongate									
	Elongate + Square									
	Square	12	20	5	1	1	10	1		1
	Oval								1	
Seed strophiole	Present	12	20	5	1	1	10	1	1	1
	Absent									

Character	Level	*C. latisepalus*	*C. lineatus*	*C. marmoratus*	*C. mollis*	*C.platycarpus*	*C. reticulatus*	*C. rugosus*	*C. scarabaeoides*	*C. sericeus*	Total
Life cycle	Annual			1		17			100	4	128
	Perennial	1	10		6		5	2			70
Growth habit	Climber										31
	Creeper			1	6	17		2	100		127
	Erect						1			4	5
	Spreading		10				4				27
	Semi-Spreading	1									8
Stem color	Brown	1					1				4
	Dark brown						1				1
	Green		10	1	6	6	2	2		4	79
	Purple										1
	Green + purple					9			58		68
	Purple + green					2			42		44
Leaf color	Dark green		10			16	1				57

Table 9 continued

Character	Level	*C. latisepalus*	*C. lineatus*	*C. marmoratus*	*C. mollis*	*C.platycarpus*	*C. reticulatus*	*C. rugosus*	*C. scarabaeoides*	*C. sericeus*	Total
	Green			1	6	1	3	2	100	4	138
	Light green	1									2
Leaflet shape	Elliptic				6						6
	Lanceolate		10								28
	Oblanceolate									4	4
	Obovate							2	100		124
	Ovate	1				17	5				25
	Rhomboidal			1							11
Leaf hairiness	1					17		2			65
	2	1		1	6		5		100	4	123
	3		8								8
	4		2								2
Flowering pattern	NDT	1	10	1	6	17	5	2	100	4	198
Flower base color	Yellow	1	10	1	6	17	5	2	100	4	198
Flower streak color	No streaks		9	1	6	17	1	2	12	4	63
	Purple										2
	Red	1	1				2		86		129
	Red + no streaks								2		2
Flower streak pattern	Dense streaks								1		17
	Few streaks		1				1		36		51
	Medium streaks	1					1		49		63
	Medium streaks + no streaks								2		2
	No streaks		9	1	6	17	1	2	12	4	63
Pod color	Green	1	10		6	16	2	2	100	4	190
	Mixed			1		1					5
Pod shape	Cylindrical									4	6
	Flat	1	10	1	6	17	2	2	100		189

Table 9 continued

Character	Level	*C. latisepalus*	*C. lineatus*	*C. marmoratus*	*C. mollis*	*C.platycarpus*	*C. reticulatus*	*C. rugosus*	*C. scarabaeoides*	*C. sericeus*	Total
Pod hairiness	1										32
	2										17
	3				6			2			8
	4	1	3				1		100		105
	5		5	1		17	4			2	30
	6		2							2	5
Seed color pattern	Mottled	1	10	1	1	17	3		53	4	137
	Mottled + plain								1		1
	Plain							2	42		44
	Plain + mottled										1
	Plain + Speckled				3				2		5
	Speckled								1		5
	Speckled + Plain				2				1		3
Primary seed color	Brown		1								1
	Dark gray		3		6	17			54	3	107
	Gray	1	2						29		48
	Gray brown		4	1			3		8	1	29
	Light gray								9		9
	Dark purple							2			2
Secondary seed color	Black			1	1						2
	Dark Gray										4
	Gray		10		5	17	3		48	4	122
	Gray brown								1		3
	Light gray	1							12		24
	None							2	39		41

Table 9 continued

Character	Level	*C. latisepalus*	*C. lineatus*	*C. marmoratus*	*C. mollis*	*C.platycarpus*	*C. reticulatus*	*C. rugosus*	*C. scarabaeoides*	*C. sericeus*	Total
Seed shape	Elongate					6					6
	Elongate + Square					1					1
	Square		10		6	10	3	2	100	4	186
	Oval	1		1							3
Seed strophiole	Present	1	10	1	6	17	3	2	100	4	196
	Absent										

and *C. crassus*, and close karyotype symmetry between *C. cajanifolius* and *C. crassus* are in Cluster 2. Bio-systematic studies encompassing morpho-cytological and electrophoretic analysis of *Cajanus* species by Pundir and Singh (1985b) revealed that *C. cajanifolius* is closest to *C. crassus* and *C. platycarpus*. These three species are together in Cluster 2 in the present study. The clustering in the present study is based on phenotypic traits and may not have any phylogenetic significance. Pundir and Singh (1985a) reported the crossability between *C. lineatus* with *C. scarabaeoides* and *C. scarabaeoides* with *C. sericeus*, which are together in Cluster 3 in the present study. No association of genepool classification and clustering of species based on traits under study were observed and in every cluster there were species belonging to secondary and tertiary genepool (Figs. 1, 2).

Information on character association is important in the classification of accessions and species. Highly significant positive correlation between leaflet length and width in *C. albicans*, and *C. mollis*, suggests the recording of leaflet length to assess the size of the leaf in these species (Table 6). These two species are of long duration (days to maturity ≥345 days) can serve as good forage for cattle, goats and sheep. Significant positive correlation between days to 50 % flowering and days to maturity (first 5 pods) over all species, *C. acutifolius*, *C. crassus*, *C. lineatus*, *C. platycarpus* and *C. scarabaeoides* suggests that selection for days to 50 % flowering will result in selection for maturity also (Table 6) (van der Maesen 1990). Because of strong positive association between pod length with pod width in *C. sericeus*, with seeds per pod in *C. lineatus* and with 100-seed weight in *C. platycarpus*, selection for pod length will result in selection for broad pods, more seeds per pod and large seeds in these species. Flowering and maturity duration of species determines their adaptation to various agro-climatic conditions. These associations also have implications in reducing number of traits in characterization by using easily measurable correlated traits.

Availability of adequate genetic variation in germplasm collection is a prerequisite for a successful breeding program. In addition to using the cultivated pigeonpea germplasm collection, efforts can be made to explore and harness the genetic variation present in *Cajanus* species of secondary and tertiary genepools. These species may be exploited for useful agronomic traits such as extra early flowering in *C. platycarpus*, early flowering in *C. scarabaeoides*, long pods in *C. mollis*, broad pods in *C. albicans*, more seeds per pod in *C. mollis*, *C. albicans*, *C. cinereus* and *C. scarabaeoides* and high seed protein content in *C. crassus*, *C. cajanifolius*, *C. mollis*, *C. platycarpus* and *C. albicans*, using conventional breeding methods and/or biotechnology tools (Mallikarjuna and Moss 1995). Mallikarjuna et al. (2011b) used embryo rescue technique to transfer genes of resistance to phytophthora blight from *C. platycarpus*, which belongs to the tertiary genepool, to pigeonpea. The possibility of exploiting tertiary genepool has opened up new vistas for the broadening genetic base of variation and for pigeonpea improvement. Diverse and promising accessions of different species identified for multiple traits in the present study may be used for exploitation (Tables 7, 8).

In view of changing climate scenario, results of the present study are useful to harness variation for useful traits in *Cajanus* genepool for sustainable pigeonpea improvement. The passport information on the *Cajanus* collection at ICRISAT genebank is available at http://www.icrisat.org/ICRISAT.crops.htm. The ICRISAT genebank serves as a major source for pigeonpea wild relatives' germplasm. Limited seeds of all in-trust accessions (with ITPGRFA/FAO) are available under Standard Material Transfer Agreement (SMTA) of the International Treaty for PGRFA, for utilization in research globally.

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