

Molecular Characterization of the Two Types of Fruit Colour of *Carica papaya* L

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| Received: 24.02.2020 | Accepted: 09.03.2020 | Published: 18.02.2021

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Abstract

Original Research Article

Papaya (*Carica papaya* Linn.) is an important crop with high pro-vitamin A carotenoids content. In Nigeria, the major papaya fruit flesh colours observed is the yellow and red-fleshed varieties. To differentiate the yellow and red-fleshed papaya varieties, a study carried out to genetically delineate the yellow and red-fleshed papaya varieties to establish their phylogenetic relationship and varietal differences using molecular markers as a diagnostic tool. DNA was extracted from the young leaves of the yellow- and red-fleshed varieties at the Regional Centre for Biotechnology and Bioresources Research Centre, University of Port Harcourt with ZYMO Quick-DNA™ Plant/Seed Miniprep Kit. The gDNA quantity and concentration were measured using the Nanodrop 2000c spectrophotometer and Agarose gel electrophoresis. The gDNA samples were shipped to the International Institute of Tropical Agriculture (IITA) Bioscience Center, Ibadan, for amplification using the Ribulose-1, 5-biphosphate carboxylase (*rbcL*) primers and the amplicons were sequenced using the ABI3500 capillary electrophoresis sequencer. The DNA sequence file was saved in the Bioedit file with extension .ab1. The sequence was analyzed using the Molecular Evolutionary Genetics Analysis (MEGA) version 7.0.26 software, and aligned using the Basic Local Alignment Search Tool for nucleotide (BLASTN) 2.8.0 version of the National Center for Biotechnology Information (NCBI) database. The amplified DNA sequences of the two papaya varieties yielded 580 base pairs. The result of the taxonomy report generated from the sequences of the two papaya varieties matched 64 sequences of the members of the Caricaceae family, of which 21 sequences hits matched *Carica papaya* and 23 sequences hits matched the *Vasconcella* genus. The BLAST phylogenetic tree indicated that both papaya species were grouped together with *Carica papaya* cultivars in the NCBI database. Also, the sequences of the yellow-fleshed papaya (NKECHI_RBCL_6) and red-fleshed papaya (NKECHI_RBCL_9) were 100% and 99.63% identical to *Carica papaya* clone 14 accession number KX951436 sequences deposited at the NCBI database. This finding indicates that *Carica papaya* clone 14 was probably a yellow-fleshed papaya variety. Therefore, the Ribulose-1, 5-biphosphate carboxylase (*Rbcl*) molecular marker was able to detect this difference between the yellow and red-fleshed papaya varieties. Consequently, these findings will provide information for the molecular database of papaya and will be useful for the papaya conservation and crop improvement programs.

Keywords: Papaya, Rbcl marker, evolutionary relationship, BLAST.

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INTRODUCTION

In Nigeria, *Carica papaya* Linn. Commonly referred to as 'pawpaw or papaya' (English); *Indu* (Ijo, Nembe); *Ojo* (Ikwerre); *Etihi-mbakara* (Efik) [1], is the most economically important species of the family Caricaceae. Taxonomically, the *Carica papaya* plant is usually described as a dioeciously, rarely herbaceous tree in which hermaphrodite forms do occur [2]. According to Purselove [2], papaya attains a height between 4-6m. The stem is straight, cylindrical with prominent leaf scars, hollow, about 10-30cm in diameter with spongy tissue core. The leaves are large, compound palmate, clustered near the apex of the trunk. The

phyllotaxy is spiral and the petiole is 25-100cm long, hollow and pale green or tinged purple. Papaya flowers can be grouped into three basic forms that reflect the whole plant gender: female, male or bisexual (hermaphrodite). With controlled cross-pollinations between flowers of each gender, the ratio of female, hermaphrodite and male offspring are predictable. The unisexual flowers are actinomorphic that are off-white in colour. The fruit of *C. papaya* is a fleshy berry, about 7-30cm long and about 9 kg in weight; spherical to ovoid-oblong when produced from pistillate flowers but pyriform, grooved or cylindrical when produced from hermaphrodite flowers. The skin of the fruit in all cases is thin, smooth and green turning yellow or orange when

ripe. Central cavity of the fruit is usually five (5) – angled. Seeds are numerous, parietal and attached in five rows to the interior wall of the ovary. Individual seeds are spherical, black or grey wrinkled and about 5mm in diameter.

