*Euphytica* **125:** 21–28, 2002.



# Diversity in selected wild and cultivated species of pigeonpea using RFLP of mtDNA

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Received 27 June 2000; accepted 3 August 2001

*Key words: Cajanus cajan*, diversity, mtDNA, RFLP

**Summary**

Diversity in 28 accessions representing 12 species of the genus, *Cajanus* arranged in 6 sections including 5 accessions of the cultivated species, *C. cajan*, and 4 species of the genus *Rhyncosia* available in the germplasm collection at ICRISAT was assessed using RFLP with maize mtDNA probes. Cluster analysis of the Southern blot hybridization data with 3 restriction enzymes – 3 probe combinations placed the genus *Rhyncosia* in a major group well separated from all the species belonging to the genus *Cajanus*. Within the genus *Cajanus*, the 4 accessions of *C. platycarpus* belonging to section *Rhynchosoides* formed a separate group in contrast to those in other sections of pigeonpea. In the section, *Cajanus* all the 5 accessions of *C. cajan* were grouped together and *C. cajanifolius* belonging to the same section was in a subgroup by itself closer to the main group. The four accessions of *C. scarabaeoides*, were together and the other species belonging to section *Cantharospermum* were in different subgroups. The intra-specific variation was seen even within accessions of certain pigeonpea wild species such as *C. scarabaeoides*, *C. platycarpus*, *C. acutifolius*, and even the cultivated species of *C. cajan*. This study suggests that RFLP of mtDNA can be used for the diversity analysis of pigeonpea and it gives some indications on the maternal lineage among the species. The variations in the mitochondrial DNA hybridization patterns also suggest the extensive rearrangement of the organelle genome among the *Cajanus* species.

## Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is an important legume crop in the tropics and subtropics. The crop is grown for multiple uses (food, fodder and fuel) in the semi-arid regions of India and Africa (Nene & Sheila, 1990). It is also grown in different cropping systems as it enriches the soil with nitrogen and phosphorus. Considerable yield improvement can be made in pigeonpea with the selective utilization of germplasm, comprising excellent sources of resistance to diseases and pests, and other important agronomic characteristics. The pigeonpea is a member of the subtribe *Cajaninae* of the *Phaseoleae* and it is the only member of its sub-tribe that has been domesticated (Smartt, 1990). A total of 32 species of *Cajanus* and 11 genera are now recognized in the subtribe *Cajaninae* (van der Maesen, 1986). Many wild species of *Cajanus* can cross readily with cultivated species, *C. cajan*, and these have been placed in the secondary gene pool (van der Maesen, 1990). Those species which do not hybridize easily with the section *Cajanus or Atylosia* are included in the tertiary gene pool represented by species such as *C. platycarpus, C. mollis*, *C. rugosus*, and other genera such as *Rhyncosia*, *Dunbaria* and *Erisema*. The taxonomic classification of *Cajanus* has been based on crossability, evolution of form, chemotaxonomy, and biochemistry and very little molecular studies have been carried out in this genus. It is important to understand the genetic diversity available in this crop and its wild relatives for a planned and better utilization of germplasm in pigeonpea breeding.

Several methods are available to study the genetic diversity in a crop species which includes isozyme profiles, (Ahmad et al., 1992) and protein analysis

by electrophoresis (Singh et al., 1991) as well as e DNA- based methods (Miller & Tanksley, 1990; Devos & Gale, 1992; Hongtrakul et al., 1997;Wang et al., 1998). A simple method like Random amplified polymorphic DNA (RAPD) has been used to study the diversity among the wild species of crops (Gonzalez & Ferrer, 1993; Jain et al., 1994; Mackill, 1995) but there are still some inherent problems of reproducibility and other factors associated with the technology that makes the comparison of results from one lab to another rather difficult (Harris, 1999). Restriction fragment length polymorphism (RFLP) analysis using genomic single copy probes has been generally used to characterize the variation among the wild and cultivated species (Jena & Kochert, 1991, Miller & Tanksley, 1990; Gawel et al., 1992, Jarret et al., 1992). Variations in chloroplast DNA (Close et al., 1989) and mitochondrial DNA (Deu et al., 1995; Moeykens et al., 1995; Tozuka et al., 1998) have been the basis for studies on the diversity of cytoplasm in crop species like soybean. Compared to the chloroplast genome, the mitochondrial genome has been shown to vary considerably within or between closely related species (Deu et al., 1995; Close et al., 1989; Grabau et al., 1992.). In addition, various new molecular methods like simple sequence repeats (SSR) (Wang et al., 1998) and amplified fragment length polymorphism (AFLP) have been used for the diversity analysis in crop species (Hongtrakul et al., 1997). Simple sequence repeats have been used as a powerful genetic marker extensively in plant genetic analysis. The advantages of SSRs arise from their relative abundance in eukaryotic genomes, high level of allelic diversity, co-dominance, and ease of analysis using PCR. However, slipped strand mispairing and the high mutational process taking place at the SSR loci often limit the scope of SSRs for phylogenetic analysis, (Levinson & Gutman, 1987; Provan et al., 1999). AFLP is a more promising tool in genetic mapping and diversity analysis compared to other molecular methods as the number of markers produced are very high in most cases and these can be manipulated by choosing the right kind of primers and changing their selective bases (McGregor et al., 2000). Further, though SSR and AFLP may be efficient methods for DNA fingerprinting other considerations like cost, level of skills required, reliability and reproducibility are deciding factors in the selection of the right molecular method. DNA sequences from ribosomal genes (rDNA) have also been used in understanding the inter-specific and inter-generic relationships. DNA sequences from the rapidly evolving 18-26 S internal transcribed spacer (ITS) particularly are employed for diversity analysis both in animal and crops species (Hershkovit et al., 1999).

