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COMPARATIVE STUDY ON GENOME SEQUENCE OF BOTH MALE AND FEMALE PLANT OF PAPAYA USING SANGER SEQUENCING

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ABSTRACT: - *Carica papaya* associated with the family of *Carricaceae* which is grown in three different sexes- Male, female and hermaphrodite. In order to explore the similarities and variations between the male and female papaya, the DNA was extracted from the papaya flower. The extracted DNA was elongated by using W1 primer for male and T1 primer for female. The amplified male DNA concentration is 351.7ng/µl and the female DNA concentration is 375.5ng/µl. Sanger sequencing was performed for establishing the sequence of male and female plant of papaya, which provide the sequence carrying 726 nucleotide for male and 1061 nucleotide for female. Because of high GC content in male flower, it harder to amplify, thus, PCR product of male sample consist less amount of nucleotide than female sample. The pairwise local alignment was performed by Matcher tool which provides 55.6% similarities in between the male and female papaya flower. SNP identification using Geneious prime software shows 19.4% SNP and 9.3% indels were identified from the alignment of male and female papaya flower. Totally, 28.86% variations projected in between male and female papaya flower. These variations in DNA sequences are responsible for the gender difference in papaya plant.

KEYWORDS:- *Carica papaya*, Polymerase chain reaction, Sanger sequencing, Single Nucleotide polymorphism**,** Bioinformatic tools.

I. INTRODUCTION

Carica papaya is commonly known as papaya or pawpaw. Papaya is come under the family of *Caricaceae*. The scientific classification of papaya is described in Table-1. Papaya can be cultivated throughout the tropical and subtropical world. Papaya fruit consist more amount of vitamin A and C[1]. Because of its high nutritive and medicinal value, papaya is considered as a commercially important fruit. The papaya have lot of medicinal properties like anticancer activity, Anti-inflammatory properties, Heart health, Anti-ageing, etc.,

Table-1: Scientific Classification of papaya

Scientific Classification	
Kingdom	Plantae
Subkingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Dillenidae
Order	Brassicales
Family	*Caricaceae*
Genus	Carica L
Species	*Carica papaya*

According to statistics, the annual production of papaya fruit in India is estimated at 6 million tonnes.

Fig-1: Female papaya flower

Papaya was grown in 3 different sexes – Male, Female and Hermaphrodite. Male papaya plant never give fruit, but it have the capable to change their gender as hermaphrodite while increasing the temperature. In the case of female papaya plant, once pollinated, it will produce the fruit. In hermaphrodite papaya plant, it will self pollinate and produce the fruit. Sanger sequencing method is designed for determining the sequence of nucleotide bases in a piece of DNA.

Fig-2: Male papaya flower

II. MATERIALS AND METHODS:

The flower of male papaya plant (Fig-1) and the flower of female papaya plant (Fig-2) was collected from the Mogappair, Chennai, Tamil Nadu, India. The collected sample was confirmed by Ms.T.Shanthi, M.Sc.Biotechnology, Production head at OPRL Bioscience, Chennai. The DNA was extracted from the male and female flower of papaya using Pro TechEx – Ultrapure Genomic DNA Spin Miniprep Kit[2]. To confirm that the DNA was extracted, the Agarose gel electrophoresis was performed. Then the extracted DNA was amplified using W_1 primer (for male) and T_1 primer (for female) in PCR machine[3] (Fig-3).

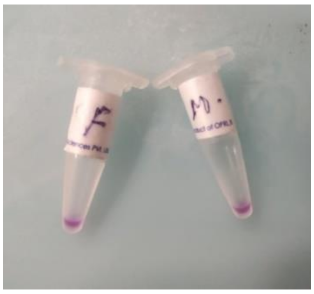


Fig-3: PCR Reaction Mix for Male (W_1 primer) and Female papaya (T_1 primer)

The reaction mix was prepared according to the component mentioned in the Table-2.

Making enough copies of DNA for analysis by gel electrophoresis typically requires from 25 to 40 cycles.

