

Carlavirus Isolation and RNA Extraction

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1. Introduction

All members of the genus are known to be transmitted mechanically, with the majority also being transmitted in a nonpersistent manner by aphids (1), though one confirmed carlavirus is known to be transmitted by whiteflies (2). Carlaviruses are noted for their narrow host range and tendency to induce little or no symptoms. This has led to many of the common names of carlaviruses, including carnation latent (CLV), American hop latent (AHLV), and lily symptomless virus (LSV). Although most carlaviruses do cause mild symptoms, there are a number of viruses that cause serious diseases on their own, for example, potato virus S (PVS) and M (PVM), blueberry scorch virus (BBS_{Sc}V), poplar mosaic virus (PMV), and a number of others that cause serious disease in mixed viral infections (with other viruses).

The virus particles of carlaviruses are slightly flexuous, with a typical length of 660 nm and diameter of 12 nm (1). These particles, which consist of a single species of protein of ca. 34 kDa organized with helical symmetry, have sedimentation coefficients of approx 157 *S*, a buoyant density in CsCl of 1.322 g/cm³, an extinction coefficient at 260 nm of 2.88 cm²/mg¹, and a nucleic acid content of ca. 6% (1,3).

The genomic RNA of a range of carlaviruses has been estimated by agarose gel analysis to be in the size range of 7.3–7.7 kb. However, the recent reports of two full-length genomic RNA sequences have indicated a genome size of 8534 nucleotides for PVM (4), and 8512 for BBS_{Sc}V (5). In addition, a wide range of carlaviruses have been sequenced in their 3' terminal region, including PVS, HeLV_S, CLV, LSV, chrysanthemum virus B, and cowpea mild mottle virus (2). All show a similar genome organization, with similar sized open reading