Protein and isozyme analyses (Ladizinsky & Hamel, 1980; Krishna & Reddy, 1982) and molecular methods like RFLPs and RAPDs (Nadimpalli et al., 1993 and Ratnaparkhe et al., 1995) have successfully elucidated the phylogenetic relationship of pigeonpea and its wild relatives. These authors have not covered a number of sections in the genus *Cajanus* and also used different molecular methods for the diversity analysis. We have used RFLPs of mtDNA to assess the level of genetic diversity in selected accessions of pigeonpea germplasm available at ICRISAT and also compare it with the molecular methods used earlier.

## Materials and methods

### *Plant material*

The 28 accessions representing 12 species of the genus, *Cajanus* arranged in 6 sections including 5 accessions of the cultivated species *C. cajan*, and 4 species of the genus *Rhyncosia* available in the germplasm collection at ICRISAT used in this study are listed in Table 1. Plants were grown in the field and young leaf samples were collected for DNA extraction.

### *DNA extraction*

DNA was extracted from pigeonpea leaves as described by Sivaramakrishnan et al. (1997). The final pellet was dissolved in TE buffer (Tris pH 8.0, 10 mM, EDTA 1 mM) and the quality of DNA was checked by running samples on agarose gels.

### *Clones used*

Because of the conserved nature of mitochondrial genome across species and the strong hybridization signal that were obtained with maize mtDNA probes on pigeonpea (Sivaramakrishnan et al., 1997) we selected a few of these clones in the present study for diversity analysis using RFLP. Maize *atp*6 clone (ATPase subunit 6, Dewey et al., 1985). Maize clones *cox*I (cytochrome oxidase subunit I, Isaac et al., 1985), and *atp* $\alpha$ (Isaac et al., 1985) were provided by C.J. Leaver, Department of Plant Science, University of Oxford, Oxford, UK.

*Table 1.* List of cultivated and wild species of pigeonpea used for diversity analysis

| Section | Genus/Species | ICRISAT Genebank Accession Nos |
|---|---|---|
| *Cajanus* | *C. cajan* (L.) Millsp.-1 | ICP 7119 |
| | *C. cajan*- 2 | ICP 7220 |
| | *C. cajan*- 3 | ICP 2376 |
| | *C. cajan*- 4 | ICP 6443 |
| | *C. cajan*- 5 | ICPL 87 |
| | *C. cajanifolius* (Haines) van der Maesen | ICPW 31 |
| *Atylosia* | *C. sericeus* (Benth.ex Bak.) van der Maesen | ICPW 161 |
| *Fruticosa* | *C. acutifolius* (F.v.Muell.) van der Maesen-1 | ICPW 4 |
| | *C. acutifolius*-2 | ICPW 12 |
| | *C. latisepalus* (Reynolds and Pedley) van der Maesen | ICPW 39 |
| *Cantharospermum* | *C. albicans* (W. and A.) van der Maesen-1 | ICPW 18 |
| | *C. albicans*-2 | ICPW 24 |
| | *C. rugosus* (W. and A.) van der Maesen | ICPW 301 |
| | *C. scarabaeoides* (L.) Thouars var. *scarabaeoides*-1 | ICPW 97 |
| | *C. scarabaeoides*-2 | ICPW 106 |
| | *C. scarabaeoides*-3 | ICPW 126 |
| | *C. scarabaeoides*-4 | ICPW 101 |
| | *C. goensis* Dalz. | ICPW 32 |
| *Volubilis* | *C. mollis* (Benth.) van der Maesen | ICPW 55 |
| | *C. volubilis* (Blanco) Blanco | ICPW 168 |
| *Rhynchosoides* | *C. platycarpus* (Benth.) van der Maesen-1 | ICPW 61 |
| | *C. platycarpus*-2 | ICPW 62 |
| | *C. platycarpus*-3 | ICPW 64 |
| | *C. platycarpus*-4 | ICPW 66 |
| Other species of genus *Rhyncosia* | *R. minima* DC. | ICPW 537 |
| | *R. edulis* Griseb | ICPW 337 |
| | *R. sublobata* (Schumach.) Meikle | ICPW 519 |
| | *R. verdcourtii* | ICPW 529 |

