Genet Resour Crop Evol (2012) 59:411–417
DOI 10.1007/s10722-011-9691-8

RESEARCH ARTICLE

Differences between *Cajanus cajan* (L.) Millspaugh and *C. cajanifolius* (Haines) van der Maesen, the progenitor species of pigeonpea

Nalini Mallikarjuna · Kulbushan Saxena · Jhansi Lakshmi · Rajeev Varshney · Sandhya Srikanth · Deepak Jadhav

Received: 4 November 2010 / Accepted: 21 March 2011 / Published online: 19 April 2011

Abstract A study was undertaken to know the difference/diversity between pigeonpea and its closely related wild species *C. cajanifolius* by studying their morphology, crossability, cytology of the hybrid between the two, and molecular studies. Studies revealed that there are at least 5–6 traits that separate the two species such as flower morphology, pod color and morphology, pod constriction, seed color and strophiole, 100 seed weight that separate *C. cajan* from *C. cajanifolius*. Molecular studies revealed that a genetic dissimilarity index value ranging from 0.81 to 0.94 exists between the two species.

Keywords *Cajanus cajanifolius* · Cytology · Morphology · Molecular diversity · Pigeonpea · Proginator species

Introduction

In the genus *Cajanus* with 32 species and 11 related genera, *Cajanus cajan* (L.) Millspaugh is the only species cultivated throughout Asia and Africa for its leguminous proteins. Although many of the closely related wild species easily cross with *C. cajan*, various studies have shown that pigeonpea originated from its closest wild relative *C. cajanifolius* (Haines) van der Maesen (Ladizinsky and Hammel 1980; van der Maesen 1980, 1986; Krishna and Reddy 1982; Pundir and Singh 1985; Panigrahi et al. 2007), most probably in India and later it spread to the continents of Africa and Australia, where some wild relatives of pigeonpea still exist.

There are many published reports indicating that *C. cajanifolius* is the proginator species of cultivated pigeonpea (van der Maesen 1980; Panigrahi et al. 2007). It would not only be interesting but useful to know the difference/diversity between the two. Studies revealed that there are at least 5–6 traits that separate the two species such as flower morphology, pod color and morphology, pod constriction, seed color and strophiole, and 100 seed weight that separate *C. cajan* from *C. cajanifolius*. Molecular studies using SSRs markers showed that a genetic dissimilarity index value ranging from 0.81 to 0.94 exists between the two species.

Materials and methods

Plant morphology

Four accessions of *C. cajanifolius* namely ICPW 28, 29, 30 and 31 together with pigeonpea cultivar ICPL

N. Mallikarjuna (✉) · K. Saxena · J. Lakshmi · R. Varshney · S. Srikanth · D. Jadhav
International Crops Research Institute for Semi Arid Tropics, Patancheru 502 324, Andhra Pradesh, India
e-mail: N.Mallikarjuna@CGIAR.ORG

85010 were grown in a glasshouse. Morphological parameters such as branching pattern, growth habit, plant height, number of primary branches, stem color, leaf shape, color of basal petal, pattern of streaks on the petal, pod color, pod constriction, pod size, seed color, seed shape, 100-seed weight and presence/absence of strophiole were studied on randomly selected plants.

Crossability

Emasculations followed by pollinations were carried out in the morning using *C. cajan* cultivar ICPL 85010 as the female parent and *C. cajanifolius* accessions as the pollen donor. Care was taken to allow only cross pollinated pistils to remain and grow in an axil, removing all other self pollinated pistils or immature buds. Application of gibberellic acid (75 mg/l), as a cotton swab wrapped around the pistil soon after pollinations, increased pod set. Mature pods were harvested upon maturity.

