ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 11| Issue: 11| November 2025|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2025: 8.691 || ISI Value: 1.188

DETECTION OF THE GENETIC DIVERSITY AMONGST PAPAYA VARIETIES IN ODISHA USING THE MORPHOLOGICAL AND MOLECULAR MARKERS

Manini Dash
Lecturer in Botany, Panchayat Degree College, Kantamal, At/Po- Kantamal, Dist: Boudh, Pin: 762017, Odisha

ABSTRACT
Papaya is a prevalent and economically vital fruit tree that comprises of several varieties worldwide. Basically, four varieties of papaya cultivated in Odisha namely Coorg Green, Washington, Honey Dew and Ranchi selection were analyzed at the morphological and the molecular level in order to understand their genetic diversity. Morphological characters applied for the comparison of species included the mature papaya leaves, inflorescences, petioles and fruits. For this molecular study, genomic DNA was amplified using the RAPD-PCR reaction. This RAPD primers yielded adequate polymorphic bands revealing variances between the papaya varieties with which a dendrogram was made.

KEYWORDS: *Papaya, RAPD markers, Dendrogram, Morphological characters*

1. INTRODUCTION

Papaya is an exciting plant producing fruits for several uses and it promulgates, grows in addition to produces fruits under tropical and subtropical situations in less than a year. Carica papaya L. is a member of the family Caricaceae connected to the Passifloraceae (Morton, 1989). Papaya is a profligate growing, semi-woody herb which shows apical dominance infrequently branching. The palmately-lobed leaves are large and gathered spirally at the crown. Usually, papaya fruits are borne by both female and the hermaphrodite trees, but their shapes are differing.

In the 16th century this plant was transported to India and was spread all around the country (Purseglove, 1968). There are several cultivars of papaya present worldwide, however in Odisha the most known varieties are Coorg Green, Washington, Honey Dew, Pusa Nanha, Pusa Delicious and Uganda selection. Varieties that have consistency of shape, delicate taste, great texture over and above flavor, resistance to pests and diseases are favored for commercial use and contain varieties Solo and Eksotic. The excessive demand for papaya has continuously increased over the past years as daily people are charming more conscious approximately the countless benefits of this fruit. In India and also in Odisha the Government is inspiring people to grow fruits far away as it epitomizes great scope for minimal processing, transformation and value adding product. Currently, administered product of papaya fruit for instance dehydrated and crystallized papaya, pickles, juices, papaya juice and Tutti Frutti are existing. The Government wants to seize all chances on the regional front to grow India into an agrobusiness hub. Moreover, it is expected that by 2015, some 8000 ha now under sugarcane, would be existing for agricultural produce which comprise the production of fruits. Above and beyond, the tourists that visit our country like this tropical fruit; hence the papaya is unceasingly in demand by hotels.

Plant taxonomy was conventionally dependent upon the proportional external morphological characters (Baxy, 2009). Nevertheless, these are not really specific and informative because gene countenance in different environments causes wide inconsistency of phenotypic characters. Consequently, molecular signs are being operated for plant identification as they are visible in all tissues and independent of environmental change. A molecular study contributes a prodigious deal in the detection, characterization and assessment of genetic diversity (Tapia, 2005) and the only in print data available on the molecular studies of some papaya varieties in India.

2. MATERIALS AND METHODS

Morphological Characterization

The morphologic characterization of the 4 different papaya varieties were based upon the IBPGI papaya signifier (1988). This study was grounded on the characterization and appraisal of papaya varieties based on stem, leaf petiole, leaf, inflorescence and the fruit characters.

Gathering of the Leaf Samples

The leaves that were selected were healthy, unbruised, tender, necrosis free, pest and virus free. These were selected in the morning and set aside among moist tissue paper in the plastic bag which was endangered from the sun. Leaf models from apiece variability were kept in dissimilar bags and labeled for that reason. All experimentations were agreed out from the August 2024 to March 2025 in this Molecular Biology laboratory, Faculty of Agriculture, The University of Agriculture, Odisha.

