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In Slilico Methods for Eradication of Papaya Leaf Curl Disease from Carica Papaya

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Abstract: ***Carica papaya is a common fruit found in India. It has various medicinal properties. several viral diseases of papaya cause huge lose to agricultural economy to a large scale. The most commonly reported is Papaya leaf curl disease which is caused by Papaya leaf curl virus. Papaya leaf curl virus belongs to the genus Begomovirus i.e. has bipartite genome. Its C2(L2) gene in segment A of genome functions as transcription activator. To remove the infection of this virus from papaya gene silencing feature was considered. Out of several methods for gene silencing, RNA interference was studied in detailed to remove Papaya leaf curl disease through in silico. AGO1, QDE-2, and RDE-1are the related proteins required for transcriptional gene silencing in plants. These protein belong to Argonaute family and play central role in RNA silencing processes. RISC is the RNA induced silencing complex responsible for gene silencing. GW(Genome Wide Micro RNAs proteins) stand for (miRNA). It guides argonaute proteins to target mRNAs leading to gene silencing. The maximum homology of Papaya leaf curl virus was found to be with Tobacco Curly Shoot Virus. It has been found from literature that Carica papaya shares common ancestor with Arabidopsis Thaliana. The related proteins i.e. AGO1, QDE-2, and RDE is common protein found in both the species. These proteins are required for transcriptional gene silencing in plants. This suggests that these proteins can help in transcriptional gene silencing in Carica papaya too. This transcriptional gene silencing will repress the gene C2(L2) and thus stop further infection of virus. Bemisia tabaci is the insect vector of Papaya leaf curl virus. Controlling the population of this insect vector can reduce the chances of this virus infecting Carica papaya. Dimethoate is an insecticide used to kill this insect vector and is hazardous for human life. Anatoxin is a naturally occurring organophosphate isolated from blue-green algae and can be used as an efficient insecticide against Bemisia Tabaci.***

Keywords: Carica Papaya, Papaya Leaf Curl Virus, Gene silencing, Dimethoate, Bemisia Tabaci

I. INTRODUCTION

Carica papaya is a common fruit found in India. *Carica papaya* is the botanical name of papaya fruit which belongs to the *Caricaceae* family. It is having various medicinal properties. It is also known as paw-paw plant and it is a pilule tropical fruit. It has commonly orange-red, yellow-green and yellow-orange hues with abundant orange pulp. Its whole plant parts have antifungal, antibacterial and antiviral properties.

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It contains high content of vitamin A, B, C, E, proteolytic enzymes such as papain and chymopapain. Carpaine is an alkaloid found in papaya which reduces the heart rate in humans and reduce blood pressure. These enzymes provide antibacterial properties of papaya.

Carica papaya is neutraceutical. It is an exquisite source of Carotene. It lowers the cholesterol level too as it is a fine source of fiber. As mentioned above it has vast role in curing many diseases due to medical properties. Problems such as blisters, corns, sinuses, eczema, glandular tumors, blood pressure, fecal impaction, general infirmity, dyspepsia and other digestive problems can be cured by papaya extract. Currently, a kidney transplant tolerant in London was dealt with of a post-agent virus by putting pieces of papaya on the injury for 48 hours. It has a high nourishing quality, for example, Sodium 3 mg, Potassium 257 mg, Phosphorus 5 mg, Magnesium 10 mg, Iron 0.10 mg, Calcium 24 mg, vitamin C 61.8 mg, Folate 38 mg, Niacin 0.338 mg, Riboflavin 0.05 mg, Thiamine 0.04 mg, Vitamin A 328 mg, Protein 0.61 mg, Fat 0.14 mg, Dietary fiber 1.8 mg, Sugars 5.9 mg, Carbohydrates 9.81 mg. The pharmacological movement of every division of papaya is extraordinary and profitable to human wellbeing. Its leaves assume real part in relieving dengue fever, goes about as tumor cell development inhibitors, shows antimalarial and antiplasmodial movement and encourage processing as well. Its organic product contains folic corrosive required for change of homocysteine to amino corrosive cysteine along these lines voiding heart stroke. The black seeds of papaya are succulent and can be used as a substituent for black pepper. The papaya peel is used in cosmetic industry for producing sunscreen as it contains vitamin D. The peel can also be used as a home remedy to fight dandruff. The juice from the papaya roots ease urinary troubles. Its latex contains papain, chymopapain and carpaine . Along with medicinal properties Papaya has side effects like it causes allergies too. A latex fluid is released by papaya when not quite ripe which causes irritation and evoked allergic reaction. The concentration of latex in unfledged papayas is postulated to cause uterine deflations which may lead to stillbirth in an expecting women. In countries like India, Bangladesh, Pakistan, Sri Lanka and other countries women have used green papaya as an herbal medicine for contraception and abortion (Aravind. G et.al, 2013). There are several varieties of papaya found in India listed below in Table 1. Many scientists have given their contribution in irradication of disease in papaya. Some of the listed works are:

The latex sap from *Carica papaya* was studied to identify fungicidal and antifungal effect of D (+) - glu - cosamine. When candida albicans was affixed to a culture during the exponential growth phase the latex sap of Carica papaya inhibited its growth. Approximately 60 % result was achieved. The cell wall degradation due to absence of polysaccharidic constituents in the peripheral coatings of the fungal cell wall and

The cell debris emancipated was the result of fungi static effect (El-Zaher, E. H. A, 2014).

Carica papaya leaf showed antimicrobial activity when it was observed on some disease causing organisms from South West Nigeria. The biological active compound of leaf and root extracts of *Carica papaya* was extracted and tested. Using the agar diffusion method, the aqueous extracts of the root did not show eloquent activity with the methanol extracts, demonstrating the highest activity against the test bacteria(I.I Anibijuwon et.al, 2009).

Several microbial and viral diseases of Papaya were studied as shown in Table 2.

Papaya Leaf Curl Virus (PLCV) is the virus which causes Papaya leaf curl disease belongs to the genus Begomovirus. The morphology of virus was studied in detail and found that virus particles of this virus are not enveloped. Nucleocapsid is 38nm long and 15-22nm in diameter. The viral particles have basic isosahedral proportionality. There are 22 capsomeres per Nucleocapsid. The genome of the virus was studied and found that it has single stranded closed circular DNA. It has a bipartite genome i.e genome is segmented in 2 segments-DNA-A and DNA-B (table1.3). DNA-B is dependent on DNA-A for its replication. The nanonucleotide sequence that acts as origin of replication was found (TAATATTC).

Table 1: Commonly Found Papaya in Different States of India (Vinod Kumar, 2012).

S. No.	Species	Cultivated In	Characteristics
1	Coorg Honey Dew	Andhra Pradesh	Commonly called as Madhubindu Green-yellow oblong shape fruits.
2	Pusa Dwarf	Orrisa, Jharkhand	Oval shaped fruits Perfect for high density plantation Drought hardy
3	Pusa Giant	Bihar	Fruits are big in size Used in canning industry Sturdy to strong wind
4	Pusa Majesty	Bihar	Fruits are medium in size Used in papain production Yields after 146 days of planting
5	Pusa Delicious	Orrisa, Karnataka, Kerela	Grown for table purposes Yields after 8 months of planting Fruits are medium in size
6	CO.1	Ranchi	Dwarf plant Odour of papain is absent Medium sized spherical fruit
7	CO.2	Andhra Pradesh	Juicy fruit Fruits are medium in size Perfect for extraction of papain
8	CO.3	Andhra Pradesh	Fruits are large in size Fruit weight 1-1.5 kg
9	CO.5	Andhra Pradesh	Famous for high papain production
10	Washington	West Bengal, Andhra Pradesh	Fruit has few seeds, Fruits round and medium in size
11	Solo	Andhra Pradesh	Perfect for kitchen garden Used for table purposes Small sized fruit with pink pulp
12	Ranchi	Bihar	Sweet in taste Oblong fruits with dark yellow pulp
13	Taiwan-786	Andhra Pradesh	Used for processing purpose Oblong fruits with sweet pulp
14	IIHR39 and IIHR54	Bangalore	Better shelf life Medium sized fruits