Cytology

Flower buds from F_1 hybrids ICPW 28 × ICPL 85010 and ICPW 29 × ICPL 85010 were squashed in 2% aceto-carmine and well spread preparations were examined. To study different stages of meiosis such as metaphase, anaphase and tetrad, at least 20 pollen mother cells (PMCs) were examined and means were calculated from them. Pollen fertility analysis was carried out by staining mature pollen grains in 2% aceto carmine. Well stained grains were counted as fertile grains and partial to unstained grains were counted as sterile.

SSR analysis

PCR amplification of microsatellite loci using 14 fluorescent-dye-labeled 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, 1 unit of Taq DNA polymerase. Amplified products were pooled as per multiplex plan and separated on an ABI 3700 fragment analyzer. The results were evaluated using the software package Genotyper 3.7 (Applied Biosystem).

Data analysis

Analysis of the data was performed using data of 14 SSRs 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 Powermarker 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).

Results

Morphological studies

Four accessions of *C. cajanifolius* ICPW 28, 29, 30 and 31 had the presence dense small trichomes on their leaves making them velvety to touch, in comparison to cultivated pigeonpea ICPL 85010, which had trichomes on their leaves but they were not velvety to touch. Variation was observed with respect to flower color. The keel petal of ICPW 28 was more yellowish and comparable to that of ICPL 85010 than in the other three *C. cajanifolius* accessions which had more of orange tinge in them (Fig. 1). All the four accessions of *C. cajanifolius* and *C. cajan* were erect with semi-spreading branching (Table 1) pattern. Accession ICPW 31 was taller than all the other three accessions of *C. cajanifolius* and *C. cajan*. The number of primary branches varied from 3 to 4 in all accessions of *C. cajanifolius* compared to 9 in *C. cajan*. The color of the keel petal and the streaks on them also varied between *C. cajanifolius* accessions and *C. cajan*. Pod constriction was prominent on *C. cajan* whereas it was slight on all the accessions of *C. cajanifolius*. Pod size too varied from 2.9 to 4.4 cm in different accessions of *C. cajanifolius* compared to a pod size 5.4 cm of *C. cajan*. Prominent difference between the accessions of *C. cajanifolius* and *C. cajan* was the seed color (Fig. 1) and seed strophiole. Seeds of *C. cajanifolius* were ash brown to

Fig. 1 *C. cajanifolius* accessions's flower and seed morphology (from *left to right*: ICPW 28, ICPW 29, ICPW 30 and ICPW 31). **a**, **b** Flower morphology and striations on the keel petal. **c** Seed morphology and presence of strophiole on all the accessions

black in color (Fig. 1) and it was light brown (beige) in *C. cajan*. Seed strophiole was prominent in all accessions of *C. cajanifolius* and it was absent in *C. cajan*. Pod morphology varied between the accessions of *C. cajanifolius* and *C. cajan*. Pods were flat in *C. cajanifolius* compared to *C. cajan* pod. The locules between the seeds were more prominent in *C. cajanifolius* accessions with clear cut demarcations between individual locules. Pod shattering was observed in all *C. cajanifolius* accessions compared to *C. cajan* where mature dry pod did not shatter.

A dendrogram was drawn based on morphological traits and ICPW 29 and 30 showed closer relationship to each other compared to ICPW 28. ICPW 31 showed closer relationship to *C. cajan* than any of the accessions of *C. cajanifolius*. But the distance between *C. cajanifolius* accessions and *C. cajan* was distant enough for species differentiation (Fig. 2).

Molecular diversity

SSR markers were able to distinguish all the four accessions of *C. cajanifolius* (Fig. 3) from *C. cajan*. Accessions ICPW 28 showed a diversity index number of 0.44 with ICPW 30, compared to the index number of 0.61 with ICPW 29 and 0.94 with ICPW 31. This shows that accession ICPW 28 is closer to ICPW 30 than to either ICPW 29 or 31. The diversity index number between all the accessions of *C. cajanifolius* and *C. cajan* varied between 0.81 and 0.94 showing difference between *C. cajanifolius* and *C. cajan* (Table 2).