C. papaya is not only cultivated for its delicious and vitamin C-rich fruits, but also because it produces the enzyme papain, which is used in food and pharmaceutical industries [3]. The leaves and unripe fruits contain milky juice which is the protein-ferment, *papain*, collected and used as digestive salt for tenderizing meat [1]. Besides this economic value, papaya fruit provides valuable amounts of micronutrients, in particular several pro-vitamin-A carotenoids. The colour of papaya fruit flesh is determined largely by the presence of carotenoid pigments with red and yellow being the two major papaya fruit flesh colours [4]. Carotenoid pigments are indispensable for photosynthesis in plants. In many species, carotenoids can also accumulate as secondary metabolites in chromoplasts of flowers, fruits, seeds or roots to provide distinct coloration, ranging from yellow to orange and red [5]. These flesh colours are controlled by a single genetic locus with yellow being dominant over red [6]. Besides, the yellow-fleshed fruit contains β -carotene while the red-fleshed papaya fruit has high levels of lycopene and the conversion of lycopene to β -carotene is catalyzed by the lycopene β -cyclase hormone [4]. Red-fleshed papaya fruit contain lycopene, whilst this pigment is absent from yellow-fleshed fruit. The conversion of lycopene (red) to β -carotene (yellow) is catalyzed by lycopene β -cyclase. Therefore, different papaya fruit flesh colours observed could be due to differences in the carotenoids content. In tropical countries, papaya fruit represents an important pro-vitamin A source, because vitamin A-rich animal foods such as eggs and milk products are expensive for the majority of the population [7]. Therefore, papaya genotypes containing both lycopene and pro-vitamin A carotenoids may represent a valuable nutritional component of the human diet [7].

Various mechanisms have been reported for the differential accumulation of carotenoids among different varieties. There are several methods of differentiating the yellow and red-fleshed papaya varieties [5, 4]. One of such methods is the use of molecular markers. Papaya species represents an important model in genetic and genomic studies; because papaya is one of the first plant species to have its genome sequenced [8]. Knowledge of the genetic diversity within this crop species is essential to understand their origin, domestication and evolutionary relationships, and to efficiently develop strategies for the conservation of their genetic resources, and effective crop improvement [9]. According to Schweiggert *et al.* [7], carotenoid levels among a set of hybrids and lines differed significantly which supports the hypothesis of an exploitable genetic variability, and a

potential heterotic effect regarding carotenoid expression which may be instrumental in papaya breeding programs. Also, the provitamin A carotenoids and total lycopene were significantly of higher levels particularly in red-fleshed hybrids. The breadth of genetic variation readily available for papaya breeding and improvement is difficult to estimate. Therefore, the aim of this study was to genetically delineate the yellow and red-fleshed papaya varieties to establish their phylogenetic relationship and varietal differences using molecular markers as a diagnostic tool.

MATERIALS AND METHODS

Source of plant materials

To extract the genomic DNA study, ripe fruits of *Carica papaya* from the red and yellow varieties used for the study were purchased from the daily market in Choba town in Rivers State, Nigeria. (Coordinates). The seeds were extracted from the fruits, air-dried and then planted in disposable cups at the Center for Ecological Studies, University of Port Harcourt, Choba. Rivers state (4° 52' 44" North, 6° 55' 20" East).

