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Research Paper

IDENTIFICATION OF GENETIC DIVERSITY AMONG PAPAYA VARIETIES IN MAURITIUS USING MORPHOLOGICAL AND MOLECULAR MARKERS

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Papaya (*Carica papaya* L.) is a popular and economically important fruit tree that consists of various varieties worldwide. Four varieties of papaya cultivated in Mauritius namely Farc, Long Orange, Ramnan and Uganda Female were analyzed at the morphological and molecular level in order to understand their genetic diversity. Morphological characters used for the comparison of species included mature papaya leaves, petioles, inflorescences and fruits. For the molecular study, genomic DNA was amplified using RAPD-PCR reaction. The RAPD primers yielded enough polymorphic bands revealing differences between the papaya varieties with which a dendogram was generated.

Keywords: Papaya, Morphological characters, RAPD markers, Dendogram

INTRODUCTION

Papaya is an interesting plant producing fruits for various uses and it propagates, grows as well as produces fruits under tropical and subtropical conditions in less than a year (Muthukrishnan and Irulappan, 1990). *Carica papaya* L. is a member of the family Caricaceae related to the Passifloraceae (Morton, 1989). Papaya is a fast-growing, semi-woody herb which exhibits apical dominance rarely branching. The palmately-lobed leaves are large and clustered spirally at the crown. Generally, papaya fruits are borne by both female and hermaphrodite trees, but their shapes differ (Jaime *et al*., 2007).

In the 18th century this plant was brought to Mauritius and was propagated all around the island (Purseglove, 1968). There are numerous cultivars of papaya present worldwide, however in Mauritius the most known varieties are Farc, Rose Belle, Uganda Female, Uganda Red, Goodland Pink, Long Orange, Ramnan, Chinniah and Wilcox. Varieties that have uniformity of shape, exquisite taste, great texture as well as flavor, resistance to pests and diseases are preferred for commercial use and include varieties Solo and Eksotic.

The great demand for papaya has constantly increased over the past years as day by day

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people are becoming more conscious about the countless benefits of this fruit. In Mauritius the Government is encouraging people to grow fruits locally as it represents great scope for minimal processing, transformation and value addition product. Nowadays, processed product of papaya fruit such as dehydrated and crystallized papaya, pickles, juices, papaya juice and Tutti frutti, are available. The Government wants to seize all opportunities on the regional front to develop Mauritius into an agrobusiness hub. Furthermore, it is expected that by 2015, some 7000 ha now under sugarcane, would be available for agricultural produce which include the production of fruits. Besides, the tourists that visit our paradise island like this tropical fruit; hence the papaya is continuously in demand by hotels.

Plant taxonomy was traditionally dependent upon the comparative external morphological characters (Baxy, 2009). Nonetheless, these are not really precise and enlightening because gene expression in different surroundings causes wide variability of phenotypic characters. Therefore, molecular markers are being utilized for plant identification as they are detectable in all tissues and independent of environmental change (Amad *et al.,* 2004). A molecular study contributes a great deal in the detection, characterization and evaluation of genetic diversity (Tapia *et al*., 2005) and the only published data available on the molecular studies of some papaya varieties in Mauritius were carried out by Baxy (2009) and Sooneeram (2009).

MATERIALS AND METHODS

Morphological Characterization

The morphological characterization of the four different papaya varieties were based upon the IBPGI papaya descriptor (1988). This study was based on the characterization and evaluation of papaya varieties based on stem, leaf, leaf petiole, inflorescence and fruit characters.

Collection of Leaf Samples

The leaf samples were collected from Pamplemoussess. The leaves that were chosen were healthy, tender, unbruised, necrosis free, pest and disease free. These were picked in the morning and kept between moist tissue paper in a plastic bag which was protected from the sun. Leaf samples from each variety were kept in different bags and labeled accordingly. All experiments were carried out from August 2010 to March 2011 in the Molecular Biology laboratory, Faculty of Agriculture, The University of Mauritius.

Cleaning and Cutting of the Leaf Samples

The leaf samples were firstly washed with distilled water and dried with tissue paper. Secondly, 70% alcohol was used to disinfect the leaves and blot dry with tissue paper again. After disinfection the samples were kept on clean tissue paper before being measured. When cutting the leaves into pieces for measurement the thick middle margin of the papaya leaves were discarded and clean disinfected scissors were used for cutting to avoid contamination.