Table-2: Component for the reaction mix

Components	Volume
Sterile water	1.6 µl
Master Mix(10X Taq polymerase Buffer, 10mM dNTP mix, Taq polymerase)	10.0 µl
T1 Primer (Forward and reverse)	4.4 µl
Template DNA	4.0 µl
Total	**20.0 µl**

To confirm that the DNA was amplified, the agarose gel electrophoresis was performed[4].After that, the Dna was purified from the agarose gel using Progene gel extraction kit[5].The DNA sequence was determined using sanger sequencing. Then compare the sequence of the male plant of papaya and male plant of date palm. After that, by using biologic tools and in-sillico approach the sequence of both male and female papaya flower was analysed and compared.

III. RESULTS AND DISCUSSION

The concentration of extracted DNA in male papaya is 80.01ng/µl and the female papaya is 64.29 ng/µl. The eluted DNA was viewed by 0.8% agarose gel electrophoresis.

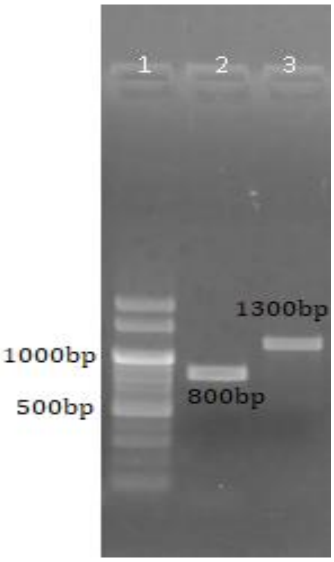


Fig-4: PCR product on AGE **Lane 1:** 100 bp DNA Ladder, **Lane 2:** Male DNA, **Lane 3:** Female DNA

The gel observed under UV transilluminator (Fig-4) and the quantity of the DNA and its purity (Table 4) was estimated by spectrophotometer. The amplified DNA concentration in male papaya is 351.7 ng/µl and the female papaya is 375.5 ng/µl.

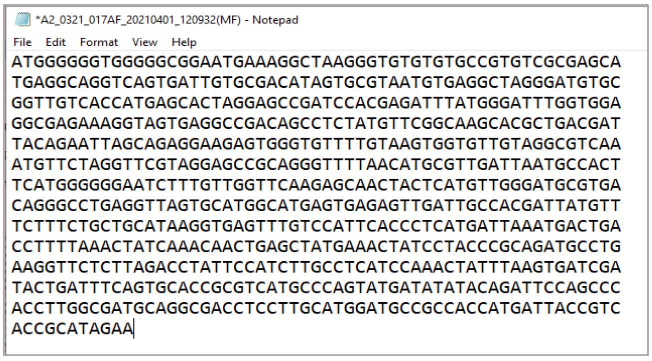
Table-3: DNA Concentration at OD 260nm (Before PCR)

S.No	Sample Name	OD at 260 nm	Concentration (ng/µl)	Purity ($\lambda_{260}/\lambda_{280}$)
1	Male flower tissue	1.600	80.01	2.11
2	Female flower tissue	1.286	64.29	1.79

Table-4: DNA Concentration at OD 260nm (After PCR)

Lane ID	Sample Type	Sample Name	Concentration (ng/µl)	Purity ($\lambda_{260}/\lambda_{280}$)
Lane 2	PCR product	Male flower tissue	351.7	1.504
Lane 3	PCR product	Female flower tissue	375.5	1.509

The sequence was analysed using Sanger sequencing. The sequence length in male papaya (Fig-5) is 726 and the female papaya (Fig-6) is 1061. The GC content is more in male papaya than female which leads more stability in male papaya and harder to amplify (Fig-7).