Molecular characterization using the Simple Sequence Repeat (SSR) marker and identification

The young leaves of the yellow- and red-fleshed *Carica papaya* were collected on ice moved to the Regional Centre for Biotechnology and Bioresources Research Centre, University of Port Harcourt (coordinates). Extraction of genomic DNA (gDNA) from *Carica papaya* was done using the ZYMO Quick-DNA™ Plant/Seed Miniprep Kit (Zymo Research Group, California, USA). The gDNA quantity and concentration were measured using the Nanodrop 2000c spectrophotometer (Thermo fisher Scientific Inc. Wilmington, Delaware, USA). The DNA purity was measured as a ratio of absorbance at 280 nanometer (nm) to that of 260 nanometer. The quality of the gDNA of was further quantified using the Agarose gel electrophoresis performed according to the modified method of Saghai-Marooof *et al.* [10] and diluted to 50 ng/ μ l. The gDNA samples were shipped to the International Institute of Tropical Agriculture (IITA) Bioscience Center, Ibadan, Nigeria for amplification and sequencing. The Ribulose-1, 5-biphosphate carboxylase (*rbcL*) primers was used; *rbcL*-F as forward primers and *rbcL*-R as reverse primers, *rbcL*-F (5'-CCACAAACAGAGACTAAAGC -3') and *rbcL*-R (5'-GTAAAATCAAGTCCACCGCG -3') were used to amplify the fragments of nuclear ribosomal DNA (rDNA). The amplicons were sequenced using the ABI3500 capillary electrophoresis sequencer. The DNA sequence file was saved in the Bioedit file with extension *.ab1*. The sequence was analyzed using the Molecular Evolutionary Genetics Analysis (MEGA) version 7.0.26 software, and aligned using the Basic Local Alignment Search Tool for nucleotide (BLASTN) 2.8.0 version of the National Center for Biotechnology Information (NCBI) database.

RESULTS

The result of the genomic DNA concentration of the yellow-fleshed and red-fleshed are presented in Table 1. The yellow-fleshed papaya (NKECHI_RBCL_6a and (NKECHI_RBCL_6b)) were 46.7ng/μl and 48.5ng/μl respectively, while the red-fleshed variety (NKECHI_RBCL_9a and

(NKECHI_RBCL_9b) were 109ng/μl to 180.9ng/μl respectively. This indicates that the concentration of the DNA was good for sequencing. The purity ratio was measured by the absorbance ratio A260/280nm which ranged from 1.59nm to 1.83nm while the A260/230nm ratio ranged from 0.69nm to 1.05nm.

Table-1: Nanodrop reading of the extracted nucleic acid (DNA) from the yellow-fleshed and red-fleshed papaya

Sample ID*	User name	Date and Time	Nucleic Acid Conc. (ng/μl)	A260	A280	A260/280	A260/230	Factor
1 NKECHI_RBCL_6a	RCBRR-UNIPORT	6/26/2018 1:02:37 PM	48.5	0.969	0.608	1.59	1.05	50
2 NKECHI_RBCL_6b	RCBRR-UNIPORT	6/26/2018 1:03:35 PM	46.7	0.933	0.567	1.65	1.03	50
3 NKECHI_RBCL_9a	RCBRR-UNIPORT	6/26/2018 1:09:04 PM	180.9	3.617	1.976	1.83	0.69	50
4 NKECHI_RBCL_9b	RCBRR-UNIPORT	6/26/2018 1:10:10 PM	109	2.181	1.26	1.73	0.71	50

*NKECHI_RBCL_6 represents the yellow-fleshed papaya and NKECHI_RBCL_9 represents the red-fleshed papaya.

The result of the amplified PCR product generated from the yellow-fleshed and red-fleshed papaya is shown in Plate 2 . The amplified DNA showed

a band on gel when observed under UV light. From the result, the ladder used indicated that the DNA sequences of the four papaya samples had over 580 base pairs.