DNA Isolation Protocol and Purification

Fresh leaf tissue (0.075 g) was ground in liquid Nitrogen to form a thin powder, which was transferred to 750 µL of cetylmethylammonium bromide (CTAB) extraction buffer (2% CTAB, 5M NaCl, 2% Polyvinylpyrrolidone (PVP), 0.5M Ethylene diamine tetra acetic acid (EDTA) pH 8, Tris-HCL (Trizma base Hydrochloric acid) pH 8 and 2% β-Mercaptoethanol in a centrifuge tube. The tube was then incubated in a water bath at 65° C for about thirty minutes with occasional swirling. 2/3 volume of chloroform: isoamyl alcohol

(24:1, v/v) was added to the tube, which was tilted several times and was centrifuged at 10 000 rpm for 10 min. The aqueous phase was transferred to a new tube. DNA was precipitated by the addition of 2/3 volume of ice-cold isopropanol and the tube was incubated at –20°C overnight. The tube was centrifuged at 13000 rpm for 30 min and the precipitated DNA was washed with 70% ice cold ethanol by centrifugation at 13000 rpm for another 15 min. The DNA pellets were then dried and re-suspended in 100 µL of sterile distilled water. RNAse treatment was carried out by adding 1 µL of RNAse to the dissolved DNA and kept overnight at 37°C.

RAPD Protocol

The RAPD reactions were always carried out on ice to prevent degradation of reagents used. The master mixes for the total number of tubes were prepared in a 1.5ml eppendorf. Each 25 µL master mix consisted of a final molarity of 1 X Reaction Buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM primer, 40 ng DNA template and 0.016 U *Taq* DNA polymerase and made up to the required final volume with nanopure water. The master mixes were aliquoted in their respective labeled PCR tubes to which the diluted DNA samples of all the varieties were already added, except the one used as negative control. The PCR tubes were centrifuged at 2000 rpm for a few seconds using the quick run function to ensure proper mixing. The RAPD – PCR reaction was carried in a Biorad thermal cycler. The cycle conditions consisted of 1 cycle involving an initial denaturation step at 95°C for 90 seconds, 40 cycles including a denaturation process at 92°C for 30 seconds, a primer annealing step at 35° C for 1 min and a step for DNA amplification at 72° C for 3 min. Thereafter, a final delay cycle for primer extension was run at 72°C for 10 min and 15°C for 5 min. These reaction products were then run on 1.5% agarose gel at 90 V and viewed under ultra violet light after staining with ethidium bromide (EtBr).

RAPD Profile Analysis

Sixty primers were used to evaluate genetic diversity and maximum polymorphism was observed with 8 random primers including OPP20, OPL05, OPD13, OPK05, OPW04, OPC03, OPC16, and OPC08. Each genotype was characterized by its banding pattern using the DNA hyperladder 2 (Bioline) as basepair ladder. The RAPD markers as viewed from the gels after electrophoresis and staining were converted into a matrix of binary data, where the presence of the band corresponded to value 1 and the absence to value 0. The statistical software NTSYS-PC (Rohlf, 2005) and DARwin 5 software (Perrier and Jacquemoud-Collet, 1996) were used to construct a UPGMA dendrogram using hierarchical clustering. Using NTSYS software, a dissimilarity matrix was calculated utilising Jaccard's (1908) coefficient. The matrix was converted to a dissimilarity matrix corresponding to the complement (dissimilarity = 1 – similarity). Cluster analysis based on the dissimilarity matrix, was performed using un-weighted pairgroup method arithmetic averages (UPGMA) (Sneath and Sokal, 1973) of the NTSYS–PC version 2.2 (Rohlf, 2005).

RESULTS AND DISCUSSION

Morphological Characterization

Selecting the morphological features for assessing the differences and similarities among the four papaya varieties were quite difficult and a better characterization would have been made from the juvenile stage until the plant has reached its final height. Many readings such as height to

first flower and fruit, sex change of flowers during growth and tree yield data could not be taken as it is a continuous assessment of about two years for a precise and accurate evaluation of the morphological characterization of the different varieties.

