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Determination of DNA, Protein and Characterization of Begomovirus in Solanum Lycopersicum

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Abstract: ***One of the most important cash crops and useful vegetables that is liked by everyone from making different dishes and having it as salads. The tomato has some medicinal properties like helping improve heart diseases and blood pressure and fighting against cancer, these properties have made me choose tomato to understand the infection that is caused in this plant which decreases the total productivity. Begomovirus is a serious threat to this crop and infects more than 60% of its total yield. In this project, I have isolated the protein and the DNA of both the healthy and the infected tomato plant leaf samples and figured out the total decrease in the percentage of both protein and DNA.***
Keywords: Begomovirus, PCR, Tomato, DNA, Protein, Agarose Gel Electrophoresis, TYLCV.

I. INTRODUCTION

The dicot plant tomato (Solanum lycopersicum.) is one of the greatest significant fruit and veggie crops, with over 182.33 million tons of tomato fruits full-grown 4.85 million per year, and more than 400 varieties. Plant viruses known as Gemini viruses contain SS spherical DNA genomes that encode genes that deviate in opposite orders from the initiation of replication in the virion component (i.e., Gemini virus genomes are ambiance).[10,12] The Gemini virus complex affects 300 plant species throughout 63 families of crops, with the Solanaceous crops such as tomato, pepper, chili, cucurbit, tobacco, and cotton being among the most significant. A double Standard circular middle is created from s-s circular DNA. In this step, the viral genomic or plus-sense DNA strand is used as a template by round DNA repair enzymes to create a complementary negative-sense strand. The viral Rep. protein cleaves the viral strand at a precise location located within the origin of replication in the following phase, the rolling circle phase, to begin replication[1,4]

DNA virus known as the (TYLCV) goes to the family of Geminiviridae and the species begomovirus. The very harmful tomato disease, (TYLCV),[8] originates in humid tropical and subtropical zones and causes significant economical losses. Bemisia tabaci, the white fly which is also known as the silver leaf fly or the sweet potato Whitefly, is the virus carried away with the help of an insect vector. It belongs to the family ALEYRODIDAE and the order HEMIPTERA. Non-enveloped, twinned (geminated), imperfect T=1 icosahedral symmetry, 22 pentameric capsids Somers consisting of 110 capsid proteins (CP), 38 .nm in length and 22 nm in width (for Maize streak virus MSV).[2,6] [5]Here the geminate particles consist of only one of a single circular ss-DNA.

Bemisia tabaci which is an insect vector transmits TYLCV in a determined-circulative non-propagative way. In the adult stage of infection, it spreads effectively.

[8] Whereas 8-24 hours is the virus's latent phage, with a short acquisition entrance period of 15-20 min. In plant-virus and vector structure, the female can transmit the virus much more easily as compared with the male fly. [5,3]According to a study, TYLCV is passed from parents to offspring for at least two generations.[9,7,12] A Tomato Yellow Leaf Curl Virus isolated from Israel can be sexually spread from one to another insect, according to a study. In this investigation, it was discovered that the virus was passed from virus-infected males to virus-infected females.

II. MATERIALS AND METHODS

A. Sample Collection

The sample collection for the experiment was taken from the agricultural field which is located at Jahangeerabad, kursi road, Lucknow, Uttar Pradesh. The time of collecting for the sample was 10 am to 12 pm approx.

FIG 1 and 2: Healthy and Infected leaves

Isolation of DNA from leaves of tomato plants PRINCIPLE: An essential attribute of the cationic detergent CTAB is its ability to precipitate nucleic acids. The CTAB extraction buffer is used to keep the DNA's surroundings from degrading while also assisting in the separation of the DNA from other cell constituents. Tris keeps its pH steady. The metal ion chelated EDTA.

