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**ESTIMATION OF DNA AND PROTEIN OF BEGOMA VIRUS INFESTED CHILLI PLANT****RAMESH CHANDRA AND SAUMYA TIWARI**

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**Received** : 24.7.15; **Accepted** : 24.9.15**ABSTRACT**

In present investigation, estimation of DNA and protein from chilli plant was carried out for healthy and infected leaves. The amount of DNA was more in healthy leaves than in infected leaves because the begomovirus damaged the DNA of infected leaves. The healthy leaves show high protein quantity, while in infected leaves the amount was less because of begomovirus.

Figures : 06

References : 10

Tables : 02

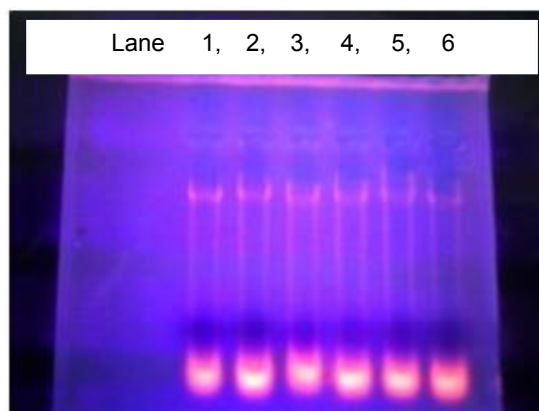
KEY WORDS : Capsaicin, *Capsicum annum*, CTAB, DNA isolation, Protein estimation.**Introduction**

Chilli, *Capsicum annum* is a member of the nightshade family Solanaceae. Chillies are indispensable and common ingredients of Indian food. In India Andhra Pradesh contributes 75% of India's export. The five domesticated species of chilli pepper are *Capsicum annum*, *C. frutescent*, *C. chinese*, *C. pubescens* and *C. baccatum*. Chilli is a rich source of vitamin C and capsaicin. Capsaicin is useful for treatment of pain, arthritis, blood sugar levels and cancer etc. Begomo viruses attack the chilli plants so that the leaves become curly and responsible for significant yield loss in wide variety of crops in India. Begomoviruses are large group of whitefly- transmitted plant virus containing single stranded circular DNA encapsulated in geminate particles. The first chilli leaf virus affecting in India, were responsible for significant yield loss in wide variety of crops<sup>9</sup>. Its infectivity was demonstrated in the natural host<sup>2</sup>. The etiology of chilli leaf curl virus was reported in 1963<sup>7</sup>. Tomato leaf curl at Joydeb pur, Bangladesh was found to be associated with chilli leaf curl disease in Punjab<sup>5, 10</sup>. Begomovirus was identified in India that is closely related to cassava mosaic virus and causes mosaic and stunting of *Jatropha curcas*. It was found that the disease was transmitted experimentally to healthy hosts by whiteflies<sup>8</sup>. The major developments in

begomovirus research was summarized in India and identified future areas<sup>1</sup>. An interesting aspect of Indian begomovirus is their overlapping host range due to polyphagous nature of vector, whitefly and mixed cropping pattern in India. Two types of DNA are known i.e. the alpha- satellite and beta-satellite, depending upon the organization of their effects on the symptoms produced by helper begomo viruses. The alpha-satellite (previously known as DNA I) encode their own replication, associated proteins and are believed to have originated from another class of single stranded viruses, the *Nano viruses*. The beta satellite (previously known as DNA-B), on the other hand, do not code for any replication associated protein, but easy and single, encoding a multifunctional protein having among others RNA splicing activity.

**Material and Methods****Isolation and purification of DNA and Protein**

The DNA was extracted from symptomatic and asymptomatic chilli sample by using CTAB (Cetyl trimethyl ammonium bromide) method<sup>3, 4</sup> and by Phenol- chloroform method. Nanodrop spectrophotometer was used for quantitative analysis and the visualization of DNA was performed by Agarose gel electrophoresis. From the fresh and infected leaves of chilli plants, the protein was estimated<sup>6</sup> and with liquid nitrogen.



