International Scholars Journals

African Journal of Virology Research ISSN 2756-3413 Vol. 14 (6), pp. 001-006, June, 2020. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Genetic variability and relatedness of the Asian and African pigeon pea as revealed by AFLP

S. Wasike, P. Okori*, and P.R. Rubaihayo

Department of Crop Science, Faculty of Agriculture, Makerere University P.O. Box 7062 Kampala, Uganda.

Accepted 21 February, 2020

Amplified fragment length polymorphism was used to study genetic variability and relatedness between African and Asian pigeon pea cultivars. Forty-one samples, 32 African and 9 Asian varieties were subjected to the analyses. The genetic data was subjected to phenetic and analysis of molecular variation (AMOVA). Phenetic analysis revealed no major clusters and indicated limited genetic variability among the samples. AMOVA at continent wide hierarchical level, revealed a significantly weak population structure (φF_{ST} = 0.05, P= 0.001) and Fishers' exact tests ($P \leq 0.05$) provided no support for population differentiation. AMOVA based on treating the cultivars as samples from a panmictic population revealed a stronger genetic structure (φF_{ST} = 0.09, P= 0.001). Estimates of average gene diversity were not significantly different among samples but were relatively higher for Indian samples. This study suggests that East Africa pigeon peas are closely related but less genetically diverse than Indian cultivars, which could also serve as source of novel traits for the latter. The study also demonstrated that AFLP is a suitable tool for DNA fingerprinting and genetic studies of pigeon pea.

Key words: AMOVA, *Cajanus cajan,* genetic diversity, genetic structure.

INTRODUCTION

Pigeon pea, *Cajanus cajan* (L) Millsp. is one of the major grain legume crops grown in the tropics and subtropics (Silim 2000, Souframanien et al., 2003). This crop is favoured for its drought tolerance and is grown mainly as an intercrop with cereals like maize and sorghum (Owere et al., 2000, Odeny, 2000). It has both short and long maturing varieties thus spreading harvest regimes through the year. Recently, the crop has regained importance in some areas of Uganda which want to bring new areas under cultivation (Odeny, 2000). India is the world's largest pigeon pea producer, though Africa has six out of the ten largest producing countries in the world, Kenya being the largest followed by Uganda (Kimani, 2000). Compared to other grain legumes such as beans and cowpea cultivated in the region, pigeon pea has received relatively little research attention and the need to do so is of utmost importance. A starting point is to determine the genetic diversity of the pigeon pea.

Determination of genetic diversity of any given crop species is a suitable precursor for improvement of the crop because it generates baseline data to guide selection of parental lines and design of a breeding scheme. In the case of pigeon pea, particularly in Africa, there is paucity of this information. In fact, the very origin of the crop has been very speculative with some authors suggesting that it was domesticated in Africa (Purseglove, 1988; Tindall, 1988), or India (van der Maesen, 1980, Souframanien et al., 2003) or in both Africa and Asia (FAO, 1988).

The early systematic studies of the genus *Cajanus* were based on phenological or morphological characters, which have been shown to have limited genetic resolution especially at species levels, as is required for pigeon pea (van der Maesen, 1980, Kimani et al., 2000). Genetic characterization especially at species level could be improved by use of neutral genetic markers that readily give good record of the recent evolutionary history of an organism (Lynch and Milligan, 1994; Weir, 1996). Genetic variability of Indian pigeon pea has been studied using neutral genetic markers such as restriction fragment length polymorphism (RFLP) (Nadimpalli et al., 1994) and random amplified polymorphic DNA (RAPD) (Ratnaparkhe

*Corresponding author. Email: pokori@agric.mak.ac.ug.

Table 1. Origin and designations of the pigeon pea cultivars used in the study.

.