Spring-cleaning and Cutting of these Leaf Samples

The leaf samples were initially washed with the distilled water and dried out with the tissue paper. Furthermore, 70% of alcohol was used to sterilize the leaves and blot dry with the tissue paper once more. After fumigation the samples were kept back on clean tissue paper beforehand being measured. When cutting this leaves into the pieces for extent the thick middle boundary of these papaya leaves were rejected and clean sanitized scissors were used for cutting to circumvent infection.

DNA Segregation Protocol and Decontamination

The fresh leaf tissue (0.075 g) was contain in liquid Nitrogen to

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 11| Issue: 11| November 2025|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2025: 8.691 || ISI Value: 1.188

form the thin powder, which was moved to 750 µL of the cetylmethyl ammonium bromide extraction buffer (5M NaCl, 2% of CTAB, 2% of Poly vinyl pyrrolidone, Trizma base Hydrochloric acid pH 8, 0.5M of Ethylene diamine tetra acetic acid pH 8, and 2% of β-Mercaptoethanol in the centrifuge tube. The tube was then gestated in the water bath at 65° C for around 30 minutes with sporadic swirling. 2/3 volm of the chloroform: isoamyl alcohol was added to this tube, which was tilted numerous times. The aqueous phase was relocated to the new tube. DNA was hastened by the accumulation of 2/3 vol^{m} of ice-cold isopropanol and this tube was nurtured at –20°C instant. The tube was centrifuged at 13000 rpm for 30 minutes and the triggered DNA was wash away with the 70% ice cold ethanol by the centrifugation at 13000 rpm for additional 15 min. The DNA pellets were then dried out and re-suspended in the 100 µL of sterilized distilled water. RNAse treatment was approved out by totaling 1 µL of RNAse to the softened DNA and set aside overnight (37°C).

RAPD Protocol

The RAPD responses were continuously carried out on ice to avert degradation of substances used. The master combinations for the overall number of tubes were ready in a 1.5ml eppendorf. Individually 25 µL master mix contained of the ultimate molarity of the 1 X Reaction Buffer, 1.5 mm of $MgCl_2$, 0.2 mm of dNTPs, 0.5 µM of primer, 40 ng DNA pattern and the 0.016 U Taq DNA polymerase and fabricated to the essential final volm with Nano pure water. The master mixtures were aliquoted in their particular labeled PCR tubes to which this diluted DNA samples of all these categories were previously added, excluding the one used as -ve control. The PCR tubes were centrifuged at the 2000 rpm for a rare seconds using this quick run function to confirm the proper mixing. The RAPD – PCR reaction was accepted in the Biorad thermal cycler. The cycle situations contained of the 1 cycle connecting a preliminary denaturation step at 95°C for the 90 seconds, 40 cycles with a denaturation method at 92°C for the 30 seconds, a primer galvanizing step at 35° C for the 1 min and a step for DNA intensification at 72° C for the 3 min. Afterward, a finishing interruption cycle for primer delay was run at 72°C for the 10 min and 15°C for the 5 min. These reaction products were at that time run on 1.5% agarose gel at 90 V and observed under ultra violet light next staining with ethidium bromide.

RAPD Summary Analysis

The sixty primers were used to assess the genetic diversity and max polymorphism was detected with 8 arbitrary primers comprising OPP20, OPL05, OPK05, OPD13, OPW04, OPC16, OPC03, and OPC08. Respectively genotype was categorized by its banding design using the DNA hyper ladder 2 as the base couple ladder. The RAPD markers as observed from this gels after electrophoresis and bruising were transformed into the matrix of the binary data, where the existence of the band agreed to value 1 and the lack to value 0. The numerical software NTSYS-PC and DAR win 5 software were used to build a UPGMA dendrogram using categorized clustering. By means of NTSYS software, a difference matrix was considered utilizing Jaccard's coefficient. The matrix was renewed to a distinction matrix corresponding to this complement (unlikeness = 1 – likeness). Cluster study based on the unlikeness matrix, was completed using unweighted pair group method arithmetic averages of the NTSYS–PC.