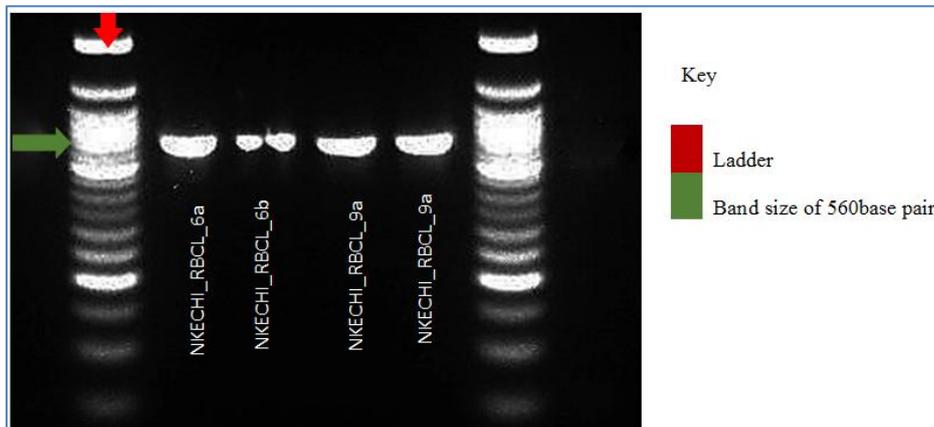


Plate-2: Amplified DNA sequence of the yellow- [NKECHI_RBCL_6] and red-fleshed [NKECHI_RBCL_9] papaya

The result of taxonomy report generated from the sequences of the yellow-fleshed and red-fleshed papaya is shown in Figure 1. The two query sequences matched 64 sequences of the members of the Caricaceae

family, of which 21 sequences hits matched *Carica papaya* and 23 sequences hits matched the *Vasconcella* genus.

Taxonomy			
Reports			
Lineage	Organism	Taxonomy	
100 sequences selected			
Taxonomy	Number of hits	Number of Organisms	Description
[-] Magnoliopsida	109	36	
[-] [-] Pentapetalae	107	34	
[-] [-] [-] Brassicales	106	33	
[-] [-] [-] [-] Caricaceae	64	28	
[-] [-] [-] [-] [-] <i>Carica papaya</i>	21	1	Carica papaya hits
[-] [-] [-] [-] [-] [-] <i>Vasconcellea</i>	23	16	
[-] [-] [-] [-] [-] [-] [-] <i>Vasconcellea cf. goudotiana</i> HCAR167	1	1	Vasconcellea cf. goudotiana HCAR167 hits
[-] [-] [-] [-] [-] [-] [-] [-] <i>Vasconcellea crassipetala</i>	1	1	Vasconcellea crassipetala hits
[-] [-] [-] [-] [-] [-] [-] [-] [-] <i>Vasconcellea monoica</i>	2	1	Vasconcellea monoica hits
[-] [-] [-] [-] [-] [-] [-] [-] [-] [-] <i>Vasconcellea sphaerocarpa</i>	2	1	Vasconcellea sphaerocarpa hits
[-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] <i>Vasconcellea cauliflora</i>	2	1	Vasconcellea cauliflora hits
[-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] <i>Vasconcellea chilensis</i>	2	1	Vasconcellea chilensis hits
[-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] <i>Vasconcellea quercifolia</i>	2	1	Vasconcellea quercifolia hits
[-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] <i>Vasconcellea parviflora</i>	2	1	Vasconcellea parviflora hits
[-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] <i>Vasconcellea microcarpa</i>	1	1	Vasconcellea microcarpa hits
[-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] <i>Vasconcellea cundinamarcensis</i>	2	1	Vasconcellea cundinamarcensis hits
[-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] <i>Vasconcellea sprucei</i>	1	1	Vasconcellea sprucei hits
[-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] <i>Vasconcellea palandensis</i>	1	1	Vasconcellea palandensis hits
[-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] [-] <i>Vasconcellea glandulosa</i>	1	1	Vasconcellea glandulosa hits
[-] <i>Vasconcellea stipulata</i>	1	1	Vasconcellea stipulata hits
[-] <i>Vasconcellea x heilbornii</i>	1	1	Vasconcellea x heilbornii hits
[-] <i>Vasconcellea candicans</i>	1	1	Vasconcellea candicans hits

Fig-1: The taxonomy report of the sequences of the yellow-fleshed and red-fleshed papaya

The result of the BLAST phylogenetic tree view is presented in Figure 2. From the phylogenetic tree, both papaya species (in yellow)

together with ten *Carica papaya* cultivars, one *Dianthus cryophyllus* and five *Vasconcellea* species in the NCBI database.

