ABSTRACT

A viral disease of Bottle gourd (*Lagenaria siceraria* (Mol.) Standl. Syn. *L. leucantha* Rusby and *L. vulgaris* Ser.) showing vein clearing, mosaic, distortion of young leaves to an eventual bleaching of the leaf lamina, fruit distortion, stunting of the plant and over all loss in productivity, was investigated and characterized.

The virus under investigation has a restricted host range infecting *Cucurbitaceae* and *Chenopodiaceae* families. The virus gives distinct local lesions on *Chenopodium amaranticolor* and shows mosaic on *Cucumis melo*, *Cucumis satives* and *Lagenaria siceraria*.

The virus under investigation was found to be transmitted by sap inoculation, contact and soil contaminated with debris of diseased plants. However no seed and aphid transmission was found. Phosphate buffer (0.01M, pH7.0) was found to be the most suitable extraction medium for retaining virus infectivity. Other buffers such as acetate, borate and citrate were found to inhibit the activity of the virus. Addition of sodium sulphite (0.1%) along with (0.1%) EDTA, enhanced virus infectivity.

The virus in crude sap lost its infectivity at a dilution of $10^{-6}$, has thermal inactivation point 90–95°C and remained infective for more than months both at room temperature as well as at 4°C respectively.
The virus was isolated by a procedure involving extraction of the virus in 0.01M 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 the 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.

The purified preparation when examined in a UV-spectrophotometer gave a spectrum typical of nucleoproteins. The nucleic acid percentage was found to be 6 as $A_{\text{max}}/A_{\text{min}}$ was 1.2. The nucleic acid was isolated by phenol chloroform method and was found to be RNA. The extinction coefficient, buoyant density and partial specific volume were 2.7573, 1.36157 g cm$^3$ and 0.7344 respectively.

SDS–PAGE electrophoresis of viral capsid proteins showed only one type of protein sub-unit with a molecular weight of 17.2KD.

The virus preparation, when negatively stained with 2% uranuyl acetate showed rigid rods measuring 280x14.5nm.

An antiserum against virus was raised and serological tests using ELISA and ISEM, showed that the present isolate is closely related to the type strain of CGMMV. The virus withstands with more than 80,000 dilutions.
RT-PCR, Multiplex RT-PCR and IC-RT-PCR further confirmed the results. The coat protein gene as well as movement protein gene was amplified using a pair of designated specific primers. The RT-PCR products ~486bp for CP and ~795 bp for MP was cloned in to PGEM-T easy vector.

The cloned coat protein as well as movement protein genes were sequenced and showed 90% to 98% homology with the Gene Bank available sequences. It indicates that CGMMV Aligarh isolate has very little variability (in coat protein gene as reported earlier) but movement protein gene was first time sequenced from India.