However, only one plant of each variety could not be taken for this analysis as the same features of the plant differ and this could be attributed to the interference of environmental as well as the agronomic factors. For a better assessment several plants of each variety were selected at random in order to get a general overview of the morphology of each.

Morphological Features of Leaves and Leaf Petiole

Healthy mature leaves of each variety were taken at random for assessment. Ramnan has the longest leaves followed by Uganda Female, however when the width were compared Uganda Female had the largest one. Long Orange had small leaves but Farc had the smallest one. The length and width of the leaves were taken on five plants for a better estimation. When comparing the shape of the leaf teeth Ramnan and Farc showed similarity and that of Long Orange resembled Uganda Female (Table 1). Nonetheless, waxiness and pubescence were absent in all the varieties. The colour of the petiole and petiole sinus shape were taken into account. Variation of the leaf petiole colour was seen in Long Orange and Farc despite that their petiole sinus shape differed (Figure 2).

Morphological Features of the Inflorescence

Only two types of flowers were observed on each plant; however female flowers were more dominant over hermaphrodite flowers. The flower size, colour of female and hermaphrodite flowers showed a lot of similarities. On the other hand, as all the plants were not of the same age and cycle, the density of inflorescence on the trunk were estimated by the number of fruits on the plant (Table 3). From Figure 1, it can be deduced that Long Orange and Uganda Female had a denser inflorescence.

Table 1: Morphological Characterization Based on the Papaya Leaf

Variety	Ramnan	Long Orange	Farc	Uganda Female
Mean length(cm)	53.2	49.2	34.8	51.6
Mean width(cm)	67.2	64.4	56.8	67.6
Shape of leaf teeth	Concave	Convex	Concave	Convex
Waxiness	Absent	Absent	Absent	Absent
Pubescence	Absent	Absent	Absent	Absent

Table 2: Morphological Characterization Based on the Papaya Leaf Petiole

Variety	Ramnan	Long Orange	Farc	Uganda Female
Colour	Green with shades of red-purple	Red-purple	Red-purple	Pale green
Shape of petiole sinus	Strongly closed	Slightly closed	Strongly closed	Slightly closed

Table 3: Morphological Characterization Based on the Papaya Inflorescence

Variety	Ramnan	Long Orange	Farc	wUganda Female
Type tree hermaphroditism	Few pistillate and hermaphrodite flowers	Many pistillate flower and few hermaphrodite flowers	Many pistillate flower and few hermaphrodite flowers	Many pistillate flower and few hermaphrodite flowers
Colour of inflorescence stalk	GreenishGreenish	GreenishGreenish		
Flower size	Intermediate	Intermediate	Intermediate	Intermediate
Colour of female flower	Yellow	Yellow	Yellow	Yellow
Colour of hermaphrodite flower	White yellow	White yellow	White yellow	White yellow
Density of inflorescence on trunk	Sparse	Dense	Sparse	Dense

Figure 1: Morphological Characteristics of the Papaya Fruit
a: Ramman Variety; b: Farc Variety; c: Long Orange Variety; d: Uganda Female Variety

