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**R**ESEARCH **P**APER

# Molecular identification of begomovirus causing leaf curl disease in potato plant through PCR

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Diseases caused by geminiviruses have long been recognized as a limitation to the cultivation of several important crops, including maize, cassava; bean, squash, potato and tomato, in tropical and subtropical regions of the world. More recently, geminivirus diseases, particularly those transmitted by whiteflies, have become an even greater threat to agriculture due to the appearance of a new and more aggressive whitefly biotype. This has renewed interest in the study of geminivirus pathogenesis and epidemiology and has stimulated work on the development of virus-resistant crop plants. Recognition of disease symptoms in field samples was done and total genomic DNA was isolated from the diseased and healthy samples. The viral genome was amplified using specific two sets of primers CP and ROJAS, was checked by agarose gel electrophoresis resulted in no amplification in case of CP and 1.2kb DNA fragments with ROJAS primer gave the confirmation of presence of DNA-A. The evidence for the presence of DNA-A was obtained from PCR amplification.

**Key words :** Geminiviruses, PCR, Leaf curl, Begomovirus

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## INTRODUCTION

The potato is one of the most important cultivated members of the family Solanaceae, along with the tomato, the eggplant, tobacco and petunia. These are the staple food for many people and they originated in the Andes Mountains of South America. Plant viruses are obligate intracellular parasites that do not have the molecular machinery to replicate without a host. These are pathogenic to higher plants (Zaitlin and Palukaitis , 2000). Plants have elaborate and effective defense mechanisms against viruses. One of the most effective is the presence of so-called resistance (R) genes (Kneller, 2006). Plant viruses are divided into more than 15 families, of which Geminiviridae constitutes the second largest family, which consists of maximum number of viruses (Fauquet *et al*., 2008). General symptoms of diseases caused by Gemini virus are curling of leaves, yellowing of veins, yellow mosaic patterns, dwarfing of leaves. These viruses are responsible for a considerable amount of crop damage worldwide and their spread has increased because of a number of factors, such as the recombination of different Gemini viruses in coinfection which enables virulent viruses to be developed, expansion of agriculture into new growing areas, transport of infected plant material to new locations and the expansion and migration of vectors that can spread the virus from one plant to another (Gray and Banerjee, 1999). Viruses belonging to Gemini viridae family are plant viruses which are obligate intracellular parasites, having no self machinery to replicate themselves (Stanley *et al.*, 2005). A begomovirus had been reported

to be associated with potato plants showing apical leaf curl symptoms in southern and northern India, using DAS-ELISA and nucleic acid hybridization technique. The causal virus had been named as potato apical leaf curl virus (Venkatasalam *et al.*, 2005). Begomovirus causes an estimated yield loss of 50–60 per cent in potato crop. The present study was concentrated on the viral disease of potato *i.e*. solanum apical leaf curl disease (SALCD) which is caused by the solanum apical leaf curl virus (SALCV) as this virus is most emerging virus complex affecting the productivity of potato crop on a large scale.

## RESEARCH METHODOLOGY

**Sample collection :**

Healthy and infected potato leaf samples were collected from the fields of Asthi region of Lucknow. The collected samples were washed and stored at -80°C for the isolation of DNA and protein.

**Isolation of DNA from potato leaves and their qualitative and quantitative analysis :**

DNA isolation from virus-infected potato leaves was done by (Sharma *et al.*, 2008).The Nanodrop ND-1000 is a full-spectrum spectrophotometer (UV and visible spectrum, 220-750nm) and was designated for measuring absorbance of DNA, RNA, proteins and microarray labeling dyes. The ratio of absorbance at 260nm and 280nm was used to assess the purity of DNA and RNA (Warshaw and Tinoco, 1966). The 260/230 ratio was used as a secondary measure of nucleic acid purity.

**Agarose gel electrophoresis of PCR products amplified by CP and ROJAS primers :**

Agarose gel electrophoresis was performed by (Wolf *et al.*, 2002) which were used to confirm the presence of viral genome in potato samples.

**Protein estimation :**

Protein was estimated by (Bradford, 1976) and standardized by Lowry *et al.* (1951).