Crossability between *C. cajanifolius* accessions and *C. cajan*

Crossability between *C. cajanifolius* accession ICPW 28, 29, 30 and 31 and *C. cajan* varied from 0.03 to

Table 1 Study of some morphological characters in some *C. cajanifolius* accessions

Identity	BRP	GH	PH	PB	FBP	PSP	PC	Pcon	PS	Sec	SCP	SS	SW	StR
ICPW 28	Semi-spread	Indeterminate	99	3.5	Yellow	Dense	Br with purple streaks	Slight	4.35	Black	Mottled	Oval	6.7	Present
ICPW 29	Semi-spread	Indeterminate	96	2.1	Yellow	Dense	Br with purple streaks	Slight	3.65	Black	Mottled	Oval	6.4	Present
ICPW 30	Semi-spread	Indeterminate	93	4.1	Yellow	Medium	Br with purple streaks	Slight	3.75	Black	Uniform	Oval	6	Present
ICPW 31	Semi-spread	Indeterminate	123	3.8	Light yellow	Sparse	Brown	Slight	2.9	Black	Mottled	Oval	6	Present
ICPL 85010	Spreading	Indeterminate	112	9.2	Yellow	Absent	Br with purple streaks	Prominent	5.4	Light brown	Mottled	Globular	12	Absent

BRP branching pattern, *GH* growth habit, *PH* plant height, *PB* primary branches, *SC* stem colour, *LS* leaf shape, *FBP* flower:colour of base of petal, *PSP* pattern of streaks on petal, *PC* pod colour (*br* brown), *Pcon* pod constriction, *PS* pod size (cms), *SeC* seed colour, *SCP* seed colour pattern, *SS* seed shape, *SW* 100 seed weight (g/100 seed), *Str* Strophiole

0.20% with the formation of mature pods as a result of cross pollinations. The response was much low when the 50 ppm GA_3 was not applied to the base of pollinated pistils soon after cross pollinations. A maximum of 9 pods were obtained as a result of 68 pollinations in the cross ICPL 85010 × ICPW 29 and a minimum of 2 pods were obtained from 45 cross pollinations from the cross ICPL 85010 × ICPW 28 (Table 3). Formation of mature pods from cross pollinations which was less than 1%, shows that the two species are closely related but distant enough as percent pod set is low compared to pollinations between accessions of *C. cajan* which ranges between 15–18%, depending upon the accessions used in cross pollinations (data not shown in the table).

Cytological analysis of the F1 hybrids

Meiocytes from the crosses ICPL 85010 × ICPW 29 and ICPL 85010 × ICPW 31 showed the formation of 11 bivalents (Fig. 4a showing total homology between the parental species. Twenty percent of the meiocytes showed the formation of 7 bivalents and 2 tetravalents showing two chromosomes which are totally homologous between the parental species (Fig. 4a, b). In the cross ICPL 85010 × ICPW 28, 10% of the meiocytes showed the presence of 2 univalents which signifies that one chromosome in each parent did not have a homologous chromosome in the other parent or the divergence of one chromosome in one of the parent. Such an anomaly was present only in 10% of the meiocytes (Fig. 4c). Tetrads were observed (Fig. 4d) in both the crosses and pollen fertility in the F_1 hybrids varied between 48–62%, showing closer relationship between the two species.

Discussion

Morphological traits such as plant morphology, leaf glabrous-ness, pod shape, pod shattering, seed shape and color, presence of strophiole are distinguishing characters between *C. cajanifolius* and *C. cajan*. These traits separate *C. cajanifolius* from cultivated pigeonpea. Morphological studies showed that ICPW 31 is closer to ICPL 85010 than the other three

