

A POTYVIRUS ISOLATED FROM COCCINIA GRANDIS(L.)VOIGT IN ALIGARH. INDIA.

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ABSTRACT

Coccinia grandis(L.)Voigt Plants collected from Aligarh showing mosaic and mottling, were naturally infected by a virus of Potyvirus group identified according to particle morphology and size, host range, physio -chemical properties, etc . The virus isolate was identified as a strain of Watermelon Mosaic Virus (WMV-2). The virus induced cytoplasmic inclusions in the form of pinwheels and scrolls and long lamellar aggregates.

Key words: Potyvirus, Watermelon mosaic virus(WMV-2), cytoplasmic inclusions

INTRODUCTION

Coccinia grandis (L.)Voigt being a wild plant of the family Cucurbitaceae has now been grown on large scale in many states of India for its Fruits, roots etc. *C. grandis* has not been reported to be infected from viral disease except for some reports of Bhargava *et al.* (1975) which suggests that *C.grandis* is a ready source of Watermelon mosaic virus-2 (WMV-2). Purciful *et al.*(1989) described a serologically related strain of WMV-2 on *C.grandis* and designate it as Trichosanthes Virus(TV).During the survey in and around Aligarh. *C.grandis* plants were found severely infected with mosaic and mottling symptoms on leaves. The present investigation is concerned with the identification and of strain of WMV-2 from naturally infected *C.grandis* plants.

MATERIALS AND METHODS

HOST RANGE

Infected *C.grandis* leaves were ground in 0.1M Phosphate buffer pH 7.0 and the extract was mechanically inoculated on the following plants of the families:

Acanthaceae (*Ruellia tuberosa* L.),
Amaranthaceae (*Amaranthus caudatus*

L.),Apiaceae(*Daucus carota* L.cv pusa kesar, *Ammi majus* L.,*Apium graveolens* L. cv. White solid, *Petroselinum crispum*(Mill.) Mansf.cv. Imperial curie,Asteraceae(*Centaurea cyanus* L., *Ageratum maxicanum* Sims W.Blue Mink., *Bellia perennis* L.cv Sutton Dwarf White,*Cosmos bipinnatus* Cav.cv. Double Mixed, *Dahlia rosea* Cav.cv. Dwarf mixed, *Lactuca sativa*L. Brassicaceae (*Brassica campestris* L., *C.amaranticolor* Coste & Reyn, *C.murale* L.,*C. quinoa* L.,*Spinacea oleracea* L.cv.pusa Jyoti and Palang sag, *Beta Vulgaris* L., Cucurbitacea (*Cucumis sativus* L., *Cucurbita maxima* L., *Lagenaria vulgaris* Ser., *Benincasa hispida* Savi., *Trichosanthes anguina* L., Lamiaceae (*Salvia occidentalis* Swartz), Solanaceae (*Capsicum annum* L., *Nicotiana debneyi*. *N.glutinosa* L.,*N.occidentalis*, *N.palmeri* Gray., *N. tabacum* L.cv Anand-2 Bidi type, *Bhopali pakra*, Harrison Special and Jayshree.

PURIFICATION

The virus was purified by a method involving the extraction of the virus in 0.1M Phosphate buffer Ph 7.0 in presence of 30% n-butanol and 0.1% thioglycolic acid together with EDTA (0.01Mm) in 6% PEG and 0.1% NaCl. This

was followed by two cycles of differential centrifugation. Further purification of partially purified virus was done by centrifugation in sucrose density gradients as described by Brakke (1969).

ELECTRON MICROSCOPY

Negatively stained preparations were made from purified virus by using 2 % (M/V) aqueous uranyl acetate solution. The average dimensions of the particle were determined from stained preparations.

The detailed schedule of the technique given by Ronald (1978) was followed to study the ultra structure of inclusions induced by the virus and its insitu localization. The treatment was given to young leaves of experimentally infected *N.glutinosa* L. plants.

SEROLOGY

Five weekly injections of 1.5ml of the purified virus preparations were administered intravenously through the marginal ear vein of the rabbit. After one week of the last intravenous injection, one booster dose of 1ml of virus emulsified with equal amount of incomplete Freund,s adjuvant was injected subcutaneously. The rabbit was bled fifteen days after the booster dose and antiserum titer was measured by tube precipitin tests.

Ouchterlony,s double diffusion tests were performed to identify the virus up to strain level. Purified virus (antigen) was deposited in the central well and antisera of zucchini yellow Mosaic (ZYMV). Bean Common Mosaic (BCMV) and

Potato Virus Y (PVY) were deposited in the peripheral wells. Diffusion was allowed overnight.