## RESEARCH FINDINGS AND ANALYSIS

The survey was conducted to observe the natural occurrence of Leaf curl disease in *Solanum tuberosum* in several regions of Lucknow. Healthy and infected samples of *Solanum tuberosum* were collected in which infected showed the symptoms of leaf curl disease-upward curling, puckering and reduced size of leaves. Severely affected plants were stunted. DNA isolation from healthy and infected leaf samples was done by the method mentioned earlier. After the DNA isolation, quality and quantity of DNA was checked.

**Quantitative analysis through nanodrop spectrophotometer :**

Nanodrop spectrophotometer was used to determine the quantity of DNA in the given potato samples. The results obtained were as shown in Table 1 and Fig 1.

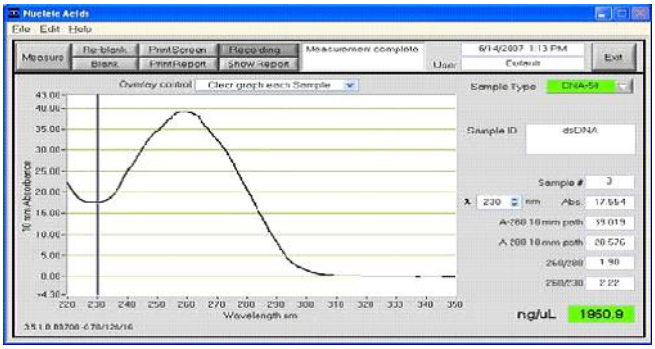


**Fig. 1 : Ideal graph showed by nanodrop spectrophotometer for pure DNA**

**Qualitative analysis of potato DNA samples by using agarose gel electrophoresis of the samples :**

Agarose gel electrophoresis was done to analyse

**Table 1: Quantification of DNA**

| Sample I.D. | Absorbance (A230nm) | Absorbance (A260nm) | Absorbance (A280nm) | 260/280 | 260/230 | Conc. (ng/µL) |
|---|---|---|---|---|---|---|
| Healthy potato | 44.306 | 74.009 | 41.265 | 1.79 | 1.67 | 3700.4 |
| Healthy potato | 32.196 | 59.582 | 31.864 | 1.87 | 1.86 | 2979.1 |
| Healthy potato | 36.832 | 70.525 | 37.949 | 1.86 | 1.91 | 3526.3 |
| Infected potato | 36.973 | 69.729 | 37.781 | 1.85 | 1.89 | 3486.4 |
| Infected potato | 40.743 | 65.625 | 36.861 | 1.78 | 1.62 | 3281.3 |
| Infected potato | 40.926 | 69.566 | 38.999 | 1.78 | 1.70 | 3478.3 |

the quality and quantity of the DNA of healthy and infected potato samples.The results are shown in Fig.2 and Table 2.

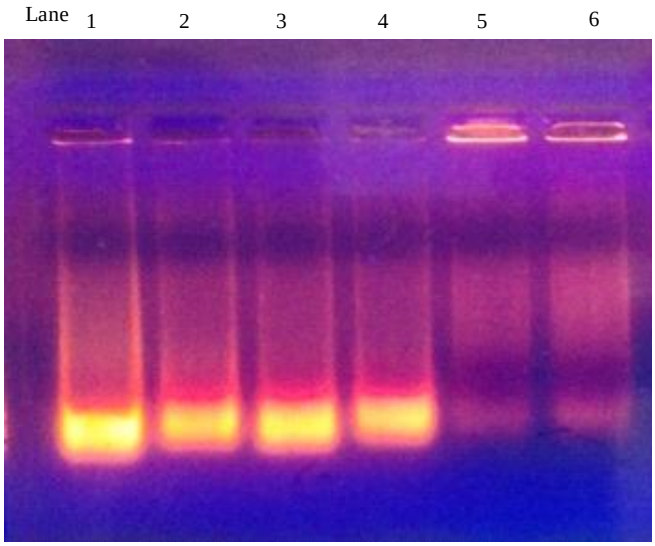


**Fig. 2 : Visualization of DNA bands through agarose gel electrophoresis**

## Agarose gel electrophoresis of PCR products amplified by CP and ROJAS primers :

PCR was done to amplify the viral genome sequence by using CP and ROJAS primer, which were used to confirm the presence of viral genome in potato samples. Results with CP primer are shown below in Fig. 3 and Table 3 in which no amplification of DNA was obtained and the results with ROJAS primer are shown in Fig. 4 and 5 and Table 4 in which amplification of DNA was obtained (Table 6).