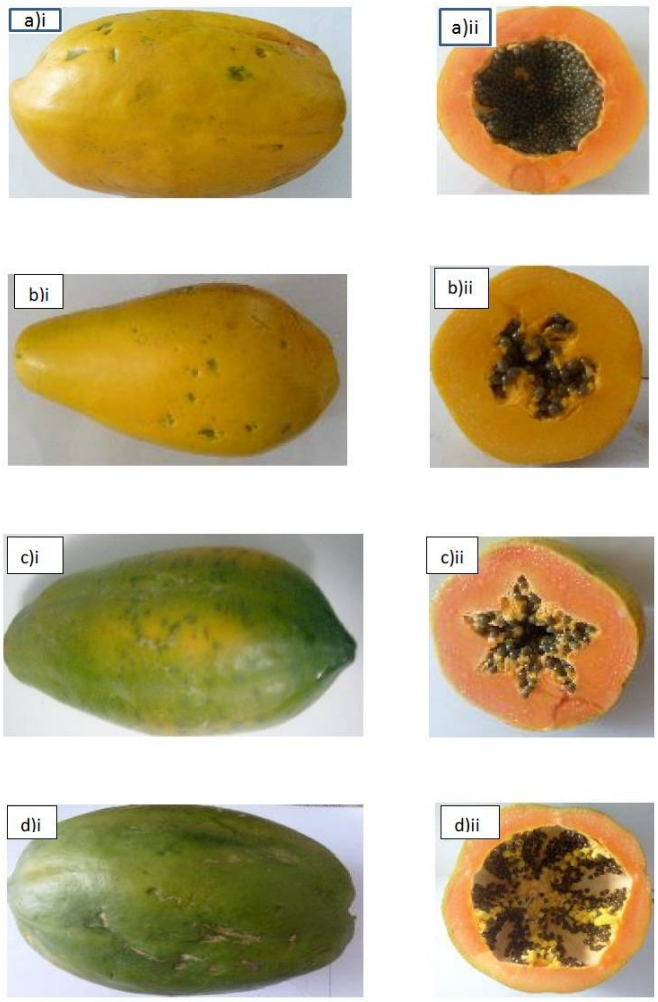


Table 4: Morphological Characterization Based on the Papaya Fruit

Variety	Ramnan	Long Orange	Farc	Uganda Female
Shape	Lengthened- cylindrical	Plum-shaped	Elliptic	Oblong-ellipsoid
Ripe skin colour	Yellowish green	Yellowish green	Yellowish green	Yellowish green
Flesh colour	Reddish-orange	Scarlett	Bright yellow	Deep yellow to orange
Stalk end fruit shape	Flattened	Flattened	Pointed	Depressed
Mean weight (kg)	2.06	1.26	1.22	1.46
Mean diameter (cm)	15	11.4	10.2	15
Mean length (cm)	28.4	27.4	15.2	21
Shape of central cavity	Round	Slightly star shaped	Irregular	Angular
Flesh aroma	Strong	Mild	Mild	Intermediate

Morphological Features of the Fruit

The fruit shape and weight showed a lot of dissimilarity among varieties. The skin colour were the same for all ripe papaya, nevertheless the flesh colour and taste were distinct. The biggest fruits were from Ramnan while the smallest one was that of Farc. The central cavity of each variety was poles apart with different seed arrangement and quantity. All the fruits showed desired characteristics, some had exquisite taste and aroma such as Ramnan while the flesh of Uganda Female were very firm and Long Orange has a great fruit uniformity (Table 4).

Molecular Characterization

RAPD Amplification

Out of sixty RAPD primers screened, 8 produced distinct amplification products and some of these gave significant polymorphism. A number of

Figure 2: PCR Amplification Products with Primers OPC 03 (Right) and OPW 04 (Left)

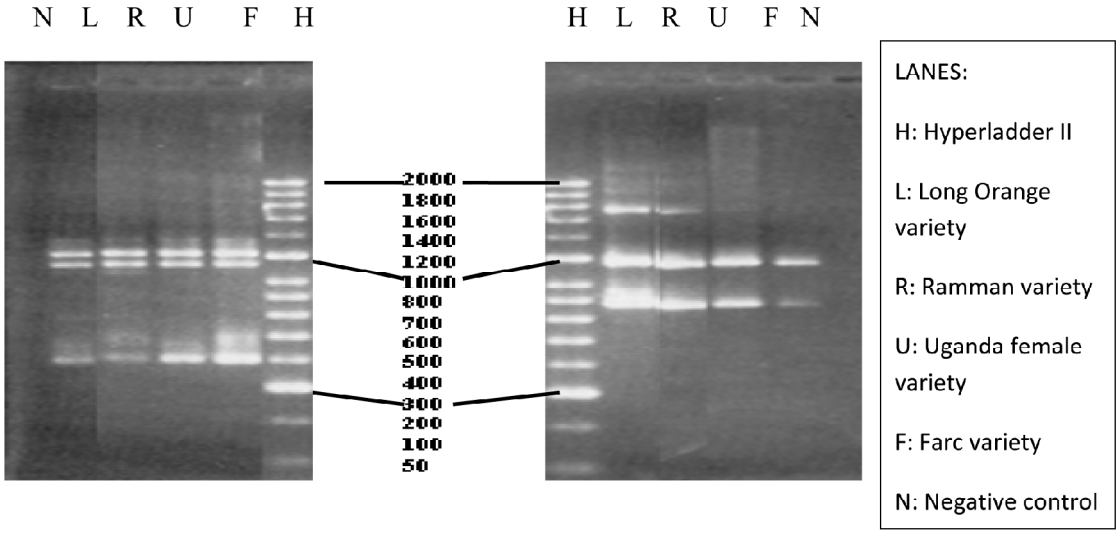


Figure 3: PCR Amplification Products with Primers OPD 13 (Right) and OPK 05 (Left)