*RFLP analysis*

Total DNA (about 10 ug) was digested with 30 units of the restriction enzyme (*Eco* RI, *Hind* III, or *Eco* RV) as per the manufacturer's protocols and the fragments were separated on 0.8% agarose gels by electrophoresis in TBE buffer (0.089 M Tris-borate, 0.002 M EDTA, pH 8.0). The fragments were viewed under UV light after staining with ethidium bromide (0.5ug/ml). After washing the gels in water, the DNA fragments were transferred onto Nylon membrane (Hybond N) by vacuum transfer (Amersham, UK). DNA was cross-linked to the membrane by exposure to UV light as per the manufacturer's protocol (Stratagene, Germany).

The random primed labeling method of Feinburg & Vogelstein (1983) was used for the preparation of $^{32}$P-labeled probes. Southern blots were prehybridized with 30 ml of prehybridization solution containing 7% SDS, 1% BSA, 0.5M $Na_2HPO_4$ and 20ug /ml sheared and denatured salmon sperm DNA per two blots (20×15 cm). Hybridization was carried out by adding the labeled probe to the prehybridization solution and incubated for 16 h at 65 °C in standard bottles (30×3.5 cm) in a hybridization oven (Hybaid, UK). The blots were washed three times in 3 × SSC containing 0.1% SDS at 65 °C for 30 min each. Autoradiography of the blots was carried out at –70 °C for varying periods using X-AR film (Kodak, USA). The fragment sizes were determined using Lambda

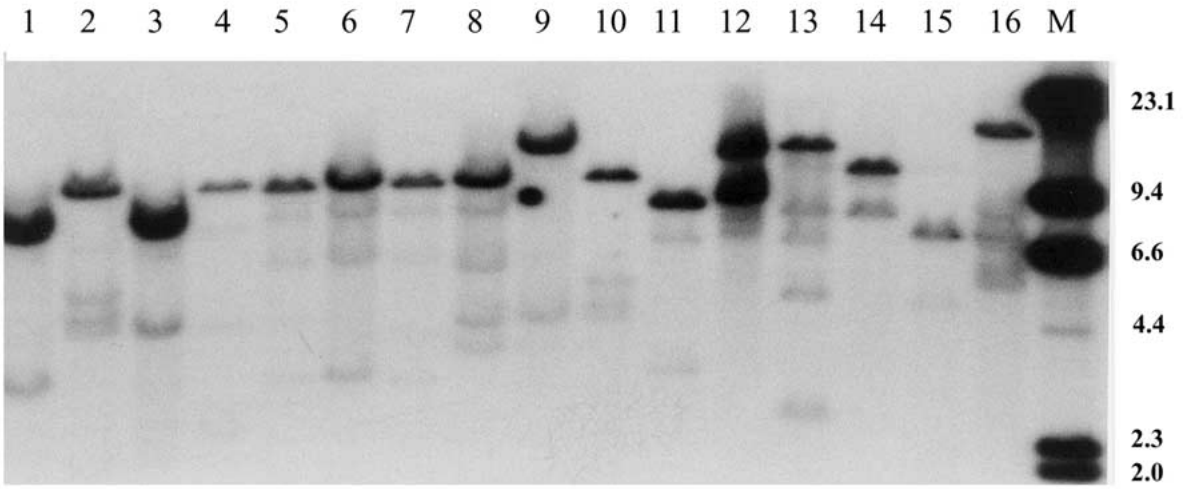


*Figure 1a.* Autoradiogram showing the diversity in pigeonpea wild species from the Southern blot hybridization of total DNA digested with *Eco*RV and probed with maize *atp* 6 clone.

Lane

1. *C. acutifolius*-2
2. *C. goensis*
3. *C. latisepalus*
4. *C. mollis*
5. *C. scarabaeoides*-1
6. *C. scarabaeoides*-2
7. *C. scarabaeoides*-3
8. *C. scarabaeoides*-4
9. *C. sericeus*
10. *C. volubilis*
11. *C. rugosus*
12. *R. sublobata*
13. *C. albicans*-1
14. *R. minima*
15. *R. edulis*
16. *R. verdcourtii*

M. λ*Hind* III DNA

DNA cut with *Hind* III standard markers. The strongly hybridizing bands in the autoradiogram were scored manually and their molecular sizes were determined from the molecular weight markers run along side the samples in each of the gel.