Table 2. List of Several Viral Diseases of Carica Papaya along with their Symptoms, Mode of Transmission and Characteristics. (Jagdish Chandra and L.Duleep Kumar Samuel-1999)

S. No.	Disease	Symptoms	Transmission	Characterstics
1	Papaya leaf curl	Severe curling, wrinkle, distortion of leaves, vein clearing, leaf size reduction, leaf become leathery	Transmitted by whitefly under normal conditions	It showed 89.9% homology with the Indian Cassava Mosaic Virus
2	Papaya leaf wrinkle	Zig-zag stem, wrinkled leaf, thick and opaque veins	No insect vector transmission	Phytoplasma is suspected to cause disease First reported from Tamil Nadu(Thomas et.al, 1939)
3	Leaf distortion disease	Leaf distortion, filiform leaves	Mechanically sap transmitted	Coat protein of virus consists of major polypepetide with MW of 26.2ka
4	Papaya ringspot virus (Purcifull, 1972)	Fail in fruiting and flowering (Lokhande et.al,1992), mottling, curling, blisters, defoliation, vein clearing	Insect transmission-Aphid vectors-A.gossypii, A.malvoe, A.medicaginis, A.citricola, A.nerii and many more; Dodder transmission-Cascuta Reflexa Roxb;Bird transmission-Saltator Coerulescens	Genetic organization similar to other pot viruses except MT protein in pot viruses

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II. MATERIAL AND METHODOLOGY

A. Papaya Leaf Curl Virus-Genes and Function

In an attempt to identify the main genes of the virus which cause Papaya Leaf Curl Disease total eight genes were identified from the literature. All these genes functions along with infection causing ability were studied. (Table 3)

Table 3. Genes Found in Segment A and Segment B of Papaya Leaf Curl Virus along with their Function and Orientation (Pita JS, et.al,2001).

SEGMENT A			
GENE	ORIENTATION	FUNCTION	WEIGHT
V1(R1)	Positive orientation	Coat protein	29.7 kDa
V2	Positive orientation	Movement protein	12.8 kDa
C1(L1)	Negative orientation	Replication initiation	40.2 kDa
C2(L2)	Negative orientation	Transcription Activator	19.6 kDa
C3(L3)	Negative orientation	Replication enhancer	15.6 kDa
C4	Negative orientation	May determine symptom expression	12 kDa
SEGMENT B			
V1(R1)	Positive orientation	Nuclear shuttle protein	33.1 kDa
C1(L1)	Negative orientation	Movement protein	29.6 kDa

To remove the infection of papaya, gene silencing feature was considered. Methods for gene silencing are RNAi, CRISPR and siRNA. Out of them RNA interference was studied in detail to remove Papaya Leaf Curl Disease through in silico. RNA interference is a gene silcencing method used for knocking down the expression of an individual gene of interest. To stop the infection of virus through RNA interference, Post Transcriptional Gene Silencing can be taken as a methodology.

AGO1, QDE-2 and RDE-1 are the specific proteins required for transcriptional gene silencing in plants.

Post Transcriptional Gene Silencing (PTGS) is a phenomenon in which leads to distinct mortification of RNAs that are similar to the transgene transcribed sequence in plants, annihilating in fungi and RNAi in animals. SGS2, QDE1 and EGO1 are the set of related protein required for RNAi and PTGS quelling. It was reported that Arabidopsis mutants which were impaired in PTGS which are affected at the AGO1 locus. QDE is required for quelling and RDE1 is the protein required for RNAi and these two proteins have similarity with AGO1. It was revealed that one amino acid essential for PTGS is also present in QDE2 and RDE1 in a very highly conserved motif through sequencing of AGO1. Putting together these results prove the hypothesis that the expression of invading nucleic acid molecules at the post transcriptional level is controlled by these practices derived from a common ancestral mechanism. RDE1 and QDE2 mutants are viable. There are several abnormalities which are displayed by AGO1 mutants. These results increase the possibility that PTGS and some of its elements can participate in the regulation of gene expression during the developmental period in plants (Mathilde Fagard, et.al. PNAs; 2000)