III. PROCEDURE OF HEALTHY AND INFECTED TOMATO LEAF

1) Then the leaf midrib and veins were taken out with the help of a blade and the leaves are allowed to get dried with the help of blotting paper. The leaves were weight about 150mg and dipped in 5ml absolute alcohol for 1 hour. Then the leaves were dried again with the help of blotting paper. Then I put the leaf carefully and with the help of mortar and pestle the leaf was gridded.
2) Now CTAB buffer was slowly heated and the 2ul beta mercapto ethanol was added slowly 1ml to per sample and with the help of mortar and pestle ground well. The 1ml of the slurry is added to each Eppendorf tube and the samples are allowed to incubate. Now I have added the 667ul 24:1 chloroform: isoamyl alcohol in each of the Eppendorf tubes. The gentle invert of the tubes for 15 min Then centrifuged at 10000 rpm for 10 min. Then the supernatant was piped out and the cold isopropanol alcohol was added and mixed. thoroughly.
3) Then the samples were allowed to incubate at -20 degree Celsius for 1 hour (for the best result, an overnight incubation is suggested). Now I washed the pellet obtained with 70 percent of ethanol 500ul. Then I centrifuge it at 10000 Rpm for 5 min. then Deccan and dried the pallet. 50ul 0.1X TAE buffer was added to the sample and incubated at 37’ degree Celsius for 1 hour. Now with the help of NanoDrop quantification of the DNA is done. The visualization of DNA bands was performed on the Agarose gel electrophoresis.

IV. RESULTS

OBSERVATION: The quantification of healthy and infected tomato leaves is done through a Nanodrop Spectrophotometer.

TABLE 1 (HEALTHY)

S. NO.	230nm	260nm	280nm	Ratio. 260/280	Ratio. 260/230	Conce. (ng/ul)
1.	19.017	42.944	20.722	2.07	2.07	2147.2
2.	22.512	46.758	22.323	2.09	2.08	2337.9
3.	2.991	42.936	20.546	2.09	2.04	2146.8
4.	17.851	36.958	17.741	2.08	2.07	1847.9

TABLE 2 (INFECTED)

S. NO	230nm	260nm	280nm	Ratio 260/280	Ratio 260/230	Conc. ug/ul.
1.	7.469	11.304	6.271	1.80	1.50	565.2
2.	6.732	10.834	5.668	1.91	1.61	541.7

3.	6.073	8.912	4.832	1.84	1.47	445.6
4.	5.724	9.191	4.748	1.94	1.61	459.6
5.	3.952	5.469	2.920	1.87	1.38	273.5
6.	10.723	15.146	8.495	1.78	1.41	757.3

OBSERVATION: The qualitative analysis of healthy and infected tomato leaves through Agarose gel electrophoresis.

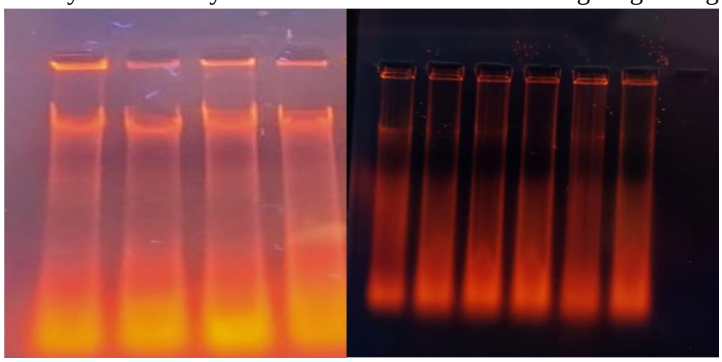

FIG 3 and 4: Healthy and Infected bands were observed.

V. ESTIMATION OF TOMATO LEAVES PROTEIN BY LOWRY'S METHOD

• 0.1M S. Phosphate Buffer (PH-7.4), 80% Acetone,10% T.C.A (trichloro acetic acid),0.1 N sodium hydroxide

PROTEIN ESTIMATION REAGENTS:

B.S.A standard solution (25 mg/25 ml DW) and other protein estimation agents. Bovine serum albumin (BSA) is the accepted industry reference for calculating total protein concentrations using colorimetric assays. BSA values are meticulously created at a concentration of 2 mg/ml and are meant to be diluted several times to create precise standard curves.

REAGENTS.

REAGENT-A-50 ml of 2% sodium carbonate (two grams in one hundred milliliters of distilled water) combined with 0.4 grams of 0.1 N NaOH solution, REAGENT-B-1% Sodium Potassium Tartaric Acid in Water (0.1 g in 10 ml Distilled Water), REAGENT-C-0.05 gram in 10 ml of distilled water, which contains 0.5% copper sulfate., REAGENT D - Make critical reagents combining 2 ml of B, 2 ml of C, and 96 ml of A. This is well-known as REAGENT-D and is newly made at the time of the testing. Reagent e-Ciocalteau reagent solution (2N), by Folin. On the day of usage, dilute commercial reagent (2N) with an equivalent volume of water (2 ml of commercial reagent plus 2 ml of distilled water).

OD AND CONCENTRATION OBSERVED THROUGH DOUBLE BEAM SPECTROPHOTOMETER

BSA standard solution.