*Data analysis*

Similarity index matrices were generated based on proportion of common restriction digestion fragments between two accessions (Nei & Li, 1979) using

$$F = \frac{2\,Mxy}{Mx + My}$$

where 'F' is the similarity index, Mx is the number of bands in accession x, My the number of bands in accession y, and Mxy the number of bands common to both x and y. Cluster analysis of the similarity index data generated from the Southern blot hybridization patterns in the autoradiograms was carried out using the statistical software package Clustan$^{TM}$, and a dendrogram was constructed.

## Results and discussion

All the three maize mtDNA probes in combination with three different restriction enzymes detected high levels of polymorphism among the different accessions of the wild and cultivated species of pigeonpea. The representative autoradiograms showing the hybridization patterns with two different enzyme-probe combinations, *Eco*R V/*atp*6 and *Eco*R V /*cox* I are shown in Figures 1 a and b. In most instances a single strongly hybridizing band was seen whereas in others such as *R. sublobota* (Figure 1a, lane 12) two bands were seen suggesting the presence of additional copies of the *atp*6 gene in this species. A similar difference in hybridization banding patterns was seen with DNA digested with *Eco*R V and probed with *cox* I. In majority of the species two strongly hybridizing bands were observed and in *C. albicans* and *C. cajanifolius* (Figure 1b lanes 2 and 3 respectively) 4 bands were seen. Such differences in the number of strongly hybridizing bands were observed with other restriction enzyme- probe combinations as well.

Cluster analysis of the similarity index data revealed the clear separation of the two genera, *Cajanus*

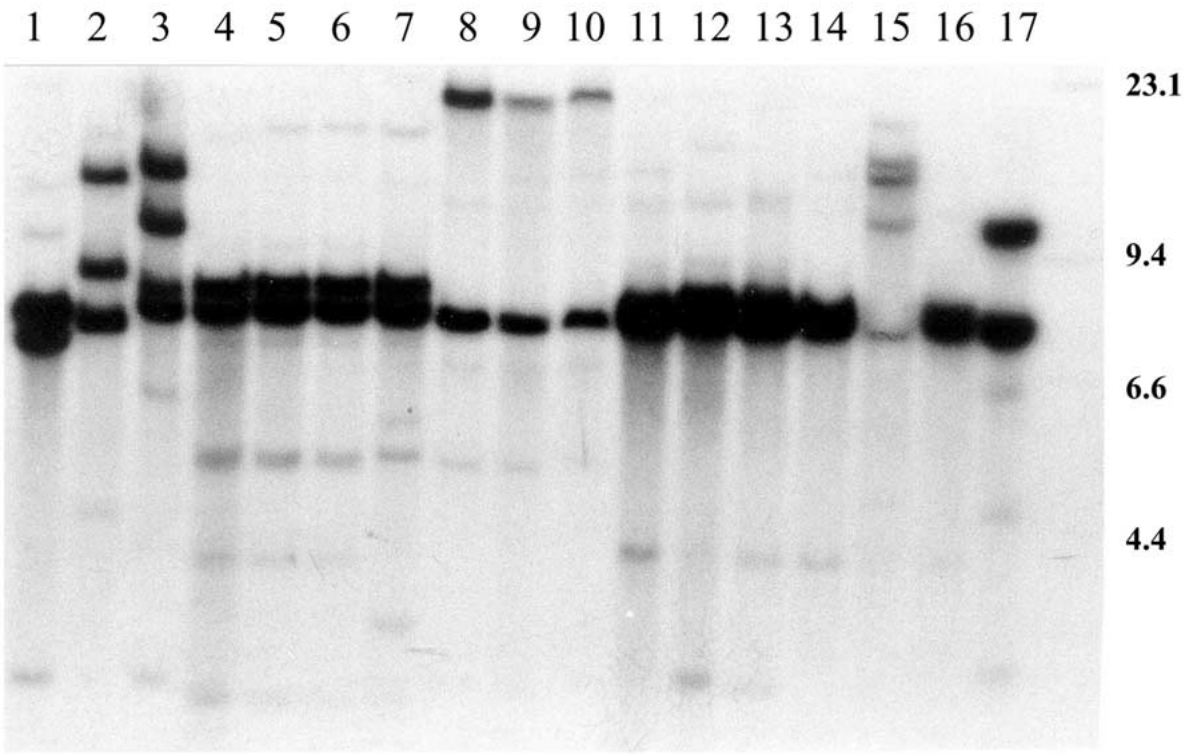


*Figure 1b.* Autoradiogram showing the diversity in pigeonpea wild species from the Southern blot hybridization of total DNA digested with *Eco* RV and probed with maize *cox* I clone.