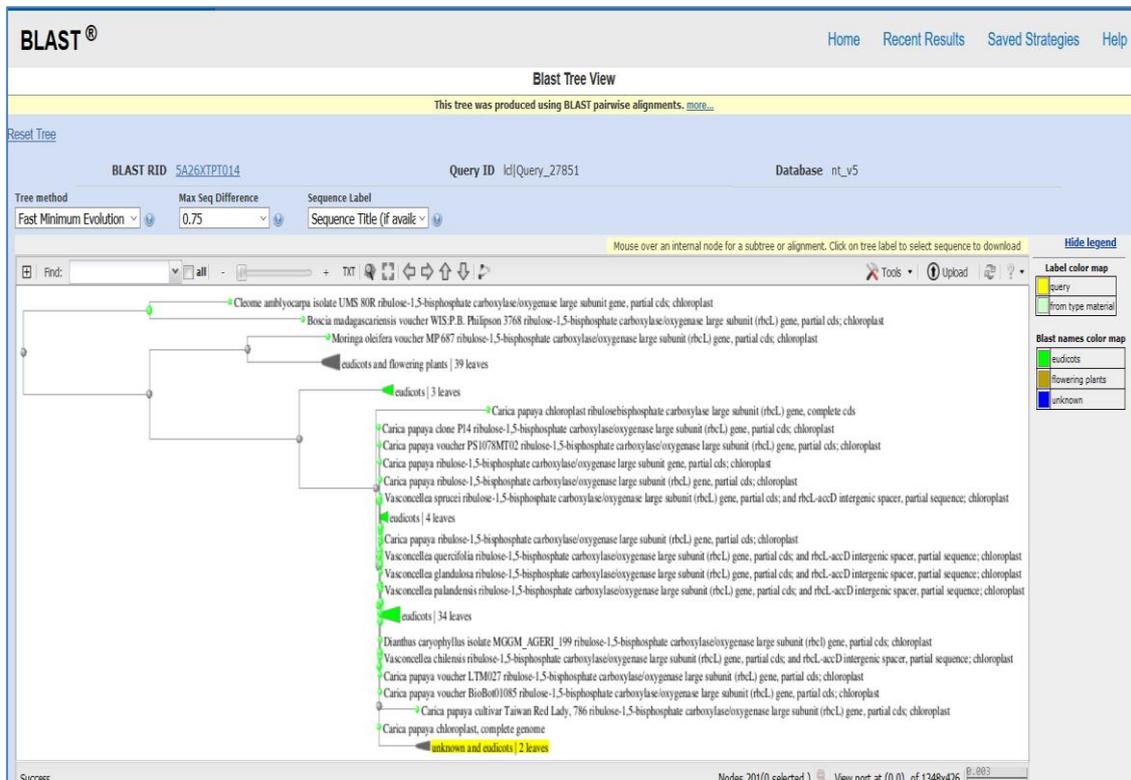


Fig-2: The BLAST phylogenetic tree view

The result of the sequences producing significant alignments are presented in Figure 3A and 3B. The yellow-fleshed papaya (NKECHI_RBCL_6) and red-fleshed papaya (NKECHI_RBCL_9) had query

length of 570 and 569 base pairs respectively and was 100% and 99.63% identical to *Carica papaya* clone 14 accession number KX951436 sequences deposited at the NCBI database.

