Identification and characterization of a virus isolate from *Coccinia grandis* (L.) Voigt.

**ABSTRACT**

A mosaic disease of *Coccinia grandis* (L.) Voigt, which caused mosaic and mottling followed by leaf distortion and reduction in leaf size with retarded vegetative growth of diseases was investigated and characterized.

The virus under investigation has a restricted host range, infecting some 24 species belonging to 10 families. Most of the hosts are distributed in families-Chenopodiaceae, Cucurbitaceae and Solanaceae. The plants belonging to Chenopodiaceae and Cucurbitaceae generally showed necrotic and chlorotic local lesions, while those of Solanaceae showed varied type of mosaic on the leaves and stunting of the plants.

The virus under investigation was found to be readily transmitted by usual mechanical sap inoculation and by three species of aphids viz. *Aphis crassivora* Koch., *A. gossypii* Glov., and *Myzus persicae* Sulz. The virus could not be transmitted by white flies (*Bemisia*
*taba*ci Genn.), dodder (*Cuscuta reflexa* Roxb.), soil and seeds. However, the disease was transmitted by grafting.

Phosphate buffer (0.1M, pH 7.0) was found to be the most suitable extraction medium for retaining virus infectivity. Other buffers such as acetate, borate, citrate and tris. HCl were found to inhibit the activity of the virus. Addition of sodium sulphite also inhibited the virus infectivity in crude sap.

The Virus in crude sap lost its infectivity at a dilution of $10^{-5}$. It withstood heating up to $55^\circ C$ for 10 min and remained active for 6 days when stored at room temperature ($20 \pm 5^\circ C$) and up to 10 days at $4^\circ C$. The Virus attained maximum concentration in *N.glutinosa* L. 14 days after mechanical inoculation. The concentration then slowly declined.

The Virus was isolated by a procedure involving extraction of the virus in 0.1M phosphate buffer pH 7.0; clarification by 30% chilled butanol; precipitation of the virus by 6% PEG in presence of 0.1% NaCl followed by two cycles of differential centrifugation. Removal of host contaminants was achieved by rate- zonal density gradient centrifugation on linear sucrose columns. The Virus exhibited a single light scattering band in sucrose
columns and infectivity was found associated with this band.

Purified preparations gave a spectrum typical of nucleo-proteins in UV- spectrophotometer ($A_{\text{max}}$ at 260 nm and $A_{\text{min}}$ at 240 nm). The nucleic acid was isolated by phenol- chloroform method and was found to be RNA by Orcinol test. The infectivity was found to be 10% of that of intact virus. RNA constituted about 6.02% of the total particle weight. The ratio of $A_{260} / A_{280}$ for nucleoprotein was 1.1838.

The extinction coefficient of the virus determined by linear regression line and the equation (Gibbs and Harrison, 1976) was 2.7651. The buoyant density and partial specific volume of the virus determined using quadratic regression line and the equation (Gibbs and Harrison, 1976) were $1.3024 \text{ g/cm}^3$ and $0.7678$, respectively.

SDS- PAGE electrophoresis of viral capsid protein showed only one type of protein subunit having a molecular weight of c. 36,000 daltons.

The classified preparation of virus negatively stained with 2% uranyl acetate showed flexuous filamentous particles measuring c.760 × 12 nm.
Ultrathin section of infected *N. glutinosa* L. leaves showed three types of inclusion bodies scattered in the cytoplasm of cells viz. long lamellar aggregates, scrolls and pinwheels typical of potyviruses.

An antiserum was raised against the virus having a titre of 1:2048 and the virus end point was 1:512 as determined by tube precipitin test. In Ouchterlony gel double diffusion tests, the virus showed serological relationship with ZYMV (Zucchini Yellow Mosaic Virus).

In ISEM (Immunosorbent electron microscopy), the virus showed close relationship with ZYMV as moderate decoration and trapping were recorded with the antiserum of this virus.

The virus under investigation differed in some respect from members of Potyvirus group with respect to host range, and biophysical properties but particle dimension, morphology, induction of inclusion bodies are more or less the same as that of potyviruses.

On the basis of serological relationship with ZYMV and other properties studied, it is concluded that the virus under investigation is a member of potyvirus group and is a strain of Watermelon Mosaic Virus-2 (WMV-2).