**Fig. 1 : Bands of DNA of Chilli leaves**

#### Restriction enzyme activity

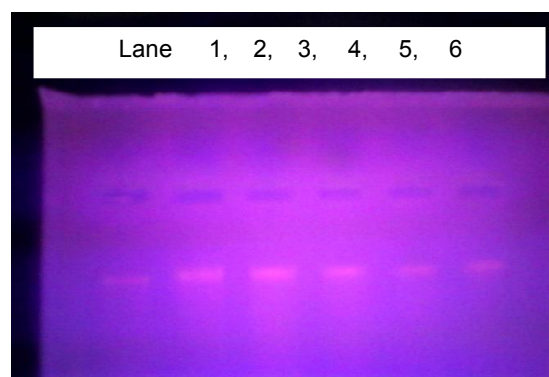
The vials containing restriction enzyme (EcoRI and Hind I) were placed on ice. Thaw the vials containing substrate (Lambda DNA) and assay buffer. Prepared two different reaction mixtures using Reaction1 (EcoRI digestion)-  $\lambda$ DNA 20 $\mu$ l, 2XAssay Buffer 25 $\mu$ l and EcoR I 3 $\mu$ l, Reaction2 (Hind III digestion) - $\lambda$ DNA. 20 $\mu$ l, 2XAssay Buffer 25 $\mu$ l and Hind III 3 $\mu$ l. Incubated the vial at 37 $^{\circ}$ C for 1hour and electrophoresed the samples at 50-100V for 1-2hours. Meanwhile, prepared 1% agarose gel for electrophoresis. After an hour added 5 $\mu$ l of gel loading buffer to vials.

Loaded the digested samples, 10 $\mu$ l of control DNA and 10 $\mu$ l of marker. Stained the agarose gel with 1x staining dye and destained to visualized the DNA bands.

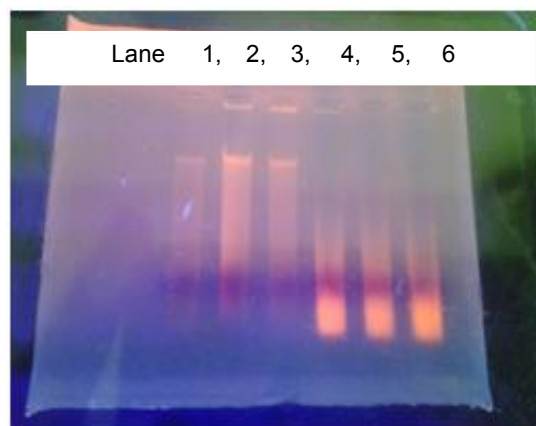
### Result and Discussion

#### Observations of DNA before purification

The observations were made during



**Fig. 2 : Bands of DNA of Chilli sample**



**Fig. 3 : Comparison of DNA bands from CTAB and Phenol Chloroform methods**

quantification by nanodrop Spectrophotometer .

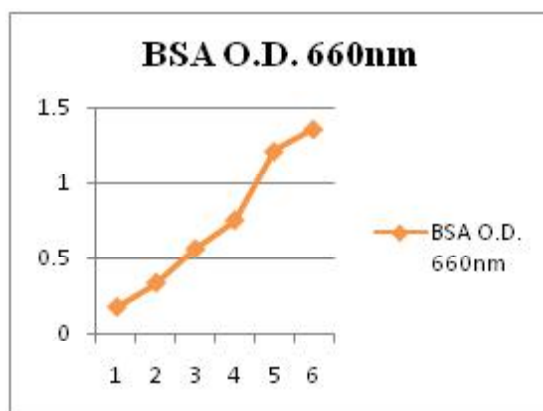
The Lane 1, 2, 3, 4, 5, 6 under UV-Trans-Illuminator with the help of Agarose Gel-Electrophoresis) (Ratio 260/280) was upto the level in all samples. All the samples showed same result in terms of DNA purity ratio but concentration of DNA was varying.

#### Observations of DNA after purification

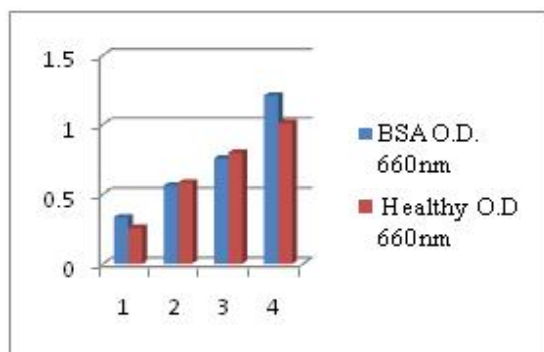
The similar observations were made during quantification by nanodrop spectrophotometer as before purification. All the samples showed same results in terms of DNA purity ratio.