3. OUTCOMES AND DISCUSSION

Morphological Characterization

Choosing the morphological structures for considering the differences and resemblances among the 4 papaya varieties were fairly hard and a better classification would have been made from this juvenile stage until the plant has grasped its final height. Many analyses such as height to the first flower and fruit, sex change of the flowers through growth and tree yield data could not be occupied as it is a continuous assessment of around 2 years for an exact and correct evaluation of this morphological classification of the dissimilar varieties. Though, only one plant of apiece variety could not be taken for this study as the similar features of this plant differ and this could be accredited to the interference of ecological in addition to the agronomic factors. For a better valuation some plants of each variety were nominated at random so as to get a common outline of the morphology of each.

Morphological Structures of Leaves and Leaf Petiole

Healthy developed leaves of apiece variety were taken at arbitrary for assessment. Ramnan has the lengthiest leaves next Uganda Female, though when the thickness were associated Uganda Female had the biggest one. Long Orange had lesser leaves but Farc had the lowest one. The length and width of this leaves were taken on 5 plants for the better assessment. When associating the shape of this leaf teeth Ramnan and the Farc displayed resemblance and that of the Long Orange look like Uganda Female (Table 1). However, waxiness and the pubescence were preoccupied in all these varieties. The colour of this petiole and the petiole sinus shape were occupied into account. Variation of this leaf petiole colour was realized in Long Orange and the Farc notwithstanding that their petiole sinus form varied (Figure 2).

Morphological Structures of the Inflorescence

Merely 2 types of the flowers were detected on each plant; though female flowers were more foremost over androgynous flowers. The flower magnitude, colour of female and hermaphrodite flowers presented a lot of likenesses. Conversely, as all the plants were not of the similar age and cycle, the thickness of inflorescence on the trunk were projected by the no. of fruits on this plant (Table 3). From the Figure 1, it can be presumed that Long Orange and the Uganda Female had a thicker inflorescence.

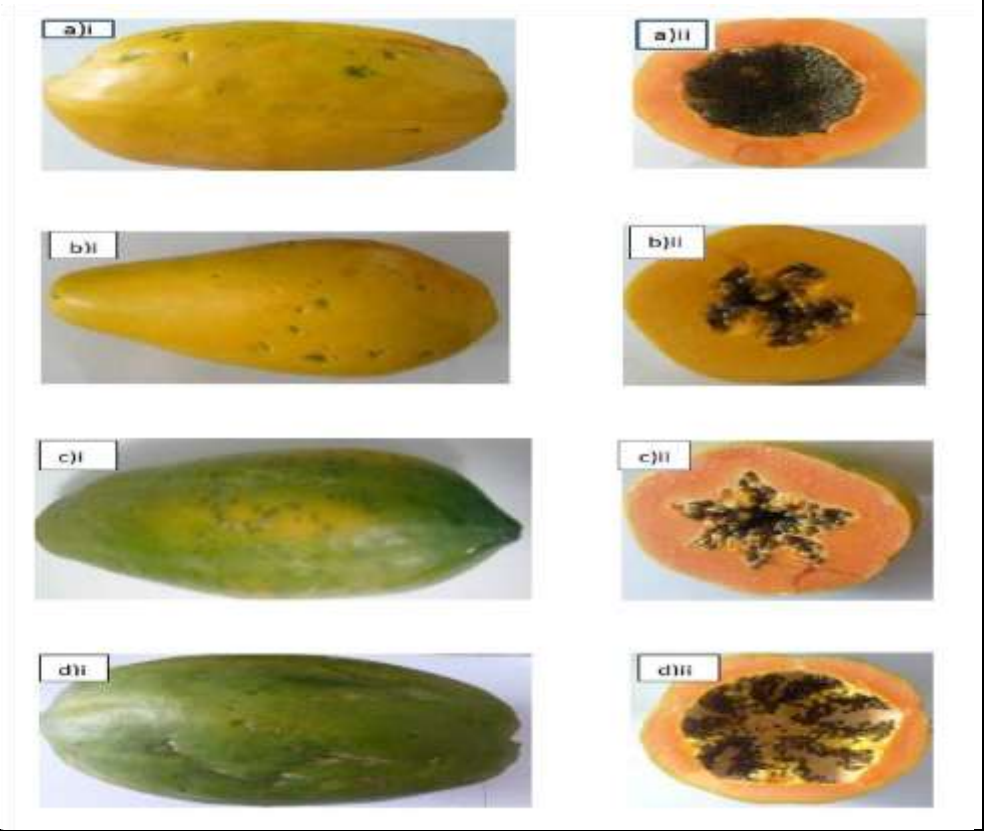
ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 11| Issue: 11| November 2025|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2025: 8.691 || ISI Value: 1.188