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ATGGGGGGTGGGGGCGGAATGAAAGGCTAAGGGTGTGTGTGCCGTGTCGCGAGCA
TGAGGCAGGTCAGTGATTGTGCGACATAGTGCGTAATGTGAGGCTAGGGATGTGC
GGTTGTCACCATGAGCACTAGGAGCCGATCCACGAGATTTATGGGATTTGGTGGA
GGCGAGAAAGGTAGTGAGGCCGACAGCCTCTATGTTCGGCAAGCACGCTGACGAT
TACAGAATTAGCAGAGGAAGAGTGGGTGTTTTGTAAGTGGTGTTGTAGGCGTCAA
ATGTTCTAGGTTCGTAGGAGCCGCAGGGTTTTAACATGCGTTGATTAATGCCACT
TCATGGGGGGAATCTTTGTTGGTTCAAGAGCAACTACTCATGTTGGGATGCGTGA
CAGGGCCTGAGGTTAGTGCATGGCATGAGTGAGAGTTGATTGCCACGATTATGTT
TCTTTCTGCTGCATAAGGTGAGTTTGTCCATTCACCCTCATGATTAAATGACTGA
CCTTTTAAACTATCAAACAACTGAGCTATGAAACTATCCTACCCGCAGATGCCTG
AAGGTTCTCTTAGACCTATTCCATCTTGCCTCATCCAAACTATTTAAGTGATCGA
TACTGATTTCAGTGCACCGCGTCATGCCCAGTATGATATATACAGATTCCAGCCC
ACCTTGGCGATGCAGGCGACCTCCTTGCATGGATGCCGCCACCATGATTACCGTC
ACCGCATAGAA
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Fig-5: DNA sequence of male papaya

The Male papaya sequence consist Adenine count-175, Guanine count-209, Cytosine count-147, Thymine count-95

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GGGCATAGGCGAGTAAGAATGCCAGTACGACCCTCTTCTTCCTCAGGTCCCTTTTCTC
CCCGTCATGCCTAAATTCACACGGATCTTGCTGAATCGTGTCTCTTATTGCCCGCGGT
ACCTTGTGCACAGCACCTACCTCACACATGCACACTACGTCGCACAGCAACCCCTTTT
TCGAGCTCTTCGAACTACGAACAGAGCCGCACATGCGACATGTGCACGGCACAGCATA
TTAGAAAAGGAAATAAACAATGAATTTAGAGAAGAAAAATTCACTTGATTGATGTATA
TTGAAATTTGAATGTATGCTACTTAGATATAAAGTGAAAGGCTAGACTAGAAAATTTA
CGTGCACTTGGATATAACAGAAAAAATAAATAATTATCTCTAATACTCCCCCTCAAGA
TGGAAAGTATATATTGATTGTACCCATCTTGCTTAAAAGTGTATGGAACTGTATAGAA
GGAAGAGCGTTAGTTATCAAATCCGCGAGTTGTATGATGCAAGAGACATGCATGATTT
AGAGTGCTCTAGCTAGTATCTTTTCTCAAACAAAATGCTCCTCGAACTCGATGTGATC
TCTTCTTTCATGATAAAACGAGATTTGCTCCTATTCGAGTAGTATTCTCATTTGTCAA
AATATCATAATGTTGGACCCTATATGTCTGACATTTCATATATTTTATTACAATTCCA
GCATCTTTACTATTTACGTGTTTTCATTTGATATAGATCAATATTCTTTTTACTGCAG
ATGATCAAGACCCAGATTTGACTGCTTCATCGTAGATGCCGTGATATGAGCGGATGAT
TCCTACATTTTTACTGTATCATATACGTAGCATCTCAGAGTTGTCAAGATCAGCCGCT
TCAGTCGGTATTCAAAAGAACACTTCTTATCTTAGAATATGAGAAACTTGCAATGGAG
AGTACTTCTCCTTGCCTGAAGTTCACTTGTTATAATGCTGTTTAGATACATTAGACAG
GCAGCTGACACAGTGAATCTTGATGCTGGACGGGATCATGCTCAGTACTGAACTTAGA
GCTCGACTATACCGAAG
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Fig-6: DNA sequence of female papaya

