

Nepovirus Isolation and RNA Extraction

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1. Introduction

Viruses of the nepovirus genus are classified within the family of *Comoviridae*, along with the comoviruses and the fabaviruses. At the time of writing, there are some 27 confirmed members of the genus, with eight or so viruses that, following further investigation, may be confirmed as members (1). These viruses infect a very wide range of plant species, producing symptoms that include mottles, mosaics, ringspots, and systemic necrosis; often infections are symptomless