Table 1: Morphological Characterization Based on this Papaya Leaf				
Variety	**Coorg Green**	**Washington**	**Honey Dew**	**Uganda selection**
Mean length(cm)	42	49.3	34.7	51.7
Mean width(cm)	38	64.5	56.7	67.6
Shape of leaf teeth	Concave	Convex	Concave	Convex
Waxiness	Absent	Absent	Absent	Absent
Pubescence	Absent	Absent	Absent	Absent

Table 3: Morphological Characterization Based on this Papaya Inflorescence				
Variety	**Coorg Green**	**Washington**	**Honey Dew**	**Uganda selection**
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	Greenish Greenish	Greenish		
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

Table 2: Morphological Characterization Based on this Papaya Leaf Petiole				
Variety	**Coorg Green**	**Washington**	**Honey Dew**	**Uganda selection**
Colour	Green with shades of the red-purple	Red-purple	Red-purple	Pale green
Shape of the petiole sinus	Strongly closed	Slightly closed	Strongly closed	Slightly closed

Figure 1: Morphological Characteristics of this Papaya Fruit a: Coorg Green Variety; b: Washington Variety; c: Honey Dew Variety; d: Ranchi selection



ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 11| Issue: 11| November 2025|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2025: 8.691 || ISI Value: 1.188

Table 4: Morphological Characterization Based on this Papaya Fruit				
Variety	**Coorg Green**	**Washington**	**Honey Dew**	**Uganda selection**
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)	0.600	1.26	1.22	1.46
Mean Diameter (Cm)	12	11.4	10.2	15
Mean Length (Cm)	26	27.4	15.2	21
Shape of Central Cavity	Round	Slightly star shaped	Irregular	Angular
Flesh Aroma	Strong	Mild	Mild	Intermediate