TABLE 3

S. NO	B.S.A. conc (mg/ml)	WATER (ml)	ALKALINE COPPER SULPHATE(ml)	LOWRY'S REAGENT(ml)	OPTICAL DENSITY(660nm)
Blank	0.0	1.0	5.0	0.5	0.0000
1.	0.1	0.9	5.0	0.5	0.3118
2.	0.2	0.8	5.0	0.5	0.5101
3.	0.4	0.6	5.0	0.5	0.8527
4.	0.6	0.4	5.0	0.5	1.1625
5.	0.8	0.2	5.0	0.5	1.3640
6.	1.0	0.0	5.0	0.5	1.6154

Healthy tomato leaves protein samples: TABLE 4

S. NO	Healthy leaf protein (mg/ml)	Water (ml)	Alkaline copper sulfate (ml)	Lowry's Reagent (ml)	Optical density (660nm)	Protein conc.(mg/ml)
Blank	0.0	1.0	5.0	0.5	0.0000	0.0000
1.	0.1	0.9	5.0	0.5	0.1501	0.0915
2.	0.2	0.8	5.0	0.5	0.2242	0.1367
3.	0.4	0.6	5.0	0.5	0.3138	0.1914
4.	0.6	0.4	5.0	0.5	0.3292	0.3292
5.	0.8	0.2	5.0	0.5	0.3690	0.3690
6.	1.0	0.0	5.0	0.5	0.4192	0.4192

Infected tomato leaves protein samples: TABLE 5

S. NO	Infected leaf protein (mg/ml)	Water (ml)	Alkaline copper sulfate (ml)	Lowry's reagent (ml)	Optical density (660nm)	Protein conc. (mg/ml)
Blank	0.0	1.0	5.0	0.5	0.0000	0.0000
1.	0.1	0.9	5.0	0.5	0.0950	0.0647
2.	0.2	0.8	5.0	0.5	0.1471	0.1002
3.	0.4	0.6	5.0	0.5	0.2793	0.1901
4.	0.6	0.4	5.0	0.5	0.3760	0.2560
5.	0.8	0.2	5.0	0.5	0.4825	0.3285
6.	1.0	0.0	5.0	0.5	0.5731	0.3901

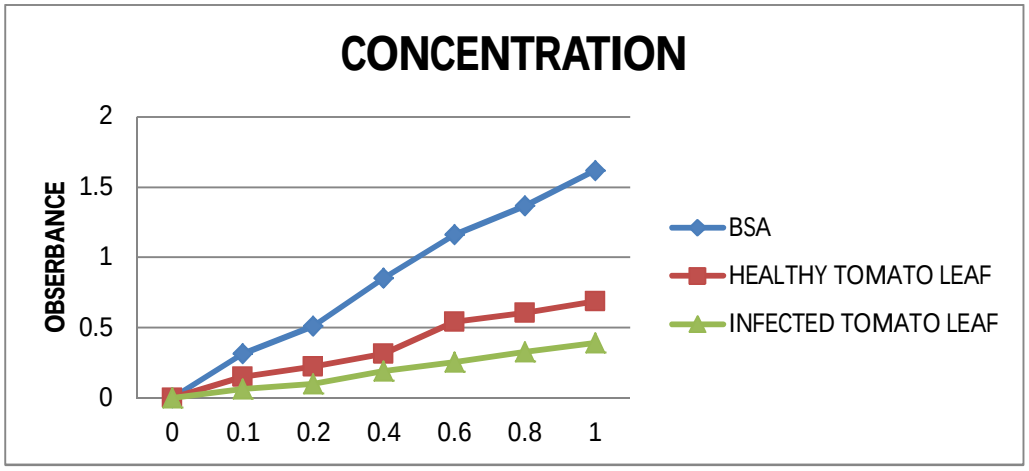

FIG 5: Graphical representation of absorbance versus conc. Of BSA, Healthy and Unhealthy tomato leaf through double beam spectrophotometer.