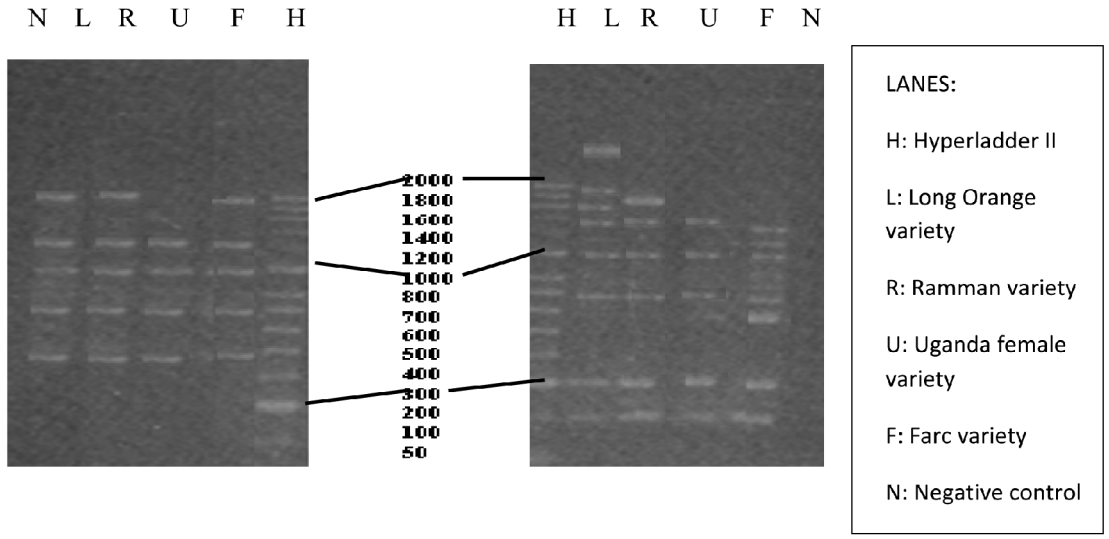
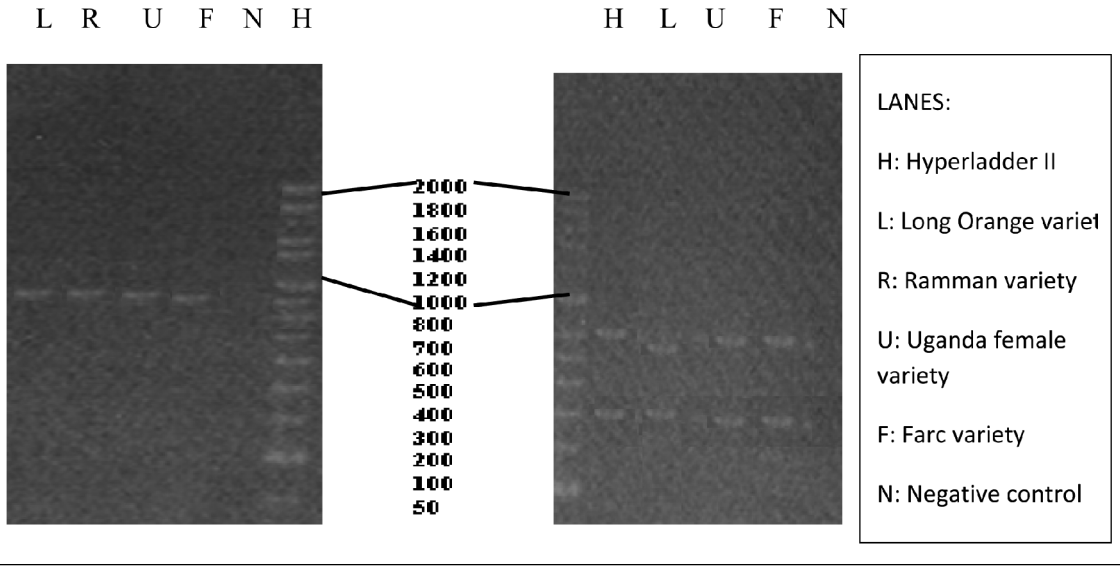