Morphological Structures of the Fruit

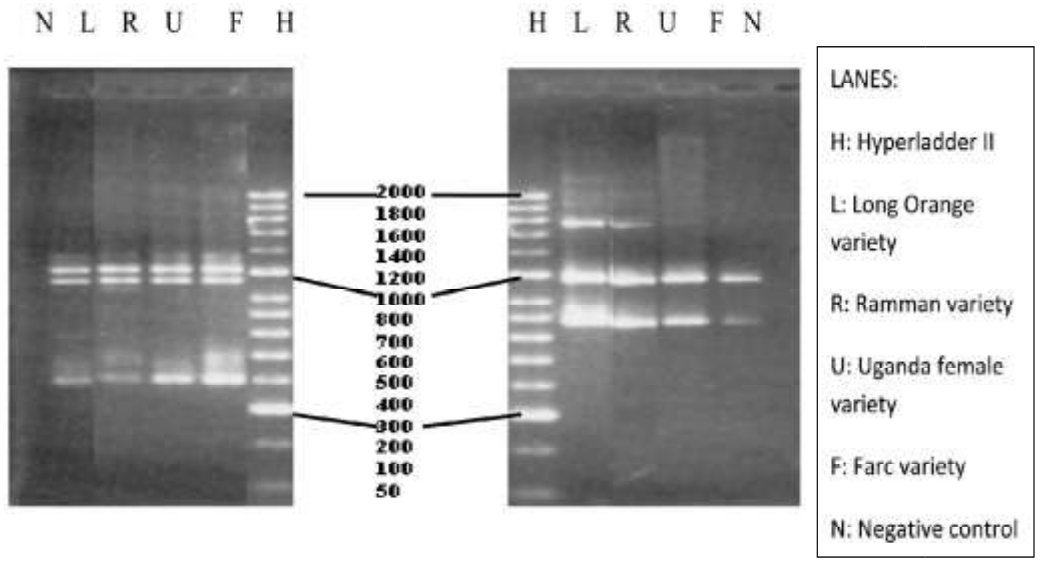
The fruit form and weight displayed a lot of difference amongst varieties. The skin colour were the similar in spite of ripe papaya, nonetheless the flesh colour and taste were separate. The largest fruits were from Ramnan while the lowest one was that of Farc. The central cavity of apiece diversity was poles separately with dissimilar seed arrangement and number. All the fruits presented wanted characteristics, some had attractive taste and aroma such as the Ramnan while this flesh of Uganda Female were very firm and the Long Orange has a great fruit consistency (Table 4).

4. MOLECULAR CLASSIFICATION

RAPD Augmentation

Out of sixty RAPD primers screened, 8 formed dissimilar amplification merchandises and some of these gave important polymorphism. A no. of bands was alike which recommended that assortments of traits are communal in those papaya varieties, OPK 05 is the idyllic primer which generated precise bands with all the selections. The polymorphic bands acquired can be used to progress exact markers such as sequence categorized amplified regions (SCARs) which will be idyllic to classify this exact variety. All these papaya varieties produced amplified products with the primer OPC 03. With OPW 04 different bands were perceived with all the varieties and two amongst those bands were alike in all diversities (Figure 2). Long Orange and the Ramnan had an added alike marker of 1600 bp. Alike bands of high strength were got with OPD 13

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



ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 11| Issue: 11| November 2025|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2025: 8.691 || ISI Value: 1.188

