

## RESEARCH ARTICLE

# Whitefly transmitted *Begomovirus* in China rose: A threat to the beauty

■ DNYANESHWAR M. PAWAR\* AND LALIT MAHATMA

Department of Plant Pathology, N.M. College of Agriculture, Navsari Agricultural University, NAVSARI (GUJARAT) INDIA

## ARTICLE INFO

**Received** : 06.07.2013  
**Revised** : 29.08.2013  
**Accepted** : 06.09.2013

## Key Words :

China rose, Begomovirus, PCR

## ABSTRACT

China rose (*Hibiscus rosa-sinensis*) is an important perennial shrub growing almost in entire country for its beautiful flowers and foliage. Hardly there would any garden which will not have the shrub. The shrub is also grown in the house, road side, hotels, etc. Apart from the aesthetic value, the flowers are also used for the worship of lord Ganesha in Hindu mythology. The plant is generally hardy and resistant to various biotic and abiotic stresses. However, a viral disease causing vein thickening, vein banding and leaf curling has been observed from the several places. Affected plants remain stunted and bear few flowers of less significance. The disease is such a prominent that around 90 per cent plant in and around the campus of Navsari Agricultural University was found affected with the virus. DNA of the infected plant was isolated by the modified CTAB method and genome of the virus was subjected to amplification. Study revealed the presence of DNA-A molecule of begomovirus. An approximately 500 to 700 bp DNA-A molecule was amplified by the different degenerate begomovirus primers amplifying the different segments of the DNA-A molecule of the virus. The disease is transmitted by the whitefly.

**How to view point the article** : M. Pawar, Dnyaneshwar and Mahatma, Lalit (2013). Whitefly transmitted *Begomovirus* in China rose: A threat to the beauty. *Internat. J. Plant Protec.*, 6(2) : 379-381.

\*Corresponding author:

Email: mavli111pawar@gmail.com

## INTRODUCTION

China rose (*Hibiscus rosa sinensis* Linn.) belonging to the family Malvaceae is an important perennial shrub growing almost in entire country for its beautiful flowers and foliage. Commonly grown in the garden, house, road side, hotels, etc. Apart from the aesthetic value, flowers are also used in the worship of lord Ganesha in Hindu mythology. The plant is generally hardy and resistant to various biotic and abiotic stresses. However, a viral disease causing vein thickening, vein banding and leaf curling has been observed from the several places. The disease is such a prominent that around 90 per cent plant in and around the campus of Navsari Agricultural University was found affected with the virus.

## MATERIAL AND METHODS

Survey was carried out in and around Navsari

Agricultural University campus. Only how many plants were found infected by viral disease and symptoms were recorded from naturally infected plants. The samples of diseased plants were collected for further study in the laboratory.

### Standardization of PCR based diagnostic protocol :

#### Isolation of viral DNA :

Viral DNA was isolated by the CTAB method given by (Doyle and Doyle, 1987) with slight modification. Different buffers and steps followed for the isolation of viral DNA from the different tissues of the plants were as followed.

### Procedure (CTAB method and modifications) :

Collect the 500 mg infected leaf tissues and crush in liquid nitrogen (N<sub>2</sub>) with the help of pastel and mortar. Crushed powder collect in eppendorf tube and added 700µl CTAB extraction buffer. Keep the eppendorf tube at 60-65°C for one

DNA extraction buffer		
Stocks	Final concentration	Quantity for buffer (10 ml)
CTAB (Cetyl trimethyl ammonium bromide)	2%	200 mg
Tris buffer stock, 1M (pH 8.0)	100 mM	1 ml
EDTA stock 0.5M (pH 8.0)	20 mM	400 µl
PVP (Polyvinyl pyrrolidone)	2 %	200 mg
2-Mercapto ethanol	1 %	100 µl
Sterile millipore water		Volume made up to 10 ml

hour in water bath. After one hour add 700 µl Chloroform: Isoamyl Alcohol (1:24) and vortex 5000 rpm for 15 min in centrifuged machine (Eltek Equipment (Bombay) Pvt. Ltd.). Later take supernatant (approx 450 µl) and same process one more time. After then take supernatant and add 700 µl Isopropyl Alcohol (Shake it vigorously) and incubate the tubes at -20°C for 2 hours. After the mixture spin at 5000 rpm for 20 min then discard supernatant and add 70% ethyl alcohol and spin at 10000 rpm for 10 min. Discard supernatant and repeat same process one more time. Then collect the pellet and dry it at room temperature. Add 50 µl TE buffer and incubate over night at 4°C. Measure the quantity of DNA with the help of spectrophotometer (Nanodrop) and test DNA quality on documented the gel by GelDoc (SYNGENE, UK).