IMMUNOSORBENT ELECTRON MICROSCOPY (ISEM)

For ISEM, the method described by Derrick (1973) and later modified by Milne and Luisoni (1977) was applied using the antis era of ZYMV, BYMV, Papaya Ring spot Virus (PRSV), PVY and homologous antis era.

RESULTS

HOST RANGE

23 out of 35 plant species or cultivars were found susceptible to WMV-2 strain. In all 25 species back inoculation onto *C. amaranticolor* gave positive results. Most of the plants of Chenopodiaceae and Cucurbitaceae were found locally infected and plants of Solanaceae specially belonging to genera *Nicotiana* produced systemic mosaic.

PURIFICATION

The purified virus preparation gave a single light scattering band in density gradient. The UV-spectrum of the purified virus was typical of nucleoproteins. A260/A280 ratio was 1.1838 and RNA% was 6.02 typical of WMV-2.

ELECTRON MICROSCOPY

The purified virus suspension revealed the presence of flexuous rod –shaped particles exhibiting typical potyvirus structure measuring 760 nm in length and 12nm in width (fig.1). The sub-cellular structure of infected *N.glutinosa* leaves showed the occurrence of cytoplasmic

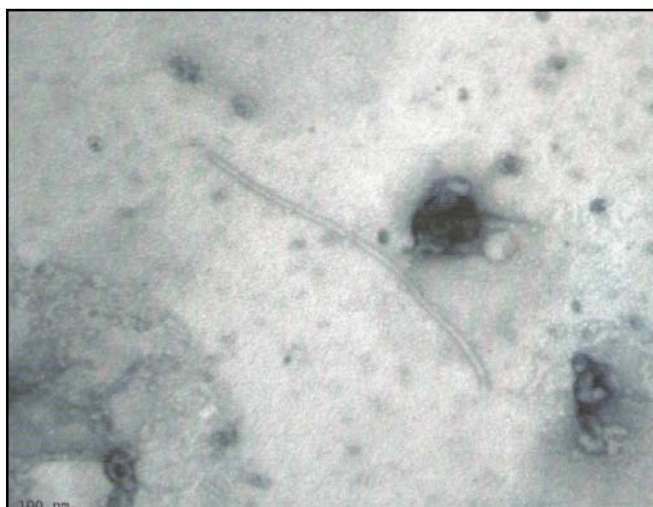


Fig 1.Flexuous rod typical of Potyvirus



Fig 2. Cytoplasmic inclusions



Fig.3. Homologous antiserum with maximum trapping pinwheel inclusions, scrolls and lamella aggregates.(fig.2).

SEROLOGY

As obtained by the precipitin test, the antigen titre was 1:512 and antiserum titre was 1:2048. In double diffusion tests, the present virus reacted strongly with the antiserum of ZYMV, as a single precipitin band was formed with the antiserum of this virus.

IMMUNOSORBENT ELECTRON MICROSCOPY (ISEM)

Homologous antiserum gave maximum trapping (fig.3). Moderate trapping was observed with antiserum of ZYMV but no trapping occurred with the antisera of BYMV, PRSV, and PVY.

DISCUSSION

Physio-chemical properties, Electron microscopy and serological studies showed that Aligarh isolate infecting *C.grandis* was a Potyvirus. The Aligarh isolate shared some of the properties of Watermelon Mosaic Virus-2 (WMV-2) as described in CMI/ABB Description of plant viruses (No.293) by Webb and Scott (1965) and Purcifull and Hiebert(1979).

The Aligarh isolate could not be compared with the strain described by Bhargava *et al.* (1975) due to paucity of information. There is much affinity between the present isolate and the virus strain. WMV-2 designated as Trichosanthes virus (TV) by Purcifull *et al.* (1989) in host range etc., but there comparison remain incomplete in the absence of information regarding particle morphology and Physio-chemical properties of

the virus, as these were not reported by Purcifull *et al.*(1989).

On the basis of properties like flexuous particles measuring c.760×12nm, RNA % of 6.02, induction of cytoplasmic inclusions like pinwheels, scrolls and lamellar aggregates, and of course serological relationship with ZYMV, it is concluded that the virus under investigation is a member of potyvirus group and appears to be a strain of Watermelon mosaic virus-2, naturally infecting *Coccinia grandis*(L.) Voigt.

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