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

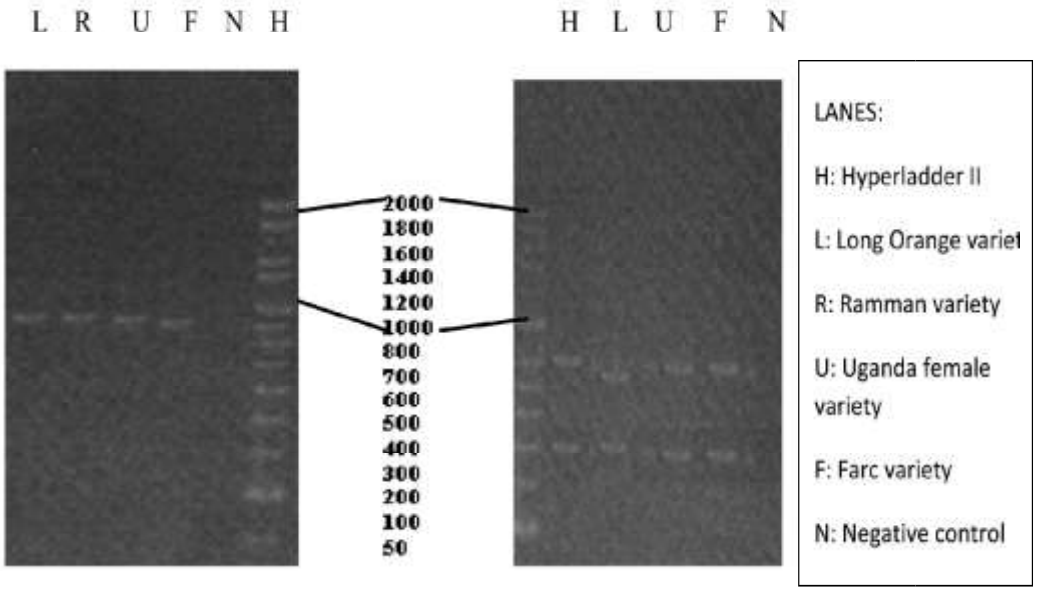
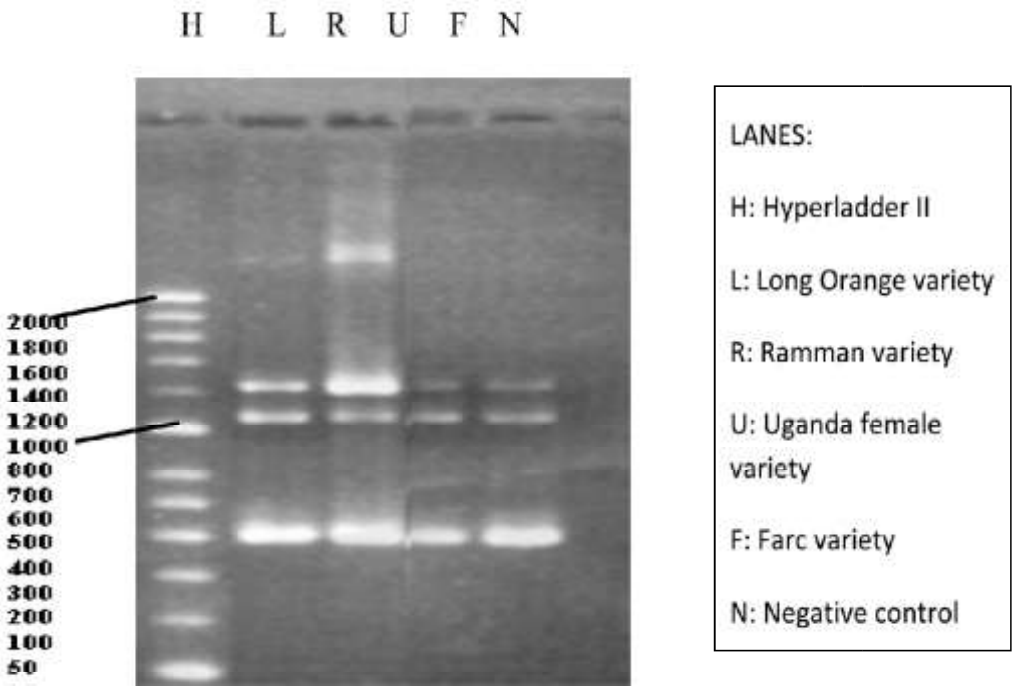
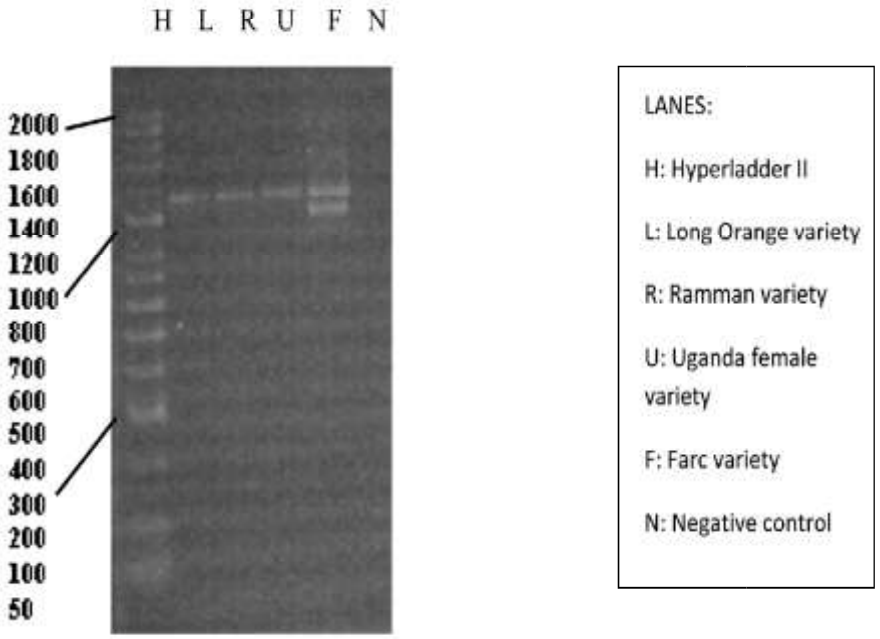