#### Primers :

The GBv74F and GBv74R pairs of specific primers for the amplification of the DNA molecule of Begomovirus were given by (Chakraborty *et al.*, 2003).

#### Polymerase chain reaction (PCR) :

Amplification was performed in a 20 µl cocktail containing forward and reverse 2.0 µl of (10 pmoles/ml) of each primer, 2.0 µl of genomic DNA, 0.6 µl of dNTPs (mixture of dATP, dGTP, dCTP and dTTP), approximately 0.6 µl of Taq polymerase (Bangalore Genei, India), 2.0 µl MgCl<sub>2</sub>, 2.5 µl of Taq Buffer (10x) and 13.3 µl of sterilized millipore water. The reaction mixture was overlaid with one drop of mineral oil, initially denatured for 2 min at 95°C, and, then, subjected to 35 cycles of 0.45 min denaturation at 94°C, 1 min annealing at 52°C, 1 min extension at 72°C; and a final extension for 5 min at 72°C uses the programme of Thermocycler.

#### TBE buffer (1 lit 5x stock solution) :

Tris base	:	53.0 g
Boric acid	:	27.5 g
EDTA	:	20 ml

#### Running of the DNA on agarose gel (Electrophoresis) :

Take the 8 g agarose and dissolve in 100 ml sterilized millipore water to prepare 0.8 gel. Melt the agarose gel in microwave oven, before pouring in the tray. Add 7 µl Ethidium bromide in the lukewarm agarose gel and pour the melted

agarose gel in the gel casting tray to make approximately 5 mm thick gel after gel tray undisturbed to uniform solidification of the gel. Then, keep the tray in the electrophoresis tank filled with TB running buffer. Gently remove the comb and fill the wells with the marker and DNA/PCR product. Connect the electrophoresis unit with the power Pac and run the unit at 80 mV (max = 100 mV) for 10-15 minutes or the tracking dye reaches to the end.

## RESULTS AND DISCUSSION

The results obtained from the present investigation are presented below :

#### Natural occurrence :

During the surveys of China rose in NAU, Navsari campus, around 450 plants out of 500 plants (90 per cent) showed typical symptoms of *Begomovirus*. Disease was seen in almost all red and orange colour flowers of the varieties/type of plants but any symptoms was not on the white flowers varieties/type of China rose.

#### Symptomatology :

In the beginning affected plant showed mild vein thickening, curling of leaves (Fig. 1), leatheryness and vein banding (Fig. 2). Gradually symptoms severity increases, inter



Fig. 1 : Vein thickening and leaf curling china rose

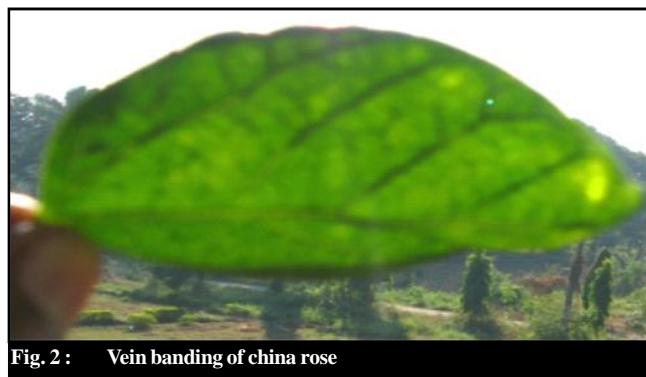


Fig. 2 : Vein banding of china rose

nodal size of the plant reduces giving bushy appearance. The symptoms were seen on calyx also. Corolla of the infected plant whither early. Infected plant bears few flowers of less significance.

