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Antigenic relationships of citrus yellow mosaic virus by immunological methods

Susmila Aparna Gaddam[1,2*] Venkata Subbaiah Kotakadi[2,3], M. N. Reddy[1] and D. V. R. Saigopal[2,3]

[1]*Department of Applied Microbiology, Sri Padmavathi Mahila Viswavidyalayam, Tirupati-517502 (A.P) India*
[2]*Department of Virology, S. V. University, Tirupati-517502 (A.P) India*
[3]*DST PURSE Centre, S. V. University, Tirupati-517502 (A.P) India*

ABSTRACT

Citrus yellow mosaic virus infecting citrus species like rangapur lime, acid lime and sweet orange was detected by different immunoassays like Agarose double diffusion test (AGDDT) and Dot Blot ELISA by using homologous polyclonal antiserum. The Citrus yellow mosaic virus infecting rangapur lime, acid lime and sweet orange was reacted positively with homologous antiserum raised against rangapur strain in AGDDT and Dot Blot ELISA. In agarose double diffusion test a precipitin line was observed with homologous antiserum and with purified virus. Positive reaction was also observed with homologous antiserum and purified virus samples of rangpur lime, sweet orange and acid lime and partially purified samples of Canna indica, maize and sorghum. The CYMV infected rangpur lime, sweet orange, acid lime, Canna indica, maize and sorghum crude leaf samples reacted positively with homologous antiserum in Dot-blot ELISA.

Keyword: Citrus yellow mosaic virus, polyclonal antibodies, AGDDT, Dot Blot Immunoassay and Antigenic relationships

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INTRODUCTION

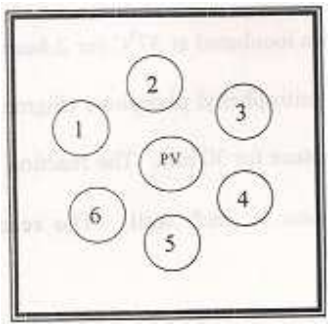
During the last two decades, a number of virus and virus - like diseases have been recorded from citrus trees in India [18]. Amongst these diseases "Citrus mosaic" caused by citrus yellow mosaic virus (CYMV), a badna virus is widely distributed in India [16, 17, 4, 5], which affects some of the important species grown in India. Citrus is considered to be one of the most remunerative fruit crops of India, having a lasting niche in the international trade and world finance. Citrus crop has significant importance in fruit economy of the country and as the second largest industry in India with respect to area and third largest with respect to production, although India ranks sixth among top citrus producing countries of the world. Rangpur lime (*Citrus limonia* (L.) Osb.) is one of the most commonly employed root stocks in India. The root stock situation in India has been reviewed adequately [6, 9, 1, 20, 11]. The overall root stock scenario in the country reveals a replacement of rough lemon with rangpur lime in some States. The virus has been named as Citrus yellow mosaic virus and considered as member of badnavirus group. Recently Huang and Hartung [12] have cloned and sequenced the citrus yellow mosaic virus (CYMV). Badnaviruses were only moderately immunogenic repeated immunization of rabbit yields antisera with homologous titre of 1:128 - 1:512 in immunodiffusion tests. Chloroplast agglutination with crude sap of mosaic infected leaves and its antiserum, shows clear agglutination indicating antigenic nature [16, 17]. The agar gel double diffusion tests clearly indicated the antigenic relationship between citrus mosaic and its own antiserum and established that the mosaic virus not related to tristeza serologically [17]. EIA and IEM indicate that there is a serological relationship between BSV and SCBV and between CSSV, SRSV and DBV. This represents the limit of serological cross - reactivity so

far demonstrated within the group. Distinct hybridization groups and serogroups exist within BSV-SCBV cluster [13]. ELISA [10] has been routinely used for detection using homologous antiserum. Dot-blot ELISA has also been adopted to allow the detection of CYMV in field infected trees. This method offers a simple, reliable procedure to test a large number of samples under laboratories, whereas inexpensive and simple test is needed. In addition, the use of immuno electron microscopy (IEM) has been proved to be successful for the detection of serologically related badnavirus [18, 23, 24]. However, IEM suffers Diagnosis of badnaviruses is often unreliable. The symptoms caused by badnavirus infection are variable, transmission to indicator plants is difficult and most badnaviruses have a narrow host range [14]. An antiserum produced from purified CSSV has been used in ELISA and IEM to allow detection and differentiation of CSSV strains [21].Not only viruses bioassays are useful in detection of toxin in drinking water[22].