Cultivar	Country of origin	Cultivar	Country of origin
UN-PI /7/2	Kenya	UN-PI/42	Kenya
UN-PI /17/3	Kenya	UN-PI/31/4	Kenya
UN-PI/16	Kenya	ICEAP 004335-S1	India
UN-PI/28/3	Kenya	UN-PI/40/6	Kenya
UN-PI/49/1	Kenya	UN-PI/31/1	Kenya
UN-PI/37/6	Kenya	UN-PI/14/5	Kenya
UN-PI/35/1	Kenya	UN-PI/51/2	Kenya
UN-PI/18/7	Kenya	ICP II298-S3	India
UN-PI/4	Kenya	ICEAP 00557-S4	India
UN-PI/34/3	Kenya	UN-PI/47/5	Kenya
UN-PI/43/4	Kenya	ICEAP 00540-S1	India
UN-PI/36/1	Kenya	UN-PI/18/8	Kenya
UN-PI/20/1	Kenya	UN-PI/30/2	Kenya
UN-PI/40/1	Kenya	UN-PI/31/7	Kenya
UN-PI/14/1	Kenya	ICEAP 00553-S1	India
UN-PI/40/3	Kenya	ICP 11298-S4	India
UN-PI/28/2	Kenya	ICPL 87091	India
UN-PI/14/2	Kenya	KAT 60/8	India
UN-PI/48/1	Kenya	ICP 6927	India
UN-PI/41/2	Kenya	Adong	Uganda
		Apio Elena	Uganda

et al.,1995). These studies have also compared genetic relationships to wild relatives and reported that cultivated pigeon pea has limited genetic variability (Nadimpalli et al., 1994). The studies also provided a basis for selection of pigeon pea lines with cytoplasmic male sterility, for subsequent use in hybrid development (Souframanien et al., 2003). No such studies have been conducted in East Africa in spite of being both the largest producer and consumer of the crop in the continent. Currently, there are many cultivars grown in different parts of East Africa, with some considered as landraces in Africa. Undertaking studies directed at elucidating the genetic variability of pigeon pea will provide a basis for informed decisions on genetic improvement of the crop. Additionally, though pigeon pea is a self-pollinated crop, it undergoes cross-pollination of 40% to 70% in some cultivars accounting for the heterogeneous nature of the crop (Gupta et al., 1980; Ratnaparkhe et al., 1995; Souframanien et al., 2003). Under such a scenario, the use of molecular markers, in particular, neutral genetic markers to unravel genetic relationships between and among cultivars and or species becomes justified. Today there is a wide array of neutral genetic markers available for use in detection of genetic variability and genetic studies in general. The amplified fragment length polymorphism (AFLP) (Vos et al., 1995) is one such technique that has been used lately in genetic characterisation of plants and plant pathogens (McDonald and Linde 1998; Okori et al., 2003). In the present investigation we studied genetic variability among African and Asian originated pigeon pea varieties. The study shows that African pigeon peas are closely related to the Indian cultivars. The African cultivars also appear to be less genetically diverse than the Indian cultivars, which could act as source of novel traits for further improvement of African germplasm.

MATERIALS AND METHODS

Plant material and DNA isolation

Young leaf samples were collected from pigeon pea accessions grown at Makerere University Agriculture Research Institute Kabanyolo (MUARIK). Only non-segregating varieties were included in the study. Professor P.M. Kimani of Nairobi University, Kenya, kindly supplied all pigeon pea varieties used in this study with exception of Apio Elena and Adong, which are local long maturing and bushy Northern Uganda land races, obtained from Ngeta Agricultural Research Development Centre in Lira District. Samples used are indicated in (Table 1).

DNA was extracted from the fresh samples (about 3 g), by modifying a hot CTAB (cetyltrimethyl ammonium bromide) method of Vroh et al. (1996). Modifications were made in the extraction buffer by reduction of -mercaptoethanol and sodium chloride concentrations, from 5% and 2 M respectively, as indicated; (2% CTAB (w/v), 1.4 M NaCl, 2% -mercaptoethanol, 20 mM EDTA, 100 mM Tris-HCL pH 8.0, 1% PVP-40 (polyvinlypyrrolidone Sigma, St. Louis, MO, USA). The use of activated charcoal was also omitted and a phenol:chloroform extraction step was included in addition to chloroform:isoamyl (24:1) extractions. These

Table 2. Description of the primers used for amplified fragment length polymorphism analysis.