## CP primer results : Lane

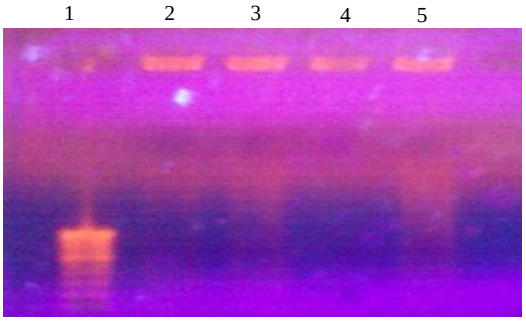


**Fig. 3 : Amplification of DNA through PCR by using CP primer**

**Table 2 : Results of agarose gel electrophoresis**

| Sr. No. | Well no. | Samples | Observation | Results |
|---|---|---|---|---|
| 1. | 1 | Healthy | High intensity bands with low smearing | Very good quality of DNA |
| 2. | 2 | Infected | High intensity band with smearing | Good quality of DNA |
| 3. | 3 | Healthy | High intensity bands with low smearing | Good quality of DNA |
| 4. | 4 | Infected | High intensity bands with low smearing | Very good quality of DNA |
| 5. | 5 | Healthy | Bands with poor intensity and high smearing | Good quality of DNA |
| 6. | 6 | Infected | Bands with poor intensity and high smearing | Good quality of DNA |

**Table 3: Results of PCR with CP primer**

| Sr.No. | Well no. | Observation |
|---|---|---|
| 1. | 1 | 10 bands from 500 to 5000bp, but low intensity |
| 2. | 2 | No amplification |
| 3. | 3 | No amplification |
| 4. | 4 | No amplification |
| 5. | 5 | No amplification |
| 6. | 6 | No amplification |

**Table 4: PCR with ROJAS primer**

| Sr.No. | Well no. | Observations | Results |
|---|---|---|---|
| 1. | 1 | 10 bands from 500 to 5000bp | Clear |
| 2. | 2 | 1 band of about 569bp, with less smearing | Infection confirmed |
| 3. | 3 | 1 band of about 564bp, with light intensity, no smearing | Infection confirmed |
| 4. | 4 | 10 bands from 500 to 5000bp | DNA marker clear |
| 5. | 5 | 1 band of about 561bp, with no smearing | Infection confirmed |
| 6. | 6 | 1 band of about 1200bp but blurred | May be infection |

**ROJAS primer :** Lane

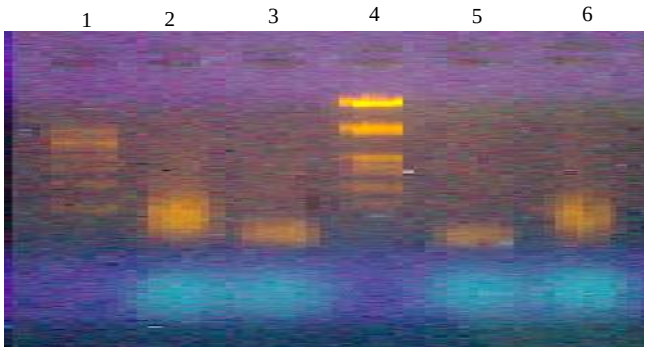


**Fig. 4 : Visualization of amplified DNA bands with ROJAS primer**

| Table 5: Final results of PCR amplification | | |
|---|---|---|
| Sample | Primers | |
| Potato samples | CP | ROJAS |
| ( Asthi) | -ve | +ve |

-ve: No amplification, +ve: Positive amplification

## Protein estimation through Lowry's method :

Lowry's method was used to measure the concentration of protein in a sample. The different concentration of different reagents were mixed in 6 test tubes as shown below in Table 6.