Lane

1. *C. acutifolius*-1
2. *C. albicans*-1
3. *C. cajanifolius*
4. *C. platycarpus*-1
5. *C. platycarpus*-2
6. *C. platycarpus*-3
7. *C. platycarpus*-4
8. *C. scarabaeoides*-1
9. *C. scarabaeoides*-2
10. *C. scarabaeoides*-3
11. *C. cajan*-1
12. *C. cajan*-2
13. *C. cajan*-3
14. *C. cajan*-4
15. *C. sericeus*
16. *C. cajan*-5
17. *C. volubilis*

M: λ DNA digested with*HindIII*

and *Rhyncosia* from each other (Figure 2). The four species of *Rhyncosia* formed one major group with three subgroups showing small variations between the species. In *Cajanus*, the four accessions of *C. platycarpus* belonging to section *Rhyncosoides* formed a separate group (Figure 2). The distinctness of *C. platycarpus* accessions observed in this study corroborates well with the earlier reports on the interrelationships of *C. platycarpus* and other wild relatives of pigeonpea as shown by the studies on esterase isozymes (Krishna & Reddy, 1982) and interspecific crossability (Reddy et al., 1981; Pundir & Singh, 1985). In a similar way, the four accessions of *C. scarabaeoides* in section *Cantharospermum,* formed a major group with 3 subgroups. The other 2 species of the same section, *C. goensis*, and *C. rugosus*, were placed in two of the subgroups within this major cluster (Figure 2). The five accessions of *C. cajan* separated together as a major group. *C. cajanifolius* of the same section taxonomically, however, clustered with section *Cantharospermum* in a separate subgroup. The clustering of *C. cajanifolius* and *C. scarabaeoides* within the same major group, but separately suggests a similarity which was also observed by Krishna & Reddy (1982) who reported a common band of esterase isozyme between the two species. Both the accessions of *C. albicans* and the single accession of *C. sericeus* were closer to *C. cajan* in a separate group. The close relationship of *C. albicans, C. sericeus, C. cajanifolius* and *C. cajan* revealed by the placing of these in closer groups in the present study is similar to those ob-

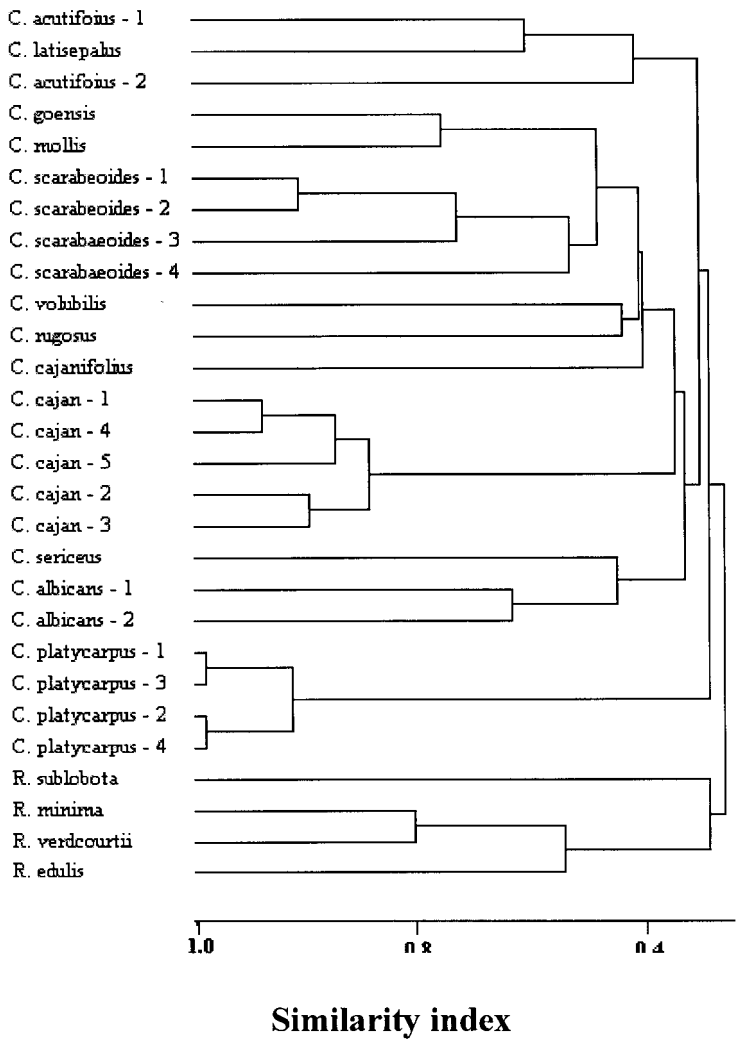


*Figure 2.* Dendrogram of 28 pigeonpea species based on the Southern blot hybridization of total DNA with maize mtDNA probes. Cluster analysis of the DNA hybridization data from the combinations of 3 restriction enzymes (*Eco* RI, *Eco* RV, and *Hind* III) and 3 maize mtDNA probes (*atp* 6, *atp* $\alpha$, and *cox* I) carried out as described under Materials and methods.