Primer	Function	Sequence
*EcoR*I-1	Adapters	5'-CTCGTAGACTGCGTACC-3'
*EcoR*I-2		3'-CTGACGCATGGTTAA-5'
*Mse*I -1	Adapters	5'-GACGATGAGTCCTGAG-3'
*Mse*I -2		3'-TACTCAGGACTCAT-5'
*EcoR*I +0	Pre-selective amplification	5'-GACTGCGTACCAATTC-3'
*Mse*I +0	Pre-selective amplification	5'-GATGAGTCCTGAGTAA-3'
EcoRI +3	Selective amplification	*MseI* +3
E_1 5'-Eseq agg-3'	Selective amplification	M_1 5'-Mseq cag -3'
E_2 5'-Eseq agc-3'	Selective amplification	M_2 5'-Mseq cac -3'
E_3 5'-Eseq act-3'	Selective amplification	M_3 5'-Mseq cac -3'
E_4 5'-Eseq agg-3'	Selective amplification	M_4 5'-Mseq cta -3'

E seq = GACTGCGTACCAATTC, *Mse*I= GATGAGTCCTGAGTAA.

modifications were performed to improve removal of impurities during DNA isolation. The isolated DNA was suspended in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0), quantified using a spectrophotometer (SmartSpec^{TM}3000, BIO-RAD, USA) and stored at -20° C until further analysis. DNA quality was also checked by ethidium bromide straining of DNA on a 1 % agarose gel (Sambrook et al., 1989).

AFLP analysis

The DNA was diluted to 500 ng/∝l and subjected to AFLP analysis using the AFLP analysis system I kit (Gibco Life technologies, California, USA). All PCR steps were performed using an iCycler^{TM} BIO-RAD, USA. The primers and adapters used in the study are indicated in Table 2. PCR products were separated using a 6% denaturing polyacrylamide gel, visualized by autoradiography using Kodak Bio Max 1 films and the films developed as recommended by the manufacturer (Eastman Kodak Company, Rochester, New York, U.S.A). Only bands that could be read unambiguously on each autoradiograph were considered for analysis.

Phenetic and population structure analyses

Each band or fragment was treated as a separate putative locus and scored using a binary system of present (1) and absent (0). The binary matrix was then subjected to phenetic analysis and tested further for a population structure. Phenetic analysis was performed using the Neighbor-joining algorithm as implemented in PHYLIP (Felsenstein, 1999). Robustness of the tree was tested by bootstrap analysis of 1000 replicates and a consensus tree drawn using Majority Rule Consensus. The PHYLIP sub-programmes SEQBOOT and CONSENSE were used for bootstrap and consensus tree drawing respectively. For further genetic analyses, the AFLP data was treated as haplotypic comprising of a combination of alleles at one or several loci (Schneider et al., 2000). The data was tested for presence of population structure by analysis of molecular variation (AMOVA) (Excoffier et al., 1992) using Arlequin version 2 (Schneider et al., 2000). For this purpose, pigeon pea accessions were structured according to continent of origin as Africa or Asia or as sub-populations from one population. The significance of the fixation indices were tested using a non-parametric permutation approach as (Excoffier et al., 1992) as implemented in Arlequin (Schneider et al., 2000). Tests for population differentiation were also performed using Fishers exact test as implemented in Arlequin. Gene diversity ($\hat{H}$) i.e. the probability that two randomly chosen haplotypes are different in a sample was also calculated. $\hat{H}$ and its sampling variance $V(\hat{H})$ were computed as:

$$\hat{H} = \frac{n}{n-1}\left(1 - \sum_{i=1}^{k} p_i^2\right)$$

$$\hat{V}(\hat{H}) = \frac{2}{n(n-1)}\left[\sum_{i=1}^{k} p_i^3 - \left(\sum_{i=1}^{k} p_i^2\right)^2\right]$$

Where n is the number of gene copies in the sample, k is the number of haplotypes and P_i is the sample frequency of the i-th haplotype (Nei 1987). The $\hat{H}$ values were compared by t-test as suggested by Nei (1987).