NKECHI_RBCL_6 (red-fleshed) was 100% identical to *Carica papaya* clone P14

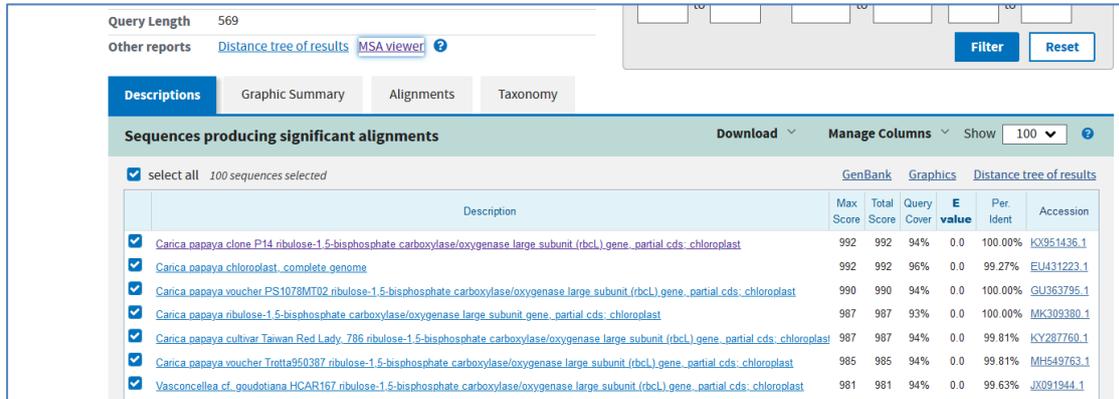


Fig-3A: The sequence alignments of NKECH_RBCL_6 sequence with NCBI database sequence

NKECHI_RBCL_9 (yellow-fleshed) was 99.63% identical to *Carica papaya* clone P14

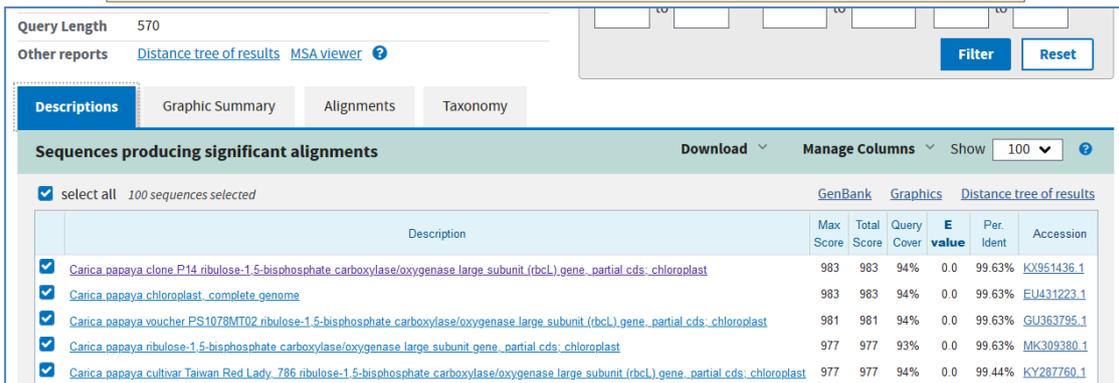


Fig-3B: The sequence alignments of NKECH_RBCL_9 sequence with NCBI database sequence

The result of the pairwise with dots for identities using the alignment view is presented in Figures 4 and 5. In Fig.4 A and B, it was observed that all nucleotides of NKECHI_RBCL_6 sequence aligned perfectly with the sequence *Carica papaya* clone 14

while all nucleotides accept the nucleotides on position 8 and 9 of *Carica papaya* clone 14 which is [TT] (red colour) was replaced with [AA] in NKECHI_RBCL_9 sequence. This finding indicates that *Carica papaya* clone 14 was probably a yellow-fleshed papaya variety.