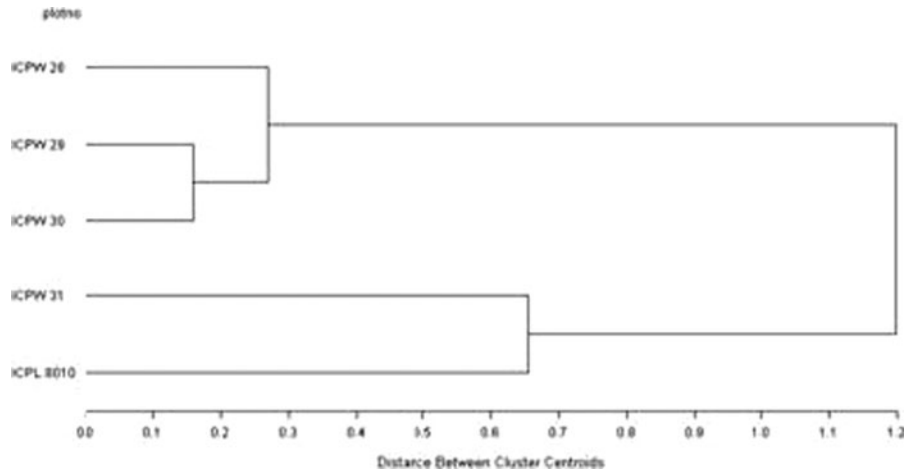

Fig. 2 Dendrogram showing diversity between *C. cajanifolius* accessions based on morphological traits

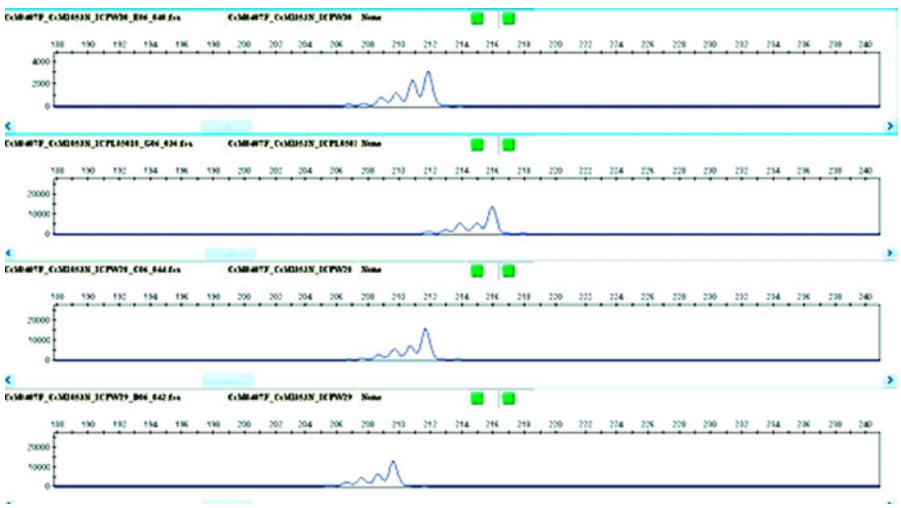

Fig. 3 Genetic diversity in *C, cajanifolius* accessions as revealed by SSR marker Ccm0407. (The *top line* is ICPW 30, *second line* is ICPW31, *third line* is ICPW 28 and the *fourth line* is ICPW 29)

Table 2 Molecular diversity between four *C. cajanifolius* accessions based on SSR markers

Identity	28	29	30	31	85010	87119
ICPW 28	0					
ICPW 29	0.61	0				
ICPW 30	0.44	0.64	0			
ICPW 31	0.94	0.94	0.92	0		
ICPL 85010	0.86	0.81	0.89	0.86	0	
ICPL 87119	0.92	0.83	0.89	0.89	0.61	0

Table 3 Summary of the crossability between pigeonpea cultivar ICPL 85010 and four accessions of *C. cajanifolius*

Cross pollinations	No. of pollinations	No. of pods (%)
ICPL 85010 × ICPW 28	45	2 (0.04)
ICPL 85010 × ICPW 29	68	9 (0.13)
ICPL 85010 × ICPW 30	52	2 (0.03)
ICPL 85010 × ICPW 31	70	4 (0.03)
Total	235	17 (0.20)

accessions and this is evident when plant height is studied. Both are taller than the other material used in the study.

