

Potexvirus Isolation and RNA Extraction

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1. Introduction

The potexvirus family has at least 30 definitive and possible members, of which potato virus X (PVX) is the type member (1–4). All potexviruses are morphologically similar, with flexuous, filamentous virions that range from 470 to 580 nm in length. Each virus particle contains a single-stranded, positive-sense RNA molecule, 5845–7015 nucleotides in length, which is encapsidated by 1000–1500 molecules of a single species of capsid protein (CP), with a mol wt ranging from 21 to 27 kDa (5). The particle has a helical structure, with 8.75 protein subunits per turn for papaya mosaic potexvirus (PMV) (6). The genomic RNA (gRNA) contains a cap structure at the 5' terminus and is polyadenylated at the 3' terminus. The N- and C-terminal regions of the capsid proteins of potexviruses are quite variable (7). The variability of amino acids at the N-terminus of potexvirus CP results in the low serological crossreactivity seen in potexviruses (8). Individual potexviruses have mol wt in the order of 35×10^6 and sedimentation coefficients ranging from 100 to 130 S.

Five principal open reading frames (ORFs) have been identified in potexviruses (Fig. 1). ORF 1 encodes a protein that contains three amino acid sequence motifs characteristic of the conserved domains of methyltransferase, NTP-binding helicases, and RNA-dependent RNA polymerases (5). ORFs 2–4 slightly overlap each other and are known as the triple gene block. The products of these three ORFs are all necessary for infectivity in the plant host, but may be dispensable for infection of protoplasts, and are believed to be involved in cell-to-cell movement of potexviruses (9). ORF 5 is the 3'-terminal ORF and encodes the CP.

Genomic RNA of potexviruses is believed to be functionally monocistronic, and only ORF 1 protein can be translated directly from the gRNA (10,11).