RESULTS

The AFLP analysis revealed an average fragment score of 75 for selective primer combinations used. Primer combination E- ACT/M- CAC yielded the highest number of fragments of 86 and E- AGG/M-CAG with the least with 61 (Figure 1). Phenetic analyses revealed no major clusters. Clusters at the major nodes all had low bootstrap support of less than 70% and are thus not indicated on the phenetic tree (Figure 2). The two indigenous Ugandan pigeon pea varieties though clustering together were distinct (Figure 2). No major clustering patterns according to country of origin were observed. Further analyses of the data using analysis of variation (AMOVA) revealed presence of a significant but weak population structure (φFST = 0.05, P= 0.001) (Table 3). When treated as subpopulations from one large population, a much stronger FST estimate was

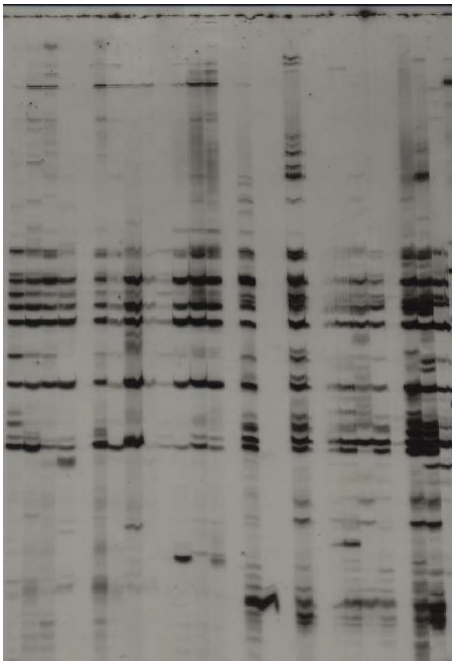

Figure 1. Amplified fragment length polymorphism profiles of East African and Indian pigeon pea cultivars used in the study. The fragments were generated using the primer pair E_1 (Eseq AGG) and M_4 (Mseq CTA) described in materials and methods.

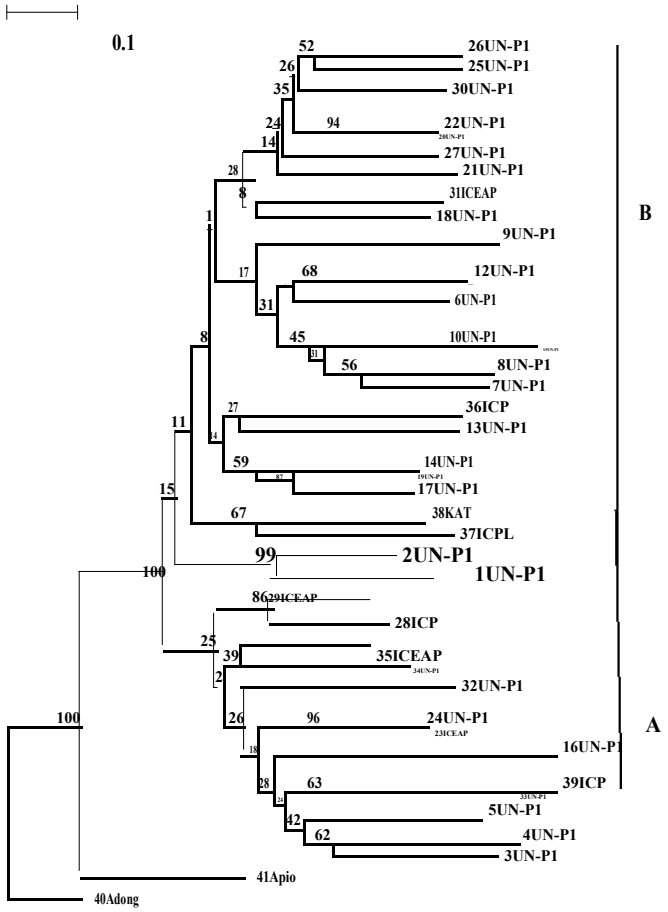

Figure 2. A dendogram showing clusters of African and Asian pigeon pea generated using Treecon 1.3b from Nie and Li Neighbour joining analysis. Two clusters A and B were generated with the latter closer to the local Ugandian varieties.

obtained (φFST = 0.09, P= 0.001). AMOVA produces estimates of variance components reflecting correlation of haplotypic diversity at different levels of hierarchical divisions and in this study; variance components at continent level were negative while estimates of samples treated subpopulations from one population were positive (Table 3). Exact tests revealed no population differentiation. Estimates of average gene diversity ( ), were moderate but not significantly different (P> 0.05), and revealed that Indian pigeon pea cultivars were more genetically diverse (Table 4). Furthermore, our work revealed no distinct clusters based upon the maturity times and morphological marker traits like flowering time and floral colour of the pigeon peas (data not shown).