Figure 5: PCR Amplification Products with the Primer OPC 16



ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 11| Issue: 11| November 2025|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2025: 8.691 || ISI Value: 1.188

Figure 6: PCR Amplification Products with the Primer OPL 05



(Figure 3). However, a marker amplification of 2000 bp product was got in all apart from for Uganda Female. Five alike amplified products of the low intensity were got with all the diversities with the primer OPK 05. A unique band was come across in Long Orange at 2000 bp while with the Farc numerous different bands of the size 700 and 1200 bp were obtained (Figure 3). The primer OPP 20 had generated only one marker of the 900 bp with all these varieties. With OPC 08, Long Orange, Uganda Female and the Farc had two alike bands of the size 500 bp and 800 bp (Figure 4). All the diversities produced bands with this primer OPC 16 (Figure 5). 3 bands of 600,100 and 1175 bp were existing in all these varieties and the Ramnan variability shaped smears above this 2000 bp marker that assuredly contained of numerous other bands. Merely one band of the 1175 bp of high intensity was got in all these varieties with OPL 05, though, with Farc an added band of the 1000 bp (Figure 6). OPK 05 formed more dissimilar polymorphic bands with all these varieties (Table 5).

Table 5: RAPD Markers and the Polymorphism

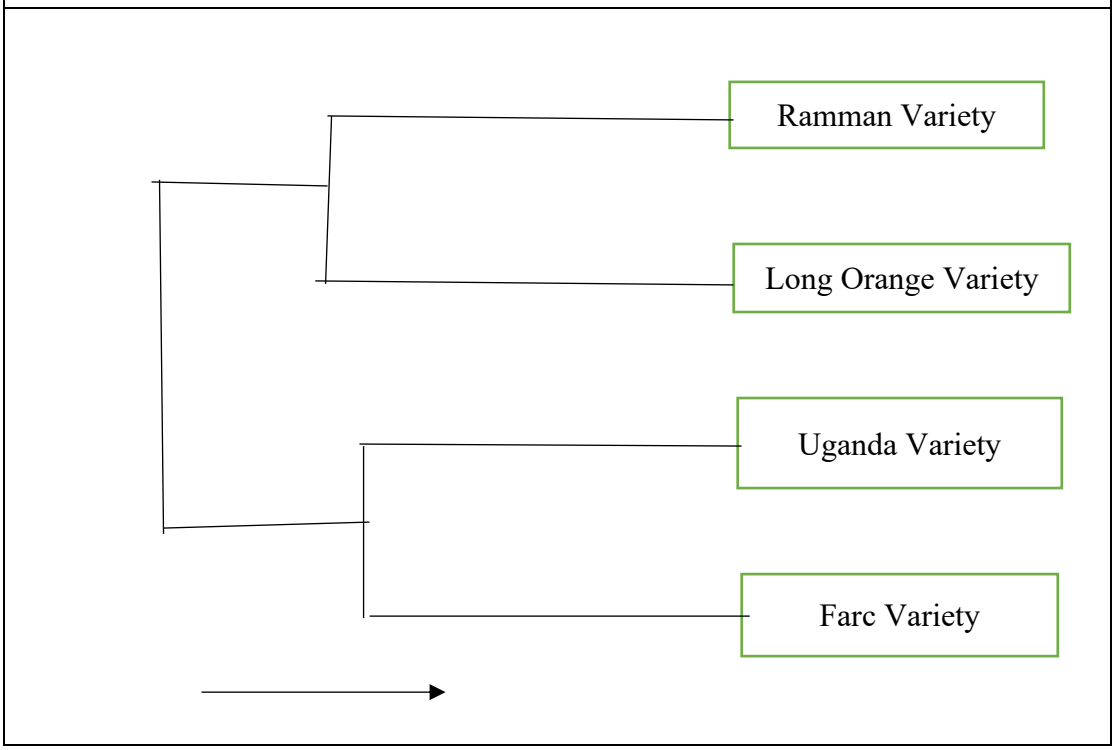
Primer	5'to 3'end sequence	Number of Markers	Number of polymorphic markers	% Polymorphism
OPP 20	GACCCTAGTC	4	0	0.0
OPC 16	CACACTCCAG	14	1	7.1
OPD 13	GGGGTGACGA	21	2	9.5
OPC 08	TGGACCGGTG	9	1	11.1
OPK 05	TCTGTCGAGG	30	5	16.7
OPL 05	ACGCAGGCAC	5	1	20.0
OPC 03	GGGGGTCTTT	21	3	14.3
OPW 04	CAGAAGCGGA	15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