#### Standardization of PCR based diagnostic protocol for the identification of causal organism and it's variant :

##### Isolation of viral DNA :

Viral DNA could be isolated from infected leaf part of the plant by CTAB method given by (Doyle and Doyle, 1987) with slight modification as given in "materials and methods." Quantity and quality of the DNA isolated was of good from the leaf tissue. This including the method given by Dellaporta *et al.* (1983) is commonly used method for the isolation of viral DNA. Similar methods have been used by Usharani *et al.* (2004) for the mungbean plants infected with MYMV.

##### Amplification of genome :

The primer pairs used in the present investigation could amplify the DNA-B molecule of the virus as per their specification. Primer pair GBv74 F + GBv74 R showed ~ 700 bp band amplification of DNA-B molecule. (Fig. 3) Since the sharpness and size of the band obtained from GBv74 was best and replicative. This primer pairs have been used by different scientists (Chakraborty *et al.*, 2003 and Hussain *et al.*, 2004) for the amplification of viral DNA. The primer pair

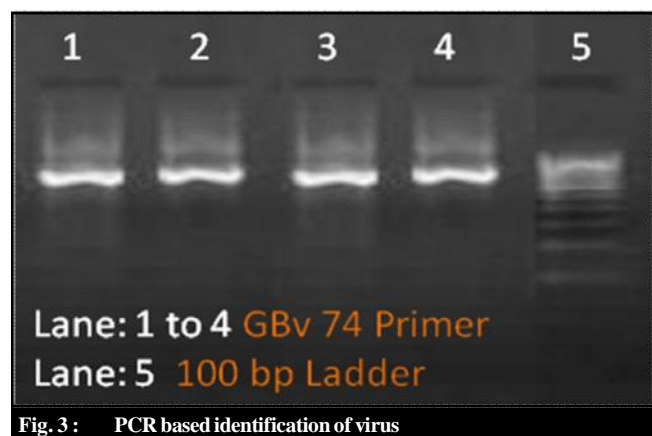


Fig. 3 : PCR based identification of virus

designed during the present investigation was designed by using primer-3 software of the NCBI. This is most versatile software and being used for the designing of primer for many molecular experiments. Apart from the China rose and many crop of the Malvaceae family are being cultivated in and around NAU campus. Virus may mutate and expand host range threatening many cultivated species.

#### Conclusion :

China rose (*Hibiscus rosa-sinensis*) is an important perennial shrub suffering from *begomovirus*. In survey was found the severe incidence of *begomovirus* on China rose about 90 per cent. The disease appeared as vein thickening, curling of leaves, leatheryness and vein banding. The PCR based diagnostic, protocol was standardized and the viral DNA could be isolated by the CTAB method given by (Doyle and Doyle, 1987) with slight modification from all the tissues. To amplify viral DNA, showed ~ 700 bp band amplification of DNA-B molecule. Since the sharpness and size of the band obtained from GBv74 was best and replicative.

#### REFERENCES

- Chakraborty, S., Pandey, P.K., Banerjee, M.K., Kaloo, G. and Fauquet, C.M. (2003). *Tomato leaf curl Gujarat virus*, a new *Begomovirus* species causing a severe leaf curls disease of tomato in Varanasi. *Indian Phytopath.*, **93** (12) : 1485-1495.
- Dellaporta, S.L., Wood, J. and Hicks, J.B. (1983). A plant DNA mini-preparation version II, *Plant Molecular Biological Report*, **1** (4) : 19-21.
- 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.
- Hussain, M., Qazi, J., Mansoor, S., Iram, S., Bashir, M. and Zafar, Y. (2004). First report of Mungbean yellow mosaic India virus (MYMIV) on mungbean in Pakistan. *Plant Pathol.*, **53** : 518.
- Usharani, K.S., Surendranath, B., Haq, Q.M.R. and Malathi, V.G. (2004). *Yellow mosaic virus* (YMV) infecting soybean in northern India is distinct from the species infecting soybean in southern and western India. *Curr. Sci.*, **86** (6) : 845-850.

6<sup>th</sup>  
Year  
★★★★★ of Excellence ★★★★★