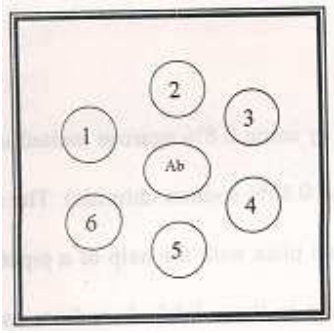
MATERIALS AND METHODS

Agarose Double Diffusion Test

Agarose double diffusion test was performed as described by Purcifull and Batchelor [19].The agarose gel was prepared by using 0.8% agarose melted in PBS (0.01 M potassium phosphate buffer pH 7.0 and 0.85 sodium chloride). The molten agarose at 50°C was poured into 5 x 5 cm glass plate with the help of a pipette and allowed to solidify. The wells (4 mm) were cut in the solidified medium using a template with a cork borer - 6 pheripheral wells at a distance of 3 mm from the edge of the central well. The agarose plugs were taken out with the help of a needle. The bottom of the wells was sealed with molten agarose to prevent seepage of the samples. To determine the antiserum relationship with purified virus, the central well was filled with 20 µl of purified virus and the peripheral wells with different bleeds of antiserum (1/ 1000, 1 / 2000, 1 /3000, 1/4000, 1/5000, 1/6000) as shown in Programme sheet - 1. The plates were incubated for 36 hrs in a moist chamber kept at room temperature.



(Programme sheet - 1)



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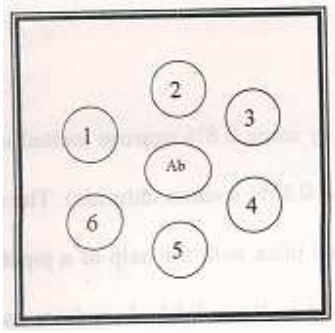

(Programme sheet - 3)

To determine the antigenic relationship, the central well was filled with 20 µl of antiserum and peripheral wells with healthy and purified mosaic infected samples of rangpur lime, sweet orange and acidlime (Programme sheet - 2); healthy and partially purified mosaic infected samples of sorghum, maize and *Canna indica* samples (Programme sheet - 3). The plates were incubated for 36 hrs in a moist chamber kept at room temperature.

Dot-blot ELISA

Dot immunobinding assay (DIBA) or Dot-blot ELISA was performed as described by Banttari and Goodwin [8]. Healthy and mosaic infected (citrus and non-citrus) leaf samples were ground in the carbonate buffer separately by using mortar and pestle. 10µl of each sample was taken and spotted on to nitrocellulose membrane by using micro syringe according to the programme sheet- 4. Then the membrane was air dried and incubated in block solution for 2 hours at room temperature. After blocking, the nitrocellulose membrane was incubated in 1:1000 dilution of homologous antiserum in antibody buffer. The membrane was washed thrice with TBS-T (each time 5 min.) and incubated for 1 ½ hour in 1:5000 dilution of horse radish peroxidase labelled goat antirabbit antibodies in antibody buffer. Then the membrane was washed thrice with TBS-T and transferred to substrate solution. Colour development was recorded visually and stopped the reaction by washing the membrane in distilled water. The results were recorded by visual observation. Dots with healthy samples were used as negative. To determine the antigenic relationship of CYMV infected rangpur lime, sweet orange, and acid lime, was performed by AGDDT and Dot-blot ELISA using polyclonal antibodies against rangpur lime.

RESULTS

Antigenic relationships by AGDDT and Dot Blot ELISA

After four successive injections of the purified virus, the test bleed was done by cutting the vein of a ear of the rabbit and collected the serum. Antiserum was collected at weekly intervals and the titre detected by performing DAC-ELISA. The positive reaction upto 1 / 5000 dilution was observed both in fourth bleed and first bleeds antisera. The reaction was observed strong with purified virus as compared with partially purified virus and also antiserum raised against CYMV infecting sweet orange. No colour was observed in buffer control.

In agarose double diffusion test a precipitin line was observed with homologous antiserum and with purified virus (Fig. 1). Positive reaction was also observed with homologous antiserum and purified virus samples of rangpur lime, sweet orange and acid lime (Fig. 2) and partially purified samples of *Canna indica*, maize and sorghum (Fig. 3).

