

Purification of Sri Lankan isolate of Chilli Veinal Mottle Virus in *Capsicum annum* L. (Chilli)

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Chilli Veinal Mottle Virus (ChiVMV) is the most prevalent virus in Chilli cultivations which cause high yield losses. In Sri Lanka, purification of ChiVMV has not been carried out previously. Several protocols were experimented with minor modifications to investigate the most suitable protocol to purify the local isolate of ChiVMV. Symptomatically ChiVMV infected Chilli leaf samples were collected from Chilli fields located at different areas of Sri Lanka. ChiVMV infection of those samples were confirmed using commercially available Double Antibody Sandwich ELISA (DAS-ELISA) kit for ChiVMV. Leaf samples which gave positive results to DAS ELISA test were used as a source of virus to propagate the virus in healthy Chilli plants. Symptomatic leaves were harvested at four weeks post inoculation and homogenized with (100 g/300 mL) 0.5 M phosphate buffer (KPB) pH 7.7 containing 0.02 M Na₂SO₃. Extraction was followed by Clarification with 10% chloroform and centrifugation. Addition of 80 g/L Polyethylene glycol along with 0.1 M Sodium Chloride to the obtained liquid fraction facilitated the pelleting of virus. The obtained pellet then layered over 30% sucrose cushion. This partial purified product was purified further in a Cesium Chloride isopycnic gradient having 1.324 g cm⁻³ initial density. The purified product was dialyzed and virus concentration was measured by spectrophotometer. Transmission electron microscope (TEM) image was obtained from the final purified product and crude sap preparations. Purified virus had a UV spectrum of a typical nucleoprotein with a maximum, minimum and a slight shoulder at 260 nm, 240 nm and 290 nm respectively. The A₂₆₀/A₂₈₀ ratio was 1.280 and A_{max}/A_{min} ratio was 1.15. Flexuous rod-shaped virus particles with a length and width of 700-750 nm, 10-15 nm were observed for both purified and crude preparations. This protocol is suitable to obtain high concentration of ChiVMV for antiserum production.

Keywords: Chilli, ChiVMV, Purification