With respect to cytology cross ICPL 85010 × ICPW 29 and ICPL 85010 × ICPW 31 showed the formation of 11 bivalents and the

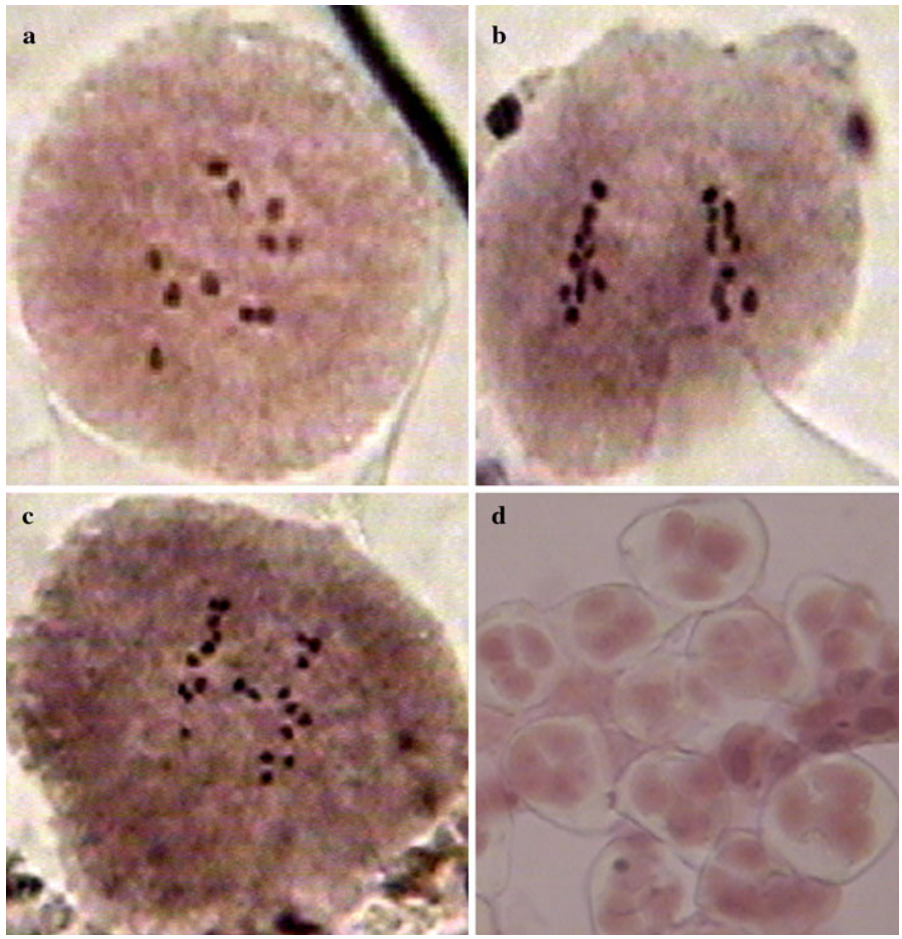

Fig. 4 Meiotic study of the F_1 hybrid between *C. cajan* × *C. cajanifolius*. **a** Eleven bivalents in F_1 hybrid from the cross ICPL 85010 × ICPW 29. **b** Anaphase from the cross ICPL 85010 × ICPW 29. **c** Anaphase showing laggards in the F_1 hybrid ICPL 85010 × ICPW 28. **d** Tetrads in the crosses ICPL 85010 × ICPW 28 and ICPL 85010 × ICPW 29

presence of two tetravalents shows that two pairs of chromosomes are probably identical to each other but might be evolving into separate pair as tetravalents were seen in only 20% of the meiocytes. In the cross ICPL 85010 × ICPW 28 the presence of two univalents in 10% of the meiocytes shows that there may be one pair of chromosome in each parent which is distinct without a partner in the other parent. It is known that as the species evolves due to crossover the pairs of chromosomes will also evolve. Pundir and Singh (1985) did not observe seed set when *C. cajan* was crossed with *C. cajanifolius* whereas Reddy et al. (1980) obtained seed set. In the present study it was observed that pod/seed set was low (<1%), nevertheless mature pod set was observed. Low mature pod set shows that although the two species are closely related there might be other extraneous factors causing low pod set. Regular meiosis between the two species shows the closer relationship between the two.