The female papaya sequence consist Adenine count– 320, Guanine count – 196, Cytosine count – 327, Thymine count – 218

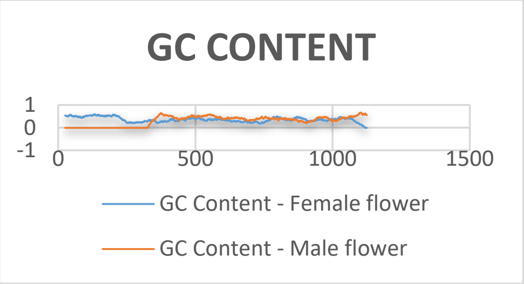

Fig-7: Graphical Representation of GC Content

The whole genome of male papaya was retrieved from NCBI database. These genome consist of 17,766 scaffolds and 47,485 contigs. The total length of genome is 370.419Mb and the protein count is 26103. By using GENSCAN, the complete genomic structure were identified. Generally, if exon score (Tscr) is above 100 which represent strong. In gene number 5 consist the internal exon (Intr) with 173.21 exon score and Initial exon (Init) with 120.84 exon score. In gene number 11 consist the single exon gene (Sngl) with 139.04 exon score.In gene number 19 consist single exon gene with 230.73 exon score. In gene number 29 consist Terminal exon (Term) with 235.93 exon score (Table-5 & Table-6).

In the comparison of the male plant of *Carica papaya* and *Phoenix dactylifera* (Fig-8) shows 79.6% similarities, 9.3% gaps and it shows the score as 500998.

The similar sequence for male papaya was identified in NCBI database Nucleotide BLAST search tool. The accession number CP010988.1, AY428939.1, AY861344.1 shows 92.31% similarity with analysed sequence of male papaya and the total score is 719.

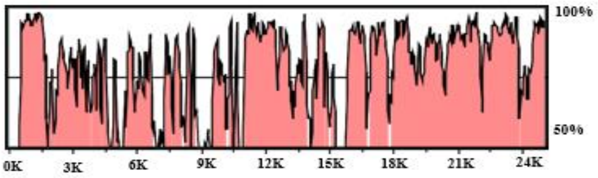

Fig-8: Conserved region of the male plant of papaya and date palms 🟥→ CNS (Conserved Non Coding Seq)

The accession number FJ011103.1 shows 92.35% and the total score is 691. The accession number AM778099, FJ011102.1, FJ011105.1, AY849325.1 shows 91.92% similarity and the total score is 708.

Table-5: GENSCAN result of male papaya

Gn.Ex	Type	S	.Begin	End…	.Len	Fr	Ph	I/Ac	Do/T	CodRg	P..	Tscr
1.01	Init	-	710	668	43	2	1	87	86	39	0.585	9.28

Table-6: GENSCAN result of female papaya

Gn.Ex	Type	S	.Begin	End…	Len	Fr	Ph	I/Ac	Do/T	CodRg	P..	Tscr
1.01	Term+	+	47	280	234	1	0	109	38	116	0.576	9.04
1.02	PlyA+	+	373	378	6							1.05
2.01	PlyA-	-	687	682	6							1.05
2.02	Term-	-	825	759	67	2	1	24	43	123	0.808	2.83

By using BLAST N, the similarity sequences were identified for female papaya. The accession number XR002539403 shows 86.13% with 148 total score. The accession number LR031879 (*Brassica oleraseae*) shows 100% similarities with 56.5 total score. The accession number LT669793.1, LT669791.1 (*Arabis alpina*), JX114756.1 (*Arabidopsis*) shows the 90.70% identity with 56.5 total score.

The *Brassica oleraseae*, *Arabis alpina*, *Arabidopsis*, these all are associated with *Brassicaceae* family. As *Brassicaceae* and *Carricaceae* family come under the Brassicales order, it both have the more identity sequence.

The sequence of both male and female plant similarity was identified by local alignment because of the variation in nucleotide length. Matcher is the tool used to identify the similarity in between these sequence.