served by others using different molecular methods (Nadimpalli et al., 1993; Ratnaparkhe et al., 1995).

In our study, *C. rugosus* was together with *C. volubilis* whereas *C. goensis* was separated out unlike the observation made by Ratnaparkhe et al. (1995). Further, this group was also reported to include other species of *Dunbaria* and *Rhyncosia* as observed by others (Nadimpalli et al., 1990; Ratnaparkhe et al., 1995). Two accessions of *C. acutifolius* (ICPW 4 and 12) and the single accession of *C. latisepalus*, which are endemic to Australia, formed a separate group (Figure 2). These two *Cajanus* species of Australian origin were always grouped together irrespective of the molecular method used to assess diversity. This observation supports the relegation of these two species under the same section, *Fruticosa* (van der Maesen, 1990) based on morphological characteristics. This grouping is in line with the taxonomic classification of these species by van der Maesen (1990).

The organelle genomes like the mitochondrial genome used in the present study are supposed to detect inter-species specific variations more efficiently than the intra-species variations (Ennos et al., 1999). But our present studies demonstrate that even intra-specific

variation could be detected efficiently *eg. C. platycarpus, C. scarabaeoides, C. acutifolius*, and *C. cajan.* The diversity in the mitochondrial genome within the cultivated species as observed in this study suggests the heterogeneity of mitochondria in the evolution of cultivated *Cajanus* species. Since the mitochondrial genome is maternally inherited, the formation of separate groups for the cultivated types only suggest that the maternal lineage of these species might be different or it might be due to internal recombination which is well known in mitochondria. In the case of soybean four distinct mtDNA hybridization patterns were seen suggesting four maternal groups among the cultivated types (Grabau et al., 1992). Such a prediction can be made in pigeonpea only after studying more number of accessions within the cultivated species.

There are two areas of diversity for the genus *Cajanus*, which now includes the former genus *Atylosia* (van der Maesen, 1990). The species belonging to the secondary and tertiary gene pools are grouped together in the present method. The six sections of *Cajanus* classified in Table 1 could easily be distinguished based on their characteristic DNA hybridization patterns. The present study also suggests that RFLPs of mtDNA could be used to distinguish the *Cajanus* species belonging to the secondary gene pool; *C acutifolius*, *C. albicans*, *C. latisepalus*, *C. scarabaeoides*, *C. sericeus*, and those belonging to the tertiary gene pools, *C. mollis*, *C. platycarpus*, *C. rugosus*, *C. volubilis*, and *Rhyncosia.* The strong mtDNA hybridization signals obtained with maize mtDNA of pigeonpea suggests that there is a high degree of conservation of the coding sequences of DNA among plant mtDNAs and so RFLPs of mtDNA could be used for the analysis of diversity among pigeonpea accessions.

One of the uses of the molecular probes is in the phylogenetic classification of plant species (Miller & Tanksley, 1990; Nadimpalli et al., 1992; Svitashev et al., 1994). Earlier studies used to rely on morphological and cytological variations for the phylogenetic and taxonomic grouping of *Cajanus* (Pundir & Singh, 1981; Reddy & De, 1983) whereas the present study as well as those of others (Nadimpalli et al., 1992; Ratnaparkhe et al., 1994) have clearly established the utility of molecular markers like RAPDs, and RFLPs in the diversity analysis of cultivated and wild species of pigeonpea accessions and deducing the phylogenetic relationships.

Tozuka et al. (1998) were able to classify the mitochondrial types of soybean cultivated in the different regions in Japan based on the RFLP pattern of mtDNA, but they also showed the variation in the cultivars from the different geographic regions in Japan. The total number of hybridization bands obtained with the different accessions of pigeonpea varied considerably – with an average of 10 different hybridization banding patterns with the different restriction enzyme-probe combinations – in contrast to the small number of bands observed for soybean by Tozuka et al. (1998) which makes it rather difficult to predict the genetic make up of the mitochondrial genome in the wild species of pigeonpea or their evolution.