DISCUSSION

The study presents for the first time genetic characterization of pigeon pea using AFLP. The study revealed a close relationship between the test cultivars in this study as shown by presence of a weak population structure and absence of any major clustering patterns from phenetic analysis. The presence of a weak population structure between the two populations, African and Asia, suggests that the cultivars in this study have not been genetically isolated from each other. Two populations deriving from the same original population have got to be reproductively and genetically isolated from each other to permit selection and fixation of unique alleles that will account for divergence and presence of genetic structures (Hartl and Clark, 1997). In this study we obtained negative variance components in AMOVA at the continent hierarchical level. AMOVA estimates variance components and φF_{ST} following a multi-allelic (multivariate analysis of variation) (Excoffier et al., 1992), as previously described by Weir and Cockerham (1984). This methodology relies on estimates of relationships between alleles in the same populations relative to alleles of different populations and is designated by the parameter Accordingly, negative variance components can result from very small but positive estimates of from data relative to the true value in nature or if is indeed negative (Weir 1996). In genetic terms, this implies that alleles are more related between than within populations (Weir and Cockerham, 1984; Weir, 1996). When treated as sub-populations of one pigeon pea population, the φF_{ST} was still weak, but much higher. This provides

Table 3. Analysis of molecular variation based on 299 AFLP fragments of pigeon pea from Africa and Asia.

Source of variation	df	Sum of squares	Variance components	Percentage of variation
[1]Continent level				
Among groups[a]	1	61.75	-9.68	-21.41
Among Populations	1	87.00	11.68	25.85
Within populations	38	1641.33	43.19	95.56
Total	40	1790.25	45.58	
ΦF_{ST} = 0.05 (P = 0.001) based on 1023 permutations				
[2]One population				
Among populations	2	148.75	3.68	7.84
Within populations	38	1641.33	43.19	92.16
Total	40	1790.10	46.87	
ΦF_{ST} = 0.09 (P = 0.001) based on 1023 permutations				

[1]Comparisons done at the continent hierarchical level, that is, Africa and Asia.
[2]All cultivars considered as samples from one large population, with samples treated as sub-populations from the Africa and Asia.
[a]Groups refers to continent of origin, that is, Africa or Asia, while populations refers to samples from Kenya, India and Uganda.

Table 4. Gene diversity and numbers of polymorphic loci of pigeon pea from Africa and Asia based on 299 AFLP loci examined.

Country of Origin	Number of polymorphic loci	Average gene diversity ($\hat{H}$)[a]
Kenya	292	0.287
Uganda	72	0.241
India	231	0.304

[a]Average gene diversity computed according to Nei (1987) and Tajima (1983).

$\hat{H}$ values are not significantly different at ($P \leq 0.05$). Tests performed according to Nei (1987).

support for our hypothesis that the pigeon pea cultivars used in this study originate from a common gene pool, and the inbred nature of the crop contributes to maintaining genetic relatedness between the two populations. Indeed, phenetic analysis revealed no major clustering, providing support for genetic relatedness between the African and Asian pigeon peas. Other workers investigating diversity in pigeon pea have reported similar findings all based on use of neutral genetic markers such as random amplified polymorphic DNA (RAPD) (Ratnaparkhe et al., 1995) or PCR restriction Fragment length polymorphism of chloroplast DNA (Lakshmi et al., 2000).

It should be noted that pigeon pea is primarily a self-pollinated crop (Ratnaparhke et al., 1995), although varying levels of natural out-crossing of up to 70% occurs in some cultivars, (Souframanien et al., 2003). This inbred nature of the crop may account for the limited genetic variability observed in this study and that reported by other workers. Using, average gene diversity or average heterozygosity, a method suitable for determining genetic variability among inbred individuals (Weir, 1996), this study revealed that Indian samples were more genetically diverse than the African cultivars. Moreover, the two farmer selected Ugandan indigenous cultivars, *Apio Elena* and *Adong* had the lowest levels of DNA polymorphism compared to all other cultivars. The two cultivars have been cultivated in northern Uganda for a long time with very limited introduction of exotic germplasm in to the country. Conversely, Kenya has a more significant influence on local germplasm through introductions and breeding, accounting for the relatively higher levels of variability observed (Kimani, 2000; Kimani et al., 2000). Earlier work based on morphological attributes proposed India as the pigeon pea centre of domestication, with Africa as a secondary centre of diversity (van der Maesen, 1980) . In this study we used a few samples of Indian origin, but a more detailed analysis using a wider genetic collection of Indian cultivars could reveal a lot more about the genetic diversity of the crop and improve our knowledge about its domestication and possible sources of germplasm for further improvement of the crop. A similar approach for African cultivars is also worthwhile.

Taken together, this study has for the first time genetically characterized African pigeon pea cultivars and made comparisons with the Indian cultivars. Our data show that the two populations are very closely related, although the Indian cultivars appear to be more genetically diverse. Thus Indian cultivars could be used as a source of germplasm for further improvement of East African pigeon peas. The small sample sizes in this study and others previously reported, however, limit the magnitude of analysis and rationalization that can be made from such data. A more logical thing to do would be to conduct more rigorous experimentation using larger samples sizes to help elucidate the general genetic variability of the crop and define primary centres of diversity of this important crop. This study also demonstrated that AFLP is a suitable tool for genetic analyses of pigeon pea since it randomly samples from different parts of the genome and generates a lot more data than RAPD, which have been previously used in pigeon pea genetic characterisation.

ACKNOWLEDGMENTS

This study was funded by a European Union grant to the third author through project INCO-DC Contract number ERBIC 18-6 CT 96-01-30. The authors thank Professor Kimani, P.M. of the University of Nairobi for supplying Pigeon pea cultivars used in the study.

REFERENCES

Excoffier L, Smouse P, Quattro J (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial restriction data. Genetics 131: 479-491.

FAO (1988). Tropical Forage Legumes. Plant Production and Protection series No 2. PJ Skerman, DG Cameron, F Riveros (eds). pp. 539-547. Food and Agric. Org. of the United Nations (FAO), Rome..

Felsenstein J. (1999). PHYLIP 3.6 (Alpha) Phylogeny inference package, Dept. of Genetics Uni. of Washington Seattle, WA, USA.

Gupta SC, Reddy LJ, Sharma D, Gree JM, Murthi AN, Saxena KD (1980). Maintenance of pigeon pea cultivars. In: Neen NL (eds), Proceedings of an Int. Workshop on Pigeon pea Vol. 2. pp. 295-301. ICRISAT, Pantancheru, India.

Hartl DL, Clark AG (1997). Principles of Population Genetics. 3rd Edition. Sinauer Associates, Inc. Publishers, Sunderland, Massachusetts, USA.

Kimani PM (2000). Pigeon pea breeding: Objectives, experiences and strategies for Eastern Africa. In: Silim SN, Mergeai G and Kimani AG (eds), Pigeon pea. Status and Potential in Eastern and Southern Africa. pp. 21- 31. Gembloux Agric. Uni., International Crop Research Institute for Semi- Arid Tropics.

Kimani PM, Mergeai G, Baudoin JP, Rubaihayo, PR, Janssens M (2000). New regional initiatives in pigeon pea improvement. In: Silim SN, Mergeai G , Kimani AG (eds), Pigeon pea. Status and Potential in Eastern and Southern Africa. Gembloux Agric. Uni. and Int. Crop Research Institute for Semi- Arid Tropics.

Lakshmi M, Senthikimar P, Parani M, Narayana M , Parida AK (2000). PCR-RFLP analysis of chloroplast gene regions in *Cajanus cajan* (leguminosae) and allied genera. Euphytic*a* 116: 243 –250.