#### Isolation of DNA from plant through phenol chloroform method

Lanes 1, 2, 3 show the bands of infected leaves and Lanes 4, 5, 6 show bands of healthy



**Fig.4 : Protein Estimation from BSA by Lowry's Method**

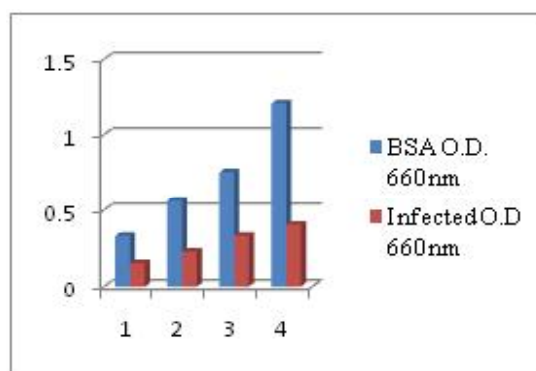


**Fig. 5 : Protein Estimation of healthy chilli plant by Lowry's Method**

leaves. The amount of DNA was more in healthy leaves and less in infected leaves in isolation of DNA, from healthy and infected leaves by CTAB methods, because the begomovirus damaged the DNA of infected leaves. On the other hand, phenol-chloroform gave less quantity of DNA in comparison to CTAB method. So, CTAB is better method for DNA isolation.

#### Estimation of protein by Lowry's Method

The different amount of protein was estimated<sup>6</sup>, Liquid nitrogen and BSA in healthy and infected leaves under different concentration of



**Fig. 6 : Protein estimation of infected leaves by Lowry's Method**

solutions.

#### Isolation and Estimation of Plant Protein

The healthy leaves show high protein quantity than the infected leaves. The amount was less because of begomovirus, but from liquid nitrogen methods, some extra bands were found.

The enzymes used in restricted digestion did not cut the DNA of healthy chilli leaves. From the present study, it is concluded that begomo plant virus used chilli plant cells as host machinery and infected the plant genetic material and proteins.

### References

1. BORAH, B.K. AND DASGUPTA. (2012) Begomovirus Research in India, A critical appraisal and the way ahead, *J. Biosc.* **37**:791-806.
2. CHATTOPADHYAY, B., SINGH, A.K., YADAV, T., FAUQUET, C.M., SARIN, N.B. AND CHAKRABORTY, S. (2008) Infectivity of the cloned components of a begomovirus: DNA beta complex causing chilli leaf curl disease in India. *Arch. Virol.* **153**: 533-539.
3. DOYLE, J.J. AND DOYLE, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* **19**:11-15.
4. DOYLE, J.J. AND DOYLE, J.L. (1990) Isolation of plant DNA from fresh tissue. *Focus*, **12**:13-15.
5. KHAN, M.S., RAJ, S.K. AND SINGH, R. (2006) First report of Tomato leaf curl New Delhi virus infecting chilli in India. *Plant Pathology* **55**: 289.
6. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A.L. AND RANDALL, R. J. (1951) Protein measurement with the Folin-Phenol reagents. *J. Biol. Chem.* **193**: 265-275.
7. MISHRA, M.D., RAYCHAUDHARI, S. D. AND JHA, A. (1963) Virus causing leaf curl of chilli (*Capsicum annuum*) *Indian J. Microbiology* **3**: 73-76.
8. RAJ, S.K., SNEHI, S.K., KUMAR, S., KHAN, M.S. AND PATHRE, U. (2008) First molecular Identification of Begomovirus in India. *Australas Plant Dis. Notes* **3**:69-72.
9. SENANAYAKE, D.M.J.B., MANDAL, B., LODHA, S. AND VARMA, A. (2006) First report of Chilli leaf curl virus affecting chilli in India. *New Dis. Rep.* **13**: 27.
10. SHIH, S.L., TSAI, W.S., GREEN, S.K. AND SINGH, D. (2006) First report of Tomato leaf curl Joydebpur virus infecting chilli in India. *New Dis. Rep.* **14**:17.