The conserved nature of the mitochondrial genome among the cereals and legumes is clearly shown by the fact that maize mtDNA could be used to differentiate pigeonpea cytoplasms using the hybridization method. It would be interesting to see if we could isolate the mitochondrial gene sequences from pigeonpea itself and compare it with those of other cereals and legumes to study the evolutionary aspect of mitochondrial diversity in pigeonpea.

## Acknowledgements

The technical assistance of Mr Brian J. Moss in the RFLP work is gratefully acknowledged.

## References

Ahmad, F., P.M. Gur & A.E. Slinkard, 1992. Isozyme polymorphism and phylogenetic interpretations in the genus *Cicer* L. Theor Appl Genet 83: 620–627.

Close, P.S., R.C. Shoemaker & P. Keim, 1989. Distribution of restriction site polymorphism within the chloroplast genome of the genus *Glycine* subgenus *Soja*. Theor Appl Genet 77: 768–776.

Deu, M.P., Hamon, J. Chantereau, P. Dufour, A. D'hont & C. Lanaud, 1995. Mitochondrial DNA diversity in wild and cultivated sorghum. Genome 38: 635–645.

Devos, K.M. & M.D. Gale, 1992. The use of random amplified polymorphic DNA markers in wheat. Theor Appl Genet 84: 567–572.

Dewey, R.E., C.S. Levings III & D.H. Timothy, 1985. Nucleotide sequence of ATPase subunit 6 gene of maize mitochondria. Plant Physiol 79: 914–919.

Ennes R.A., W.T. Sinclair, X-S. Hu & A. Langdon, 1999. Using organelle markers to elucidate the history, ecology and evolution of plant populations. In: P.M. Hollingsworth, R.M. Bateman & R.J. Gornall (Eds.), Molecular Systematics and Plant Evolution, pp. 1–19. Taylor & Francis, London.

Feinburg, A.P. & B. Vogelstein, 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 137: 266–267.

Gawel, N.J., R.L. Jarret & A. Whittemore, 1992. Restriction fragment length polymorphism (RFLP)-based phylogenetic analysis of *Musa*. Theor Appl Genet 84: 286–290.

Gonzalez, J.M. & E. Ferrer, 1993. Random amplified polymorphic DNA analysis in *Hordeum* species. Genome 36: 1029–1031.

Grabau, E.A., W.H. Davis, N.D. Phelps & B.G. Gengenbach, 1992. Classification of soybean cultivars based on mitochondrial restriction fragment length polymorphisms. Crop Sci 32: 271–274.

Harris, S.A., 1999. RAPDs in sytematics – a useful methodology? In: P.M. Hollingsworth, R.M. Bateman and R.J. Gornall (Eds.), Molecular Systematics and Plant Evolution, pp. 211–228. Taylor & Francis, London.

Hershkovitz, M.A., E.A. Zimmer & W.J. Hahn, 1999, Ribosomal DNA sequences and angiosperm systematics. In: P.M. Hollingsworth, R.M. Bateman & R.J. Gornall (Eds.), Molecular Systematics and Plant Evolution, pp. 268–326. Taylor & Francis, London.

Hongtrakul, V., G.M. Huestis & S.J. Knap, 1997. Amplified fragment length polymorphisms as tool for DNA fingerprinting sunflower germplasm: genetic diversity among oilseed inbred lines. Theor Appl Genet 95: 400–407.

Isaac, P.G., V.P. Jones & C.J. Leaver, 1985. The maize cytochrome c oxidase subunit I gene: sequence, expression and rearrangement in cytoplasmic male-sterile plants. EMBO J 4: 1617–1623.

Jain, A., S. Bhatia, S.S. Banga, S. Prakash & M. Lakshmikumaran, 1994. Potential use of random amplified polymorphic DNA (RAPD) technique to study the genetic diversity in Indian mustard (*Brassica juncea*) and its relationship to heterosis. Theor Appl Genet: 116–122.

Jarret, R.L., N. Gawel & A. Whittemore, 1992. Phylogenetic relationships of the sweet potato (*Ipomoea batatas* (L.) *Lam*.] J Am Soc Hort Sci 117: 633–637.

Jena, K.K. & G.C. Kochert, 1991. Restriction fragment length polymorphism analysis of CCDD genome species of the genus *Oryza* L. Plant Mol Biol 16: 831–839.

Krishna, T.G. & L.J. Reddy, 1982. Species affinities between *Cajanus cajan* and some *Atylosia* species based on esterase isozymes. Euphytica 31: 709–713.

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

Levinson, G. & G.A. Gutman, 1987. Slipped-strand mispairing: a major mechanism for DNA sequence evolution. Molec Biol Evol 4: 203–221.

Mackill, D.J., 1995. Classifying *Japonica* rice cultivars with RAPD markers. Crop Sci 35: 889–894.