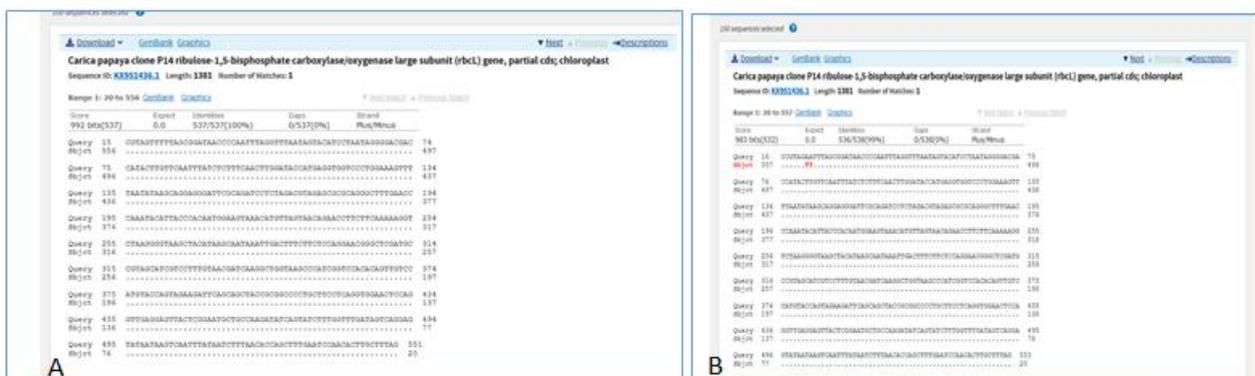


Fig-4: The alignment view of the pairwise with dots for identities of *Carica papaya* clone 14 sequence to the sequences of A. NKECHI_RBCL_6, and B. NKECHI_RBCL_9.

DISCUSSION

Genetic diversity within crop species is essential to understand their origin, domestication and evolutionary relationships, and to efficiently develop strategies for the conservation of their genetic resources, and effective crop improvement [9]. The use of molecular markers in characterization of organisms allows the comparison of DNA sequence information between known and unknown species is very much reliable than traditional methods of identification in diversity studies. According to Kumar *et al.*, [11] assessing the diversity of plants at their molecular level will provide information for the molecular database that will be useful for conservation, and crop improvement programs.

The main objective of this study was to genetically delineate the yellow and red-fleshed papaya varieties to establish their phylogenetic relationship and varietal differences using molecular markers. From this study, the taxonomy report confirmed the evolutionary relationship of the yellow- and red-fleshed papaya as belonging to the Caricaceae family and the order Brassicales. Traditionally, the position of Caricaceae within the dicotyledons has been in the order of Violales [12]. Recently however, the use of molecular techniques [13] suggest Caricaceae should be placed within the order Brassicales (Scheldeman [14]. This is confirmed in this study. Also, the use of molecular markers has been useful in determining the variability among papaya varieties and the degree of relatedness of some of these cultivars [15]. To authenticate this, the taxonomy report generated from the sequence alignment of the two yellow and red-fleshed papaya varieties, matched *Carica papaya* and *Vasconcella* genus. According to Scheldeman [14], *Vasconcella* species also known as highland papayas, are evergreen pachycaul shrubs or small trees growing 5 metres tall, native to South America and have edible fruit similar to papaya. Most species of this genus were treated as *Carica sp.*

From the result of the alignment view, it was observed that all nucleotides of NKECHI_RBCL_6 sequence aligned perfectly (100%) with the sequence *Carica papaya* clone 14 while all nucleotides except the nucleotides on position 8 and 9 of *Carica papaya* clone 14 which is [TT] was replaced with [AA] in NKECHI_RBCL_9 sequence. This finding indicates that *Carica papaya* clone 14 was probably a yellow-fleshed papaya variety. Also observed there was difference in the percentage of identity between the two papaya varieties of 0.47%. The most common explanations for this percentage difference maybe as a result of mutation in the structural gene sequences. According to a study reported by Lu *et al.* [16], it was observed that carotenogenic gene mutation occurred in six tomatoes varieties, watermelon, and loquat resulting in segregation of flesh colour.

However in this study, it was interesting that the Ribulose-1, 5-biphosphate carboxylase (*RBCL*) molecular marker was able to detect this difference between the yellow and red-fleshed papaya varieties.

CONCLUSION

Papaya (*Carica papaya* Linn.) is an important crop with high pro-vitamin A carotenoids content. In Nigeria, the major papaya fruit flesh colours observed is the yellow and red-fleshed varieties. Ribulose-1, 5-biphosphate carboxylase (*RBCL*) molecular marker was able to detect this difference between the yellow and red-fleshed papaya varieties. These findings provide information that will contribute to the molecular database of papaya and will be useful for the papaya conservation and crop improvement programs.

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