Molecular analysis showed a greater diversity ranging between 0.81 and 0.94, between *C. cajanifolius* accessions and cultivated pigeonpea, and this cannot be explained by an inversion separating the two species as suggested by Pundir and Singh (1985). Published literature show that *C. cajanifolius* is closely related to *C. cajan*, and according to the present report there were at least five traits that separated *C. cajanifolius* from *C. cajan*. This is expected as species evolve, there are bound to be differences and in case of cultivated pigeonpea domestication coupled with selection could have

resulted in the differences. Parani et al. (2000) have concluded that *C. scarabaeoides* (L.) Thouars is the progenitor species of *C. cajan* but their analysis does not include *C. cajanifolius*. Apart from the above mentioned traits that separate *C. scarabaeoides* from *C. cajan*, the trailing growth habit is an additional trait separating *C. scarabaeiodes* from *C. cajan,* showing a more distant relationship with cultivated pigeonpea. In conclusion, although *C. cajanifolius* is the progenitor species of *C. cajan*, there are at least five evident traits that separate the two species. Since molecular diversity between the two species is large, it can be concluded that there might be some minor traits which are not very evident such as leaf size and glabrous-ness etc., which might add up to the diversity between the two species.

References

Krishna TG, Reddy LJ (1982) Species affinities between *Cajanus cajan* and some *Atylosia* species based on esterase isozymes. Euphytica 31:709–713

Ladizinsky G, Hammel A (1980) Seed protein profiles of pigeonpea (*Cajanus cajan*) and some *Atylosia* species. Euphytica 29:313–317

Liu K, Muse SV (2005) PowerMarker: integrated analysis environment for genetic marker data. Bioinformatics 21(9):2128–2129

Panigrahi J, Kumar DR, Mishra M, Mishra RP, Jena P (2007) Genomic relationships among 11 species in the genus *Cajanus* as revealed by seed protein (albumin and globulin) polymorphisms. Plant Biotechnol Rep 1:109–116

Parani M, Lakshmi M, SenthilKumar P, Parida A (2000) Ribosome DNA variation and phylogenetic relationships among *Cajanus cajan* (L.) Millsp. and its wild relatives. Curr Sci 78(10):1235–1238

Perrier X, Jacquemoud-Collet JP (2006) DARwin software. http://darwin.cirad.fr/darwin

Pundir RPS, Singh RB (1985) Cytogenetics of F_1 hybrids between *Cajanus* and *Atylosia* species and its phylogenetic implications. Theor Appl Genet 71:216–220

Reddy LJ, Green JM, Sharma D (1980) Genetics of *Cajanus cajan* (L.) Mills sp. × *Atylosia* spp. In: Nene YL (ed) Proceedings of the international workshop on Pigeonpeas, vol 2. ICRISAT, Patancheru, A.P., India, pp 39–50

van der Maesen LJG (1986) *Cajanus* DC. and *Atylosia* W. & A. (*Leguminosae*). Agricultural University Wageningen papers 85–4 (1985). Agriculture University, Wageningen, The Netherlands, p 225

van der Maesen LJG (1980) India is the native home of pigeonpea. In: Arends JC, Boelema G, de Groot CT, Leeuwenberg AJM , Veenman H, Zonen BV (eds) Libergratulatorius in honorem H.C.D. de Wit landbouwhogeschool Miscellaneous paper no 19, Wageningen, Netherlands, pp 257–262