VI. PCR (POLYMERASE CHAIN REACTION) USING DEGENERATIVE PRIMER

The polymerase chain reaction (PCR), is a powerful technique with several applications in molecular biology. The PCR, which depends on the concept of enzymatic replication of nucleic acids, is a method for rapidly and efficiently amplifying DNA sequences. The study of molecular biology greatly benefits from the use of this method, which is one of the core methods for DNA analysis. The three steps of PCR are typically repeated 39 times. The quantity of synthesized DNA in the reaction, doubles after each cycle of three stages, continuing until all of the reaction's components have been consumed. The PCRs are heated and cooled repeatedly using thermal cycler equipment. In several scientific fields, including virology, PCR has become the new benchmark for identifying a wide range of templates. The technique uses two artificial oligonucleotides or primers, Each of them covers an area that will grow exponentially and hybridizes to one strand of a target's double-stranded DNA.

[5] A DNA polymerase builds a complementary strand by successively adding deoxynucleotides to the hybridized primer as a substrate. A programmed thermal cycle regulates the pace of temperature change, the time spent incubating at each temperature, and the number of times each cycle is performed.

Following Agarose gel electrophoresis can be used to separate the amplified DNA fragments, the bands are visible by Ethidium Bromide when exposed to UV lights the DNA appears orange-colored. Certain sets of primers, specific primers, and random primers are being used to amplify different target DNA, they vary in annealing temperature.

Materials used in Polymerase Chain Reaction:

Micropipettes (2-20 µl, 0.5-2.5 µl), Micropipette Tips(sterilized), PCR Tubes, Ice trays, Tube holder

For the PCR amplification, the following reagents are used and added to the PCR tube:

Working concentration (1X)

FOR 50 µl OF VOLUME IN MASTERMIX.

Table 6

1. nuclease-free water	39.5 ul
2. Taq Buffer	5 ul
3. dNTP mix.	1 ul
4. F- Primer.	2 ul
5. R- Primer.	2 ul
6. Taq DNA polymeras0e.	0.5 ul
7. Templates.	2.5 ul

A master mix with a total volume of 50 l was generated by combining nuclease-free water, taq buffer, dNTP mix, forward and reverse primers, and Taq DNA polymerase. The master mix has a total volume of 50 mL, I used a micropipette to transfer 12.5 mL of the master mix to each PCR tube before adding 2.5 mL of the separated template DNA to the master mix of each tube. Currently, each PCR tube has a total volume of 15 ul. (Amplicon size: 1.6kb)

Table 7 steps of PCR

Step	Temperature	Time	
Initial Denaturation.	94 degree C	3 minute	
Denaturation.	94degree C	45 second	30
Annealing.	52degree C	45 second	
Extension.	72degree C	1 minute	
Final extension.	72degree C	7 minute	

The total time for the test taken was around 3 hours to complete this whole cycle-After the procedure was complete, the PCR tubes were removed, a gel loading buffer or bromophenol blue was added to each tube, and the mixture was properly mixed while keeping the tubes in the ice. Now these samples, each containing 10 ul, were loaded into the agarose gel for the electrophoresis. 2% Agarose gel was prepared for the gel run and the total volume of buffer used was 20 ml, therefore I weighed 0.4 gm. of agarose for 20 ml as mentioned above.

VII. RESULT FOR PCR PRODUCT IN AGAROSE GEL

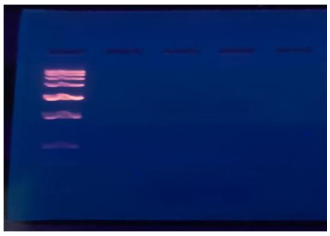

FIG 6: PCR result.

OBSERVATION

The analysis of my PCR product was that the bands were not visible as the primer could not amplify the DNA. Hence the infected tomato plant did not show any viable viral infected DNA but as per my analysis the quantity of the DNA in healthy was around 3000 ng/ul in concentration whereas in the infected I got around 800 ng/ul as the concentration, hence it proves that the plant was infected with the virus but did not carry the genetic material.

VIII. DISCUSSION

The most significant viral diseases in the Indian subcontinent are begomoviruses. One of the most destructive diseases, tomato leaf curl disease has been linked to several begomoviruses, which makes breeding for resistance more difficult. Additionally, complexity is increased by the existence of several beta satellites. The year-round existence of whiteflies is ensured by climate change and improper pesticide usage, which creates new difficulties for the control of the illness. In addition to the viral symptom, symptoms brought on by trips and whiteflies sucking mislead the breeder and make it harder to come up with a management plan. A different approach to creating a management plan is to have a full grasp of the diversity of viral complexes and the epidemiology. For the analysis of the viral genome, the samples that I have taken was infected tomato leaf that was collected from a nearby field of Jahangeerabad. This infected tomato leaf showed the symptoms of curling, and yellowing of the leaf, and had brown spots on its plant. During this process, the isolation of both the infected leaf and fresh healthy leaf was done and the concentration of protein and DNA that was isolated shows a wide decrease with the healthy leaf. The DNA concentration that was obtained from the infected leaves was around 273 ug/ul. While in healthy was around 3000 ug/ul. After the determination of DNA and protein, I also performed PCR using Degenerative Primers for my sample to detect the viral genome but the result come out to be negative though all the criteria from symptoms to the concentration were gained positive. Therefore, the interpretation of my result could be that the virus has infected the plant but the infection might be old and hence there was no presence of the viral genetic material.