Table 6: Dissimilarity Matrix Based on this Proportion of Shared the RAPD Fragments Amongst Dissimilar Papaya Varieties

	Ramman Variety	Long Orange Variety	Uganda Female Variety
Long Orange Variety	0.3332		
Uganda Female Variety	0.47	0.27	
Farc Variety	0.723	0.457	0.193

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 11| Issue: 11| November 2025|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2025: 8.691 || ISI Value: 1.188

Figure 7: Dendogram Created Using the UPGMA Cluster Analysis Presenting the Relationships and the Diversity Amongst the Papaya Varieties



RAPD Analysis

The distance matrix (Table 6) was made using this RAPD-PCR amplified products. The Jaccard's resemblance analysis showed a good degree of the genotypic diversity present in this papaya genotypes studied. The minimum and the maximum resemblance values were 0.28 and 0.807. The dendogram replicate a good genetic study which is established on amplification indications from these RAPDs proving that it is a decent marker to assess the genetic relations amongst papaya accessions as formerly reported (Stiles, 1993; Muthulakshmi, 2007; Huang, 2010). The dendogram (Figure 7) displayed closer genetic similarity amongst Uganda Female and the Farc while Ramnan gathered together with this Long Orange variety in the distinct clade.

5. CONCLUSION

Subsequently directing both the molecular and the morphological examination of the 4 papaya varieties, analysis of this morphological characters was found to be less edifying. A limited no. of morphological traits moves away, even so fruits characteristics on condition that strong evidence of their delimitation in distinct varieties. More changes could be evaluated from this molecular study and they were found to be most unswerving for the differentiation of this dissimilar papaya varieties. These initial outcomes will cover the way to more in-depth lessons on the categorization of this papaya germplasm in Odisha which will ultimately simplify breeding programme for the growth of novel cultivars using this elite local cultivars.

REFERENCES

1. *Jaccard P (1908) "New Research on the Floral Distribution", Bull. Soc. Vaud. Sci. Nat., Vol. 44, pp. 223-270.*
2. *Baxy J N (2009), "Morphological characterization of four locally available Papaya Cultivars", B.Sc. (Hons) Dissertation, UOM Library, University of Mauritius.*
3. *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.*
4. *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.*
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.*

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 11| Issue: 11| November 2025|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2025: 8.691 || ISI Value: 1.188

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.*