By using that Matcher tool, similarity in between these sequences were identified as 55.6%. The variation of sequence in between male and female plant of papaya was identified by Geneious prime software. 19.4% SNP and 9.3% indels were identified.

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Consensus      TGAKGYWAGWGNCATNGCATGAKTKAGAGTNGMTNNCYANCKAKTATSTTTNTYTCNNNNNNNNNTGCTSCWNKAASKYGAKKTKRTCYMTT   91
Female flower  TGATGCAAGAGACAT-GCATGATTTAGAGT-GCT--CTAGCTAGTATCTTT-TCTCAAACAAAATGCTCCTCGAACTCGATGTGATCTCTT   86
Male flower    TGAGGTTAGTG-CATGGCATGAGTGAGAGTTGATTGCCA-CGATTATGTTTCTTTC--------TGCTGCA-TAAGGTGAGTTTGTCCATT   80

Consensus      CWNNYTCATGATWAAAYGANNNYTGNWCCTWTTMRASTAKYANNNWWCTSAKYTRTSAAAMTATCMTAMNNNNNNNNCCSYAKATGYCTGAM  182
Female flower  CT--TTCATGATAAAACGAGATTTGCTCCTATTCGAGTAGTA---TTCTCATTTGTCAAAATATCATAATGTTGGACCCTATATGTCTGAC  172
Male flower    CACCCTCATGATTAAATGA---CTG-ACCTTTTAAACTATCAAACAACTGAGCTATGAAACTATCCTAC-------CCGCAGATGCCTGAA  160

Consensus      RKTTCWYWTAKNYYTATTMCAWNTTSCMKCATCYWWACTATTTAMGTGNNNNNANTYGATAYWGATNNCARTRYWCYKYKTMMTGNNCAGN  273
Female flower  ATTTCATATAT-TTTATTACAA-TTCCAGCATCTTTACTATTTACGTGTTTTCATTTGATATAGAT--CAATATTCTTTTTACTG--CAG-  256
Male flower    GGTTCTCTTAGACCTATTCCATCTTGCCTCATCCAAACTATTTAAGTG-----A-TCGATACTGATTTCAGTGCACCGCGTCATGCCCAGT  245

Consensus      ATGATNNAKAYMCAGATTYSASNSCYWCMTYGKMGATGCNSGYGANNNNYWTGMRYGGATGMYKCCWMCATKWTTACYGTNAYCRYATA  362
Female flower  ATGATCAAGACCCAGATTTGACTGCTTCATCGTAGATGC-CGTGA----TATGAGCGGATGATTCCTACATTTTTACTGT-ATCATATA  339
Male flower    ATGAT--ATATACAGATTCCAG-CCCACCTTGGCGATGCAGGCGACCTCCTTGCATGGATGCCGCCACCATGATTACCGTCACCGCATA  331
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Fig-9: Consensus sequence of both male and female flower

IV. CONCLUSION

The benefits of comparative study on genome sequence is that identifying the biochemical diversity among the same species and also used for identifying the evolution of species. In genome analysis of male papaya, 1,60,100 nucleotide with 28,072 coding genes was analysed. The candidate gene involved in gender determination in papaya is *SVP-like* gene[6]. According to this, the identified 28.41% variation occured in *SVP-like* gene. In future research, these variations can be manipulated by the genetic engineer, and make the male plant as hermaphrodite permanently, so that, the yield of papaya fruit will be increased. Generally the male papaya was mutated itself while increase the temperature, at that time, the male papaya plant act as hermaphrodite and produce the fruit. From the result of our project, heat (increasing temperature) work on those nucleotide variations and lead to mutation for making that male papaya plant to produce the fruit. We expect that our result will be helpful to other genome project which is related to papaya.

V.ACKNOWLEDGEMENT

I would like to thank Dr.A.Praveena and Ms.T.Shanthi for their valuable suggestions and guiding us throughout the project.

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