B. AGO1,QDE2 and RDE

These proteins belong to the Argonaute family . The argonaute protein family performs a very important role in RNA silencing techniques. RISC is the RNA induced silencing complex responsible for gene silencing. The argonaute proteins bind to individual classes of small non-coding RNAs, incompassing micro RNAs, small interfering RNAs and Piwi interacting RNAs. Small RNAs guide argonaute proteins to their specific targets through sequence complementary (base pairing) which then leads to mRNA cleavage or translation inhibition. The name of this protein family is derived from a mutant phenotype resulting from mutation of AGO1 in Arabidopsis Thaliana (Bohmert K, et.al,1998).

There are majorly four characteristic domains encoded by the argonaute family– N-terminal, PAZ domain, mid domain and a C-terminal Piwi domain. The main function of the mid domain is to site the 5'phosphate of a siRNA or miRNA (Hutvanger G, et.al, 2008).

The PAZ domain is a module that binds with RNA and recognizes the 3'end of both siRNA in a sequence independent. For cleavage or translation inhibition it targets mRNA by base pairing interaction(Tang G, 2005).

C. GW Protein

GW Proteins stands for Genome Wide Micro RNAs (miRNA), it guides Argonaute proteins to cause gene silencing by targetting mRNAs. AGO Proteins are not the true mediators of gene silencing however interact with a member of the GWI82 protein family (also known as GW proteins) which regulates all subsequent steps in gene silencing(Eulalio et.al, 2009).

A plant specialized GW protein called NERD defines an additional RNAi-dependent chromatin based pathway in Arabidopsis. This RNAi dependent chromatin based pathway encompasses both Post Transcriptional Gene Silencing and Transcriptional Gene Silencing in plants. (Dominique Pontier et.al, 2012)

III. RESULT AND DISCUSSION

A. Papaya Leaf Curl Virus – Homology Modelling

Homology modeling of Papaya Leaf Curl Virus was done using BLAST tool to find homologus viruses species. Reference sequence of papaya leaf curl virus was obtained from NCBI having sequence ID NC004147. It was observed from result that (figure 1) the homology of papaya leaf curl virus was with radish leaf curl virus, ageratum enation virus, cherry tomato leaf curl virus and croton yellow vein virus. Croton yellow vein virus has the maximum homology with Papaya leaf curl virus. Phylogenetic relationship of Papaya Leaf Curl Virus with its homologous viruses was obtained and studied the evolutionary relationship.

B. Evolutionary Significance with Arabidopsis

It has been found from literature that Carica papaya shares a common ancestor with Arabidopsis Thaliana (Ray Ming et.al ;2008). As discussed in method and material section the three related proteins i.e AGO1, QDE2, and RDE are required for Transcriptional Gene Silencing in plants. These proteins were first studied in Arabidopsis Thaliana. This suggests that these proteins can help in transcriptional gene silencing in Carica papaya too. This transcriptional gene silencing will repress the gene C2(L2) which is a transcription activator protein in Papaya Leaf Curl Virus and thus stop the further infection of the virus. The protein interaction of the protein i.e AGO1 was visualized using STRING tool (Figure 1)

Table 4: Homologues of Papaya Leaf Curl Virus with Different Varities

Virus	Identity	E-Value
Papaya Leaf Curl Virus	100%	0
Croton yellow vein virus	91%	0
Cherry tomato leaf curl virus	89%	0
Radish leaf curl virus	90%	0
Ageratum Enation Virus	89%	0