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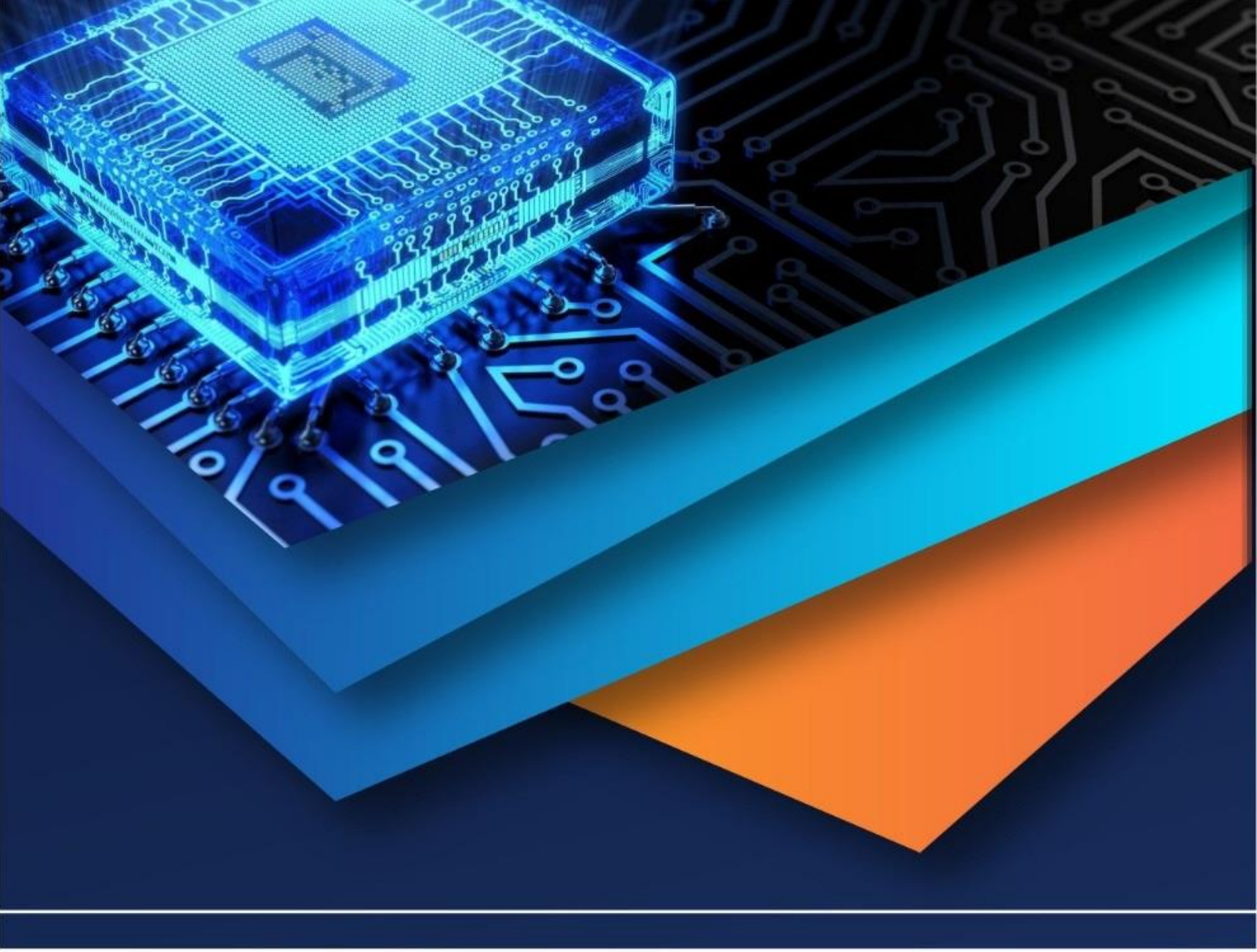

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