McGregor C.E., C.A. Lambert, M.M. Greyling, J.H. Louw & L. Warnich, 2000. A comparative assessment of DNA fingerprinting techniques (RAPD, ISSR, AFLP and SSR) in tetraploid potato (*Solanum tuberosum* L.) germplasm. Euphytica 113: 135–144.

Miller, J.C. & S.D. Tanksley, 1990. RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. Theor Appl Genet 80: 437–448.

Moeykens, C.A., S.A. Mackenzie & R.C. Shoemaker, 1995. Mitochondrial genome diversity in soybean: repeats and rearrangements. Plant Mol Biol 29: 245–254.

Nadimpalli, R.G., R.L. Jarret, S.C. Phatak & G. Kochert, 1993. Phylogenetic relationships of the pigeonpea (*Cajanus cajan*) based on nuclear restriction fragment length polymorphisms. Genome 36: 216–223.

Nei, M & W.H. Li, 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci (USA) 76: 5269–5273.

Nene, Y.L. & V.K. Sheila, 1990. Pigeonpea: Geography and importance. In: Y.L. Nene, S.D. Hall & V.K. Sheila (Eds.), The Pigeonpea, pp. 1–15. C.A.B. International.

Provan, J., N. Saranzo, N.J. Wison, J.W. McNicol, M. Margante & W. Powel, 1999. The use of uniparentally inherited simple sequence repeat markers in plant population studies and systematics. In: P.M. Hollingsworth, R.M. Bateman & R.J. Gornall (Eds.), Molecular Systematics and Plant Evolution, pp. 35–50. Taylor and Francis, London.

Pundir, R.P.S. & R.B. Singh, 1985. Cytogenetics of $F_1$ hybrids between *Cajanus* and *Atylosia* species and its phylogenetic implications. Theor Appl Genet 71: 216–220.

Ratnaparkhe, M.B., V.S. Gupta, M.R. Ven Murthy & P.K. Ranjekar, 1995. Genetic fingerprinting of pigeonpea (*Cajanus cajan* (L.) *Millsp*.] and its wild relatives using RAPD markers. Theor Appl Genet 91: 893–898.

Reddy, L.J. & D.N. De, 1983. Cyto-morphological studies in *Cajanus cajan* × *Atylosia lineata*. Ind J Genet 43: 96–103.

Reddy, L.J., J.M. Green & D. Sharma, 1981. Genetics of *Cajanus cajan* (L.) *Mills* sp. × *Atylosia* spp. In: Proceedings of the International workshop on Pigeonpeas, 15–19 Dec 1980. ICRISAT Center, Vol 2, pp. 39–50. Patancheru, AP, India.

Singh, A.K., S. Sivaramakrishnan, M.H. Mengesha & C.D. Ramaiah, 1991. Phylogenetic relations in section *Arachis* based on seed protein profile. Theor Appl Genet 82: 593–597.

Sivaramakrishnan, S., K. Seetha, A. Nageshwar Rao & Laxman Singh, 1997. RFLP analysis of Cytoplasmic male-sterile lines of pigeonpea [*Cajanus cajan* (L.) Millsp.] developed by interspecific crosses. Euphytica 93: 307–312.

Smartt, J., 1990. Grain Legumes: Evolution and Genetic Resources, pp. 278–293. Cambridge University Press, Cambridge.

Svitashev, S., T. Bryngelsson, A. Vershinin, C. Pedersen, T. Sall & R. von Bothmer, 1994. Phylogenetic analysis of the genus *Hordeum* using repetitive DNA sequences. Theor Appl Genet 89: 801–810.

Tozuka, A., H. Fukushi, T. Hirata, M. Ohara, A. Kanazawa, T. Mikami, J. Abe & Y. Shimamoto, 1998. Composite and clinal distribution of *Glycine soja* in Japan revealed by RFLP analysis of mitochondrial DNA. Theor Appl Genet 96: 170–176.

van der Maesen, L.J.G., 1986. *Cajanus* D.C. and *Atylosia* W. & A. (*Leguminosae*). Agric. University of Wageningen Papers 85-4 (1985). Agricultural University. Wageningen, The Netherlands, 225 pp.

van der Maesen, L.J.G., 1990. Pigeonpea: origin, history, evolution, and taxonomy. In: Y.L. Nene, S.D. Hall & V.K. Sheila (Eds.), The Pigeonpea, pp. 15–46. C.A.B. International.

Wang, G., R. Mahalingam & H.T. Knap, 1998. (C-A) and (G-A) anchored simple sequence repeats (ASSRs) generated polymorphism in soybean, *Glycine max* (L.) *Merr*. Theor Appl Genet 96: 1086–1096.