Fig.1

Fig. Agar gel double diffusion test
Central well : Purified virus
Peripheral wells
1. First bleed antiserum
2. Second bleed antiserum
3. Third bleed antiserum
4. Fourth bleed antiserum
5. Fifth bleed antiserum
6. Sixth bleed antiserum

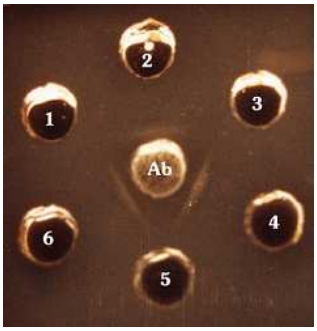

Fig.2

Fig. Agar gel double diffusion test
Central well : antibody
Peripheral wells :
1. Healhty sweet orange
2. Purified virus sweet oranage
3. Healthy Rangapur lime
4. Purified virus Rangapur lime
5. Healthy Acid lime
6. Purified virus Acid lime

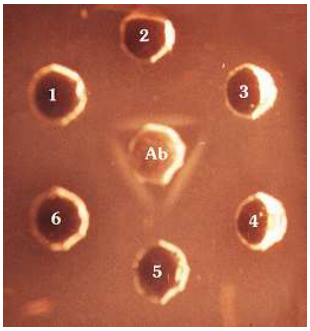

Fig.3

Fig. Agar gel double diffusion test
Central well : antibody
Peripheral wells :
1. Healthy Sorghum
2. Infected Sorghum
3. Healthy Maize
4. Infected Maize
5. Healthy Canna Indica
6. Infected Canna Indica

The CYMV infected rangpur lime, sweet orange, acid lime, *Canna indica*, maize and sorghum crude leaf samples reacted positively with homologous antiserum in Dot-blot ELISA (Fig. 4).

Programme sheet 4

1- Healthy rangpur lime leaf sample
2 - Healthy sweet orange leaf sample
3 - Healthy acid lime leaf sample
4 - Healthy *Canna indica* leaf sample
5 - Healthy maize leaf sample
6 - Healthy sorghum leaf sample
7 - Mosaic infected rangpur lime leaf sample
8 - Mosaic infected sweet orange leaf sample
9 - Mosaic infected acid lime leaf sample
10 - Mosaic infected *Canna indica* leaf sample
11 - Mosaic infected maize leaf sample
12 - Mosaic infected sorghum leaf sample

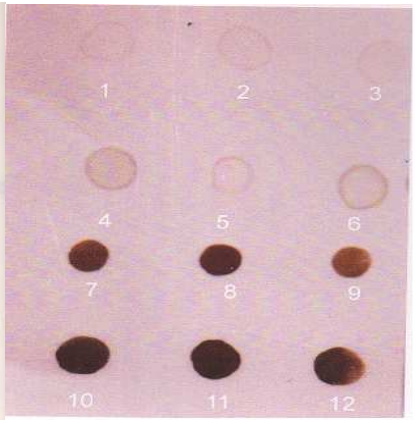

Fig.4 Dot Blot ELISA

DISCUSSION

Earlier, Pant and Ahlawat [18] produced antiserum for citrus mosaic virus. In DAC-ELISA the optimum virus detection was mentioned as antigen dilution of 1:2, antibody dilution of 1:500 and conjugate dilution of 1:2000 [18]. The results of agar gel diffusion tests clearly showed the antigen relationship between mosaic and its own antiserum[16,17]. There is a general lack of cross reactivity between members of the badna virus group and individual badna viruses which may have one or more distinct serotypes. In the present studies, in DAC-ELISA the purified and partially purified viruses reacted positively upto 1/5000 dilution both in fourth bleed and first bleed antiserum produced against purified CYMV infected rangpur lime[7]. In agarose double diffusion test a precipitate line was observed with homologous antiserum and purified virus of three isolates. These results confirmed the serological relationship between citrus yellow mosaic virus and its own antiserum.

The antigenic relationships of CYMV infected rangpur lime, sweet orange and acid lime, *Canna indica*, maize and sorghum samples were studied [7]. These samples reacted with the homologous antiserum raised against rangpur lime in DAC-ELISA, Agrose gel double diffusion test and Dot-blot ELISA. These studies show the presence of antigenic relationship among CYMV infected samples with the homologous antiserum.

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