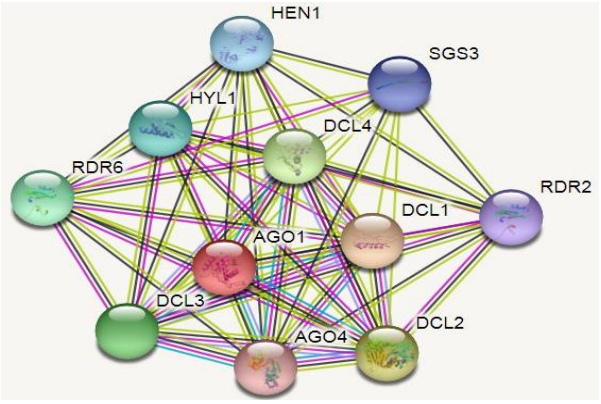

Figure 1. Interaction of Proteins in Arabidopsis Thaliana using STRING tool

Table 5: Predicted Functional Partners of AGO1 Interacting in Arabidopsis Thaliana

S. No.	Predicted Functional Partners	Function
1	DCL1	Performs major role in RNA-mediated post- transcriptional gene silencing (PTGS)
2	DCL2	Intricated in RNA-mediated post- transcriptional gene silencing (PTGS)
3	DCL4	intricated in RNA-mediated post- transcriptional gene silencing (PTGS)
4	DCL3	intricated in RNA-mediated post- transcriptional gene silencing (PTGS)
5	RDR6	Intricated in post-transcriptional gene silencing (PTGS)
6	HYL1	intricated in RNA- mediated post-transcriptional gene silencing (PTGS)
7	HEN1	Important in plant development through its role in small RNAs processing
8	SGS3	Involved in post-transcriptional gene silencing and natural virus resistance
9	RDR2	Required in transcriptional gene silencing (TGS)
10	AGO4	Involved in transcriptional gene silencing (TGS) by DNA methylation

C. Transmission and Control – Bemisia Tabaci

Bemisia tabaci also called as silverleaf whitefly is currently the most important agricultural pest and is the insect vector of Papaya Leaf Curl Virus in papaya causing Papaya Leaf Curl Disease. (Priyanka Varun et.al, 2017). Controlling the population of this insect vector can reduce the chances of this virus infecting Carica papaya. An insecticide called Dimethoate is used to kill mites and insects. It belongs to the class organophosphates. This chemical acts by intruding with the activities of an enzyme called Cholinesterase, that is essential for the efficient working of the nervous system in humans and insects both. As mentioned this chemical also affects human as it is readily absorbed by skin and easily absorbed through lungs. Also high UV light or high environment temperature enhances its toxicity. Some of the affects of this chemical on humans are running nose, chest discomfort, short breath, reproductive affects in both males and females, mutagenic effects, carcinogenic effects and organ toxicity (Ramesh C. Gupta et.al, 2014).

After detailed study it was found that Anatoxin is a naturally occuring organophosphate which which is a changeless active site-directed inhibitor of Cholinesterase(Hyde EG1, Carmichael WW - 1990). It is obtained from the freshwater cyanobacterium (blue-green alga) Anabaena flos-aquae strain NRC 525-17. As anatoxin is a naturally occuring organophosphate it is relatively less toxic and can be used as an efficient insecticde against insect vector Bemisia Tabaci.

IV. CONCLUSION

The study was done on Papaya leaf curl virus transmission and its control using computational analysis. The genes of Papaya leaf curl virus were studied in detail. Further control measure was studied using RNA interference for gene silencing of the gene C2(L2). This gene is responsible for transcription activation. This gene silencing method can reduce amount of pesticides used in agriculture. It will improve the cultivation of *Carica Papaya* to a large extent as this viral disease is the most common and devastating in view of agricultural economy. The in silico computational method is low cost as well as less time consuming. On the other hand to prevent whitefly which is insect vector for Papaya leaf curl virus, the use of anatoxin in place of dimethoate helps to control the population of *Bemisia Tabaci (whitefly).*It is beneficial to human health especially for the farmers who use this insecticide in the agricultural field. Improvement in the *Carica Papaya* crop will lead to increase in its cultivation and thus it will be able to meet the population demand at cheaper rate.

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