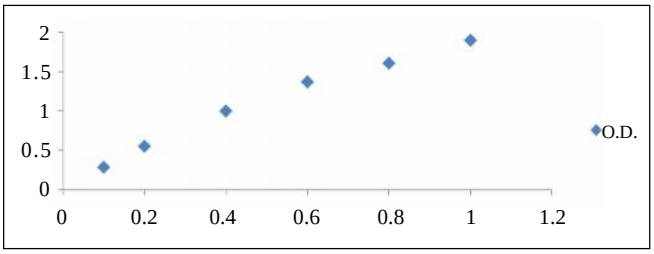


**Fig. 5 : Calibration curve for protein estimation**

O.D. of 0.2ml leaf lysate was found 0.8976, amount of protein in 0.2ml leaf lysate was 0.3600 µg and amount of protein in 2ml leaf lysate was 3.6 µg. protein in 2ml leaf lysate was the amount present in 100mg leaf sample. Thus, the conc. of protein in potato sample was 3.6 µg/ 100mg fresh weight.

Potato (*Solanum tuberosum* L.) is among the world's most popular vegetable belonging to family Solanaceae. But during past few years, viruses have been found as the most devastating disease causing agents of potato plants, causing serious losses. The epidemiology of whitefly-transmitted geminiviruses is characterized by a close correlation between disease incidence and whitefly populations which often show strong seasonal fluctuations. During a study done on the epidemiology of this disease in Sri Lanka marked fluctuations in the rate of spread of the disease was found during wet season as compared to the dry season. It was suggested that the slow spread of disease was due to reduced mobility of vector during wet season. During another study it was shown that, certain aspects of variation between B. tabaci 'types' (previously, races, strains, biotypes) may directly influence vector-mediated dispersal of geminiviruses, thereby, potentially influencing disease epidemiology, and consequently the evolution of begomoviruses (Oliveira *et al.*, 2001).

Begomoviruses are transmitted in a persistent, circulative manner by *B. tabaci* and the viral nucleocapsid is thought to constitute the sole viral determinant of transmission. However, evidence suggests that several whitefly proteins may interact with viral capsid (virions), collectively, constituting 'the transmission pathway' that confers virus-vector specificity. Elucidating the cellular and molecular interactions and understanding the precise basis for the specificity underlying whitefly-mediated begomovirus transmission will enhance the fundamental understanding of complex interactions occurring between organisms from three distinct kingdoms and further, may lead disease control by targeting key of sites of specificity in the pathway to disrupt virus transmission (Skaljac *et al.*, 2010).

So far, the importance of whitefly-transmitted geminiviruses causing leaf curl and yellowing diseases of potato has not been established since their symptoms

| Table 6: Reaction mix for Lowry's method | | | | | | |
|---|---|---|---|---|---|---|
| Sr.No. | Vol. of stock solution (BSA) (ml) | Amount of BSA (mg) | Water (ml) | ALK. $CuSO_4$ (ml) | Lowry's reagent (ml) | OD (µg/µl) |
| 1. | 0.1 | 0.1 | 0.9 | 5.0 | 0.5 | 0.2831 |
| 2. | 0.2 | 0.2 | 0.8 | 5.0 | 0.5 | 0.5507 |
| 3. | 0.4 | 0.4 | 0.6 | 5.0 | 0.5 | 0.9971 |
| 4. | 0.6 | 0.6 | 0.4 | 5.0 | 0.5 | 1.3667 |
| 5. | 0.8 | 0.8 | 0.2 | 5.0 | 0.5 | 1.6039 |
| 6. | 1 | 1 | 0 | 5.0 | 0.5 | 1.8957 |
| 7. | - | - | 1 | 5.0 | 0.5 | - |

are easily confused with similar symptoms caused by other viruses, insects, or mites. The prevalence and distribution of these viruses and their vector in subtropical, tropical and fringe temperate regions has escalated in recent years for reasons not yet understood. Changing agricultural practices (most notably the continuous cultivation of crops such as potato, cotton, beans, soybean, tomato, pepper and melon which are susceptible to the viruses and are attractive hosts for the whiteflies) certainly account for some of the increase in the severity and the vast spread of diseases caused by geminiviruses (Belaong and Bajet, 2007). This has led to an increased awareness throughout the world of the importance of these diseases and has spurred intensive research on the viruses involved.

Data embodied in this project provides knowledge on the molecular detection of a begomovirus strain causing leaf curling, yellowing and stunting symptoms in potato leaves. The data will help in understanding the phylogenetic relationship of begomovirus strain under study with other strains reported all over the world and assigning their correct taxonomic position. Data are also expected to complement to the already available information on Begomovirus at molecular level.

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