Lynch M, , Milligan M (1994). Analysis of population structure genetic structure using RAPD markers. Mol. Ecol. 3: 91-99.

McDonald B A , Linde C (2002). Pathogen population genetics, evolutionary potential and durable resistance. Annu. Rev. Phytopathol. 40: 349-3479.

Nadimpalli RG, Jarret RL, Phatak SC , Kochert G (1994). Phylogenetic relationships of the pigeon pea (*Cajanus cajan*) based on nuclear restriction fragment length polymorphism. Genome 36: 216-223.

Nei M (1987). Molecular Evolutionary Genetics. Columbia Uni. Press, New York.

Odeny, DA (2000). Inheritance of resistance to fusarium wilt in pigeon pea. In: S.N. Silim, G. Mergeai. and P.M. Kimani, (eds), Pigeon pea. Status and Potential in Eastern and Southern Africa. Pp. 43- 47. Gembloux Agric. Uni. and Int. Crop Research Institute for Semi- Arid Tropics

Okori P, Fahleson J, Rubaihayo, PR, Adipala E , Dixelius, C (2003). Assessment of genetic variation among East African *Cercospora zeae-maydis* populations using AFLP and RFLP. Afri. Crop Sci. J. 11: 75-86.

Owere L, Rubaihayo PR , Osiru DSO (2000). Cereal-pigeon intercropping systems: the Ugandan experience. In: Silim SN, Mergeai G and Kimani AG (eds), Pigeon pea. Status and Potential in Eastern and Southern Africa. Pp. 84–89. Gembloux Agric. Uni. and Int. Crop Research Institute for Semi- Arid Tropics.

Purseglove JW (1988). Tropical Crops: Dicotyledons. Longman, UK.

Ratnaparkhe MB, Gupta VS, Ven Murthy MR , Ranjekar PK (1995). Genetic fingerprinting of pigeon *Cajanus cajan* (L.) Millsp and its wild relatives using RAPD markers. Theor. Appl. Genet. 91: 893-898.

Sambrook J, Fritsch ET , Maniatis T (1989). Molecular Cloning: A Laboratory Manual. 2nd ed. Cold Spring Harbour Laboratory Press, USA.

Silim SN (2000). Strategies and experiences in pigeon pea variety development for Eastern and Southern Africa. In: Silim SN, Mergeai G , Kimani AG (eds), Pigeon pea. Status and Potential in Eastern and Southern Africa. Pages 11-20. Gembloux Agric. Uni.and Int. Crop Research Institute for Semi- Arid Tropics.

Schneider S, Roessli D , Excoffier L (2000). Arlequin version 2.000: A software for population genetics data analysis. Genetics and Biometry Laboratory, Uni. of Geneva, Switzerland.

Souframanien J, Manjaya JG, Krishna TG , Pawar ME (2003). Random amplified polymorphic DNA analyses of cytoplasmic male sterile and male fertile pigeon pea (*Cajanus cajan* (L.) Millsp. Euphytica 129:293-299.

Tajima F (1983). Evolutionary relationship of DNA sequences in finite populations. Genetics 105:435-460.

Tindall HD (1988). Vegetables in the Tropics. Macmillan International, UK.

Van der Maesen LJG (1980). Pigeon pea: Origin, history evolution and taxonomy. In: Nene NL, Hall SD and Sheila VK (eds.), The Pigeon Pea. CAB Int. Uni. Press Cambridge, Lond.

Vos P, Hogers R, Bleekers M, Rejians M, van de Lee, T, Hornes M, Frijters, A, Pot J, Peleman J, Kiuper M , Zabeau M (1995). AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res. 23: 4407-4414.

Vroh Bi I, Harvengt L, Chandelier A, Mergeai G , Du Jardin P (1996). Improved RAPD amplification of recalcitrant plant DNA by the use of activated charcoal during DNA extraction. Plant Breed. 115:205-206.

Weir BS (1996). Genetic data analysis II. Sinauer Associates Inc. Publishers, Sunderland Massachusetts, USA.

Weir BS , Cockerham CC 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358-1370.