Figure 4: PCR Amplification Products with Primers OPP 20 (Right) and OPC 08 (Left)



bands were similar which suggested that assortments of traits are common in those papaya varieties, OPK 05 is the ideal primer which yielded specific bands with all the varieties. The polymorphic bands obtained can be used to develop specific markers such as sequence characterized amplified regions (SCARs) which will be ideal to identify this specific variety.

All the papaya varieties generated amplified products with the primer OPC 03. With OPW 04 distinct bands were observed with all the varieties and two among those bands were similar in all varieties (Figure 2). Long Orange and Ramnan had an additional similar marker of 1600 bp. Similar bands of high intensity were obtained with OPD 13 (Figure 3). Nonetheless, a marker of 2000

Figure 5: PCR Amplification Products with Primer OPC 16

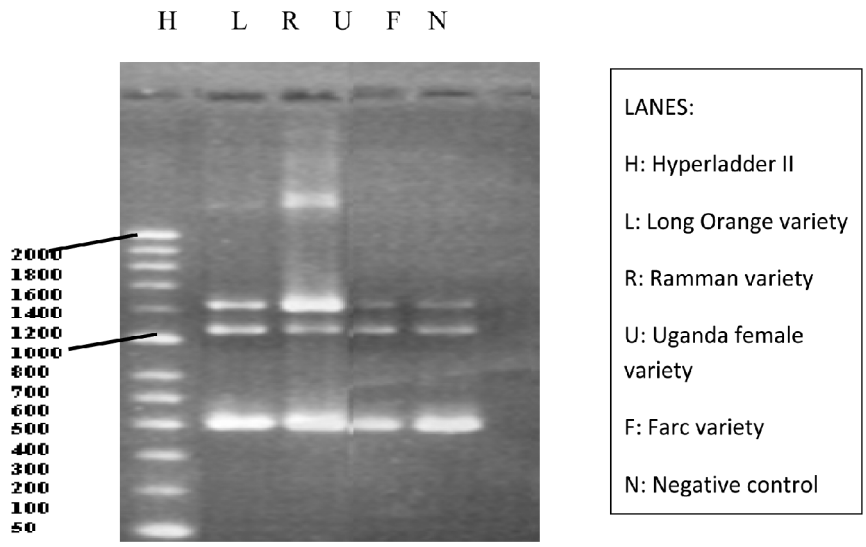
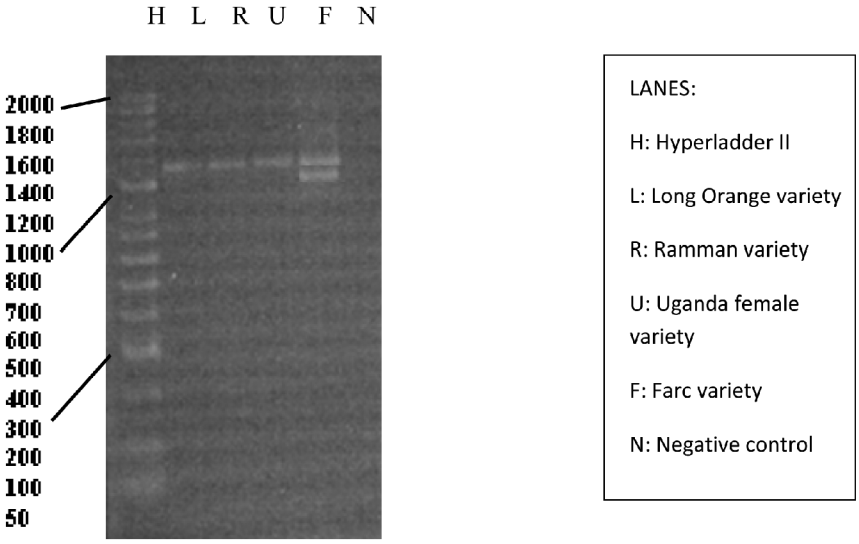


Figure 6: PCR Amplification Products with Primer OPL 05



bp amplification product was obtained in all except for Uganda Female. Five similar amplified products of low intensity were obtained with all the varieties with primer OPK 05. A unique band was encountered in Long Orange at 2000 bp while with Farc several distinct bands of size 700 and 1200 bp were obtained (Figure 3). The primer OPP 20 had generated only one marker of 900 bp with all the varieties. With OPC 08, Long Orange, Uganda Female and Farc had two similar bands of size 500 bp and 800 bp (Figure 4). All the varieties produced bands with the primer OPC 16 (Figure 5). Three bands of 600,100 and 1175 bp were present in all varieties and the Ramnan variety produced smears above the 2000 bp marker that surely consisted of several other

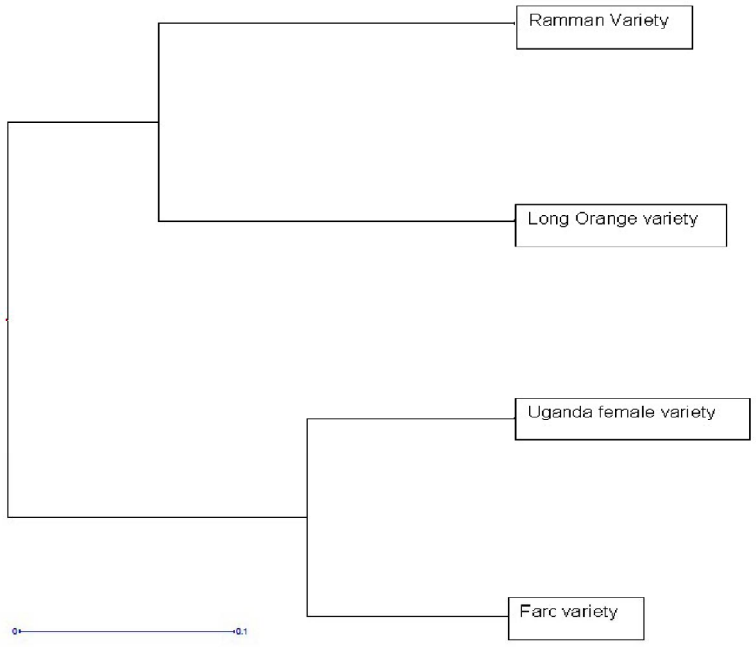
Table 5: RAPD Markers and Polymorphism

Primer	5'to 3'end sequence	Number of Markers	Number of polymorphic markers	% Polymorphism
OPP 20	GACCCTAGTC	4	0	0.0
OPC 08	TGGACCGGTG	9	1	11.1
OPD 13	GGGGTGACGA	21	2	9.5
OPK 05	TCTGTCGAGG	30	5	16.7
OPC 16	CACACTCCAG	14	1	7.1
OPL 05	ACGCAGGCAC	5	1	20.0
OPC 03	GGGGGTCTTT	21	3	14.3
OPW 04	CAGAAGCGGA	15	3	20.0
Total		149	38	25.5

Table 6: Dissimilarity Matrix Based on the Proportion of Shared RAPD Fragments Among Different Papaya Varieties

	Ramman Variety	Long Orange Variety	Uganda Female Variety
Long Orange Variety	0.3322		
Uganda Female Variety	0.46	0.26	
Farc Variety	0.722	0.456	0.194

Figure 7: Dendogram Generated Using UPGMA Cluster Analysis Showing the Relationships and Diversity Among the Papaya Varieties



bands. Only one band of 1175 bp of high intensity was obtained in all the varieties with OPL 05, however, with Farc an additional band of 1000 bp (Figure 6). OPK 05 produced more distinct polymorphic bands with all the varieties (Table 5).

RAPD Analysis

A distance matrix (Table 6) was generated using the RAPD-PCR amplified products. The Jaccard's similarity analysis depicted a good degree of genotypic diversity existing in the papaya genotypes studied. The minimum and maximum similarity values were 0.28 and 0.806. The dendogram reflect a good genetic analysis which is based on amplification signals from RAPDs proving that it is a good marker to evaluate the genetic relationships among papaya accessions as previously reported (Stiles *et al*., 1993; Muthulakshmi *et al*., 2007; Huang *et al*., 2010). The dendogram (Figure 7) showed closer genetic relatedness between Uganda Female and Farc while Ramnan clustered together with Long Orange variety in a separate clade.

CONCLUSION

After conducting both the molecular and morphological analysis of the four papaya varieties, analysis of the morphological characters was found to be less informative. A limited number of morphological traits diverge, nonetheless fruits characteristics provided strong evidence of their delineation in separate varieties. More differences could be assessed from the molecular study and they were found to be most reliable for the differentiation of the different papaya varieties. These preliminary results will pave the way to more in depth studies on the characterization of the papaya germplasm in Mauritius which will eventually facilitate breeding programme for the development of new cultivars using the elite local cultivars.

REFERENCES

1. Ahmad R, Potter D and Southwick S M (2004), "Genotyping of Peach and Nectarine Cultivar with SSR and SRAP Molecular markers", *Journal of the American Society for Horticulture Science,* Vol. 129, No. 2, pp. 204-210.
2. Baxy J N (2009), "Morphological characterization of four locally available Papaya Cultivars", *B.Sc. (Hons) Dissertation, UOM Library, University of Mauritius.*
3. Jaccard P (1908) "New Research on the Floral Distribution", *Bull. Soc. Vaud. Sci. Nat.*, Vol. 44, pp. 223-270.
4. Jaime A, Teixeira da Silva Z, Rashid Duong, Tan Nhut Dharini, Sivakumar A, Gera M, Teixeira Souza Jr. P and Tennant F (2007), "Papaya (*Carica papaya* L.) Biology and Biotechnology", *Tree and Forestry Science and Biotechnology.* Global Science Books.
5. Huang H, Desmond L R and Kubisiak T L (2003), "Molecular Characterization of Cultivated Pawpaw (Asimina triloba) Using RAPD Markers", *J. Amer. Soc. Hort. Sci.,* Vol. 128, No. 1, pp. 85-93.
6. Morton Julia F (1987), "Papaya", in *Fruits of Warm Climates*, pp. 336-346, Creative Resources Systems Inc. Miami.
7. Muthulakshmi S, Balamohan T N, Amutha R, Baby Rani W, Mareeswari P and Indira K (2007), "Molecular Studies in Papaya Carica papaya L. and Carica Candamarcensis L. and their F1 & F2 Progenies", *Research*

Journal of Agriculture and Biological Sciences, Vol. 3, No. 5, pp. 453-455.

8. Muthukrishnan C R and Irulappan I (1996), "Papaya", In *Tropical and Subtropical Fruits*", pp. 304-331.
9. Perrier X and Jacquemoud-Collet J P (2006), "*DARwin Software*", http://darwin.cirad.fr/darwin
10. Purseglove J W, (1968), "*Papayas .In: Tropical crops Dicotyledons",* pp. 45-51.
11. Rohlf F J (2005), "NTSYSpc (Numerical Taxonomy & Multivariate Analysis System) Version 2.2", Exeter Software, Applied Biostatistics Inc., New York.
12. Sneath P H A and Sokal R R (1973), *Numerical Taxonomy*, W H Freeman and Co., New York.
13. Soneeram D (2009), "Genetic Analysis of Some Economically Important Cultivar of Papaya (*Carica papaya*)", *B.Sc. (Hons.) Dissertation, UOM Library, University of* Mauritius.
14. Stiles J I, Lemme C, Sondur S, Morshidi M B and Manshardt R (1993), "Using Ramdormly Amplified Polumorphic DNA for Evaluating Genetic Relationships Among Papaya Cultivar", *Theoretical and Applied Genetics,* Vol. 85, pp. 697-701.
15. Tapia C E, Gutierrez E M A, Warbourton L M, Uriza A D and Rebellodo M A (2005), "Characterization of Pineapple Germplasm (Ananas spp) by mean AFLPs, Proceedings of the fourth International Pineapple Symposium", *Acta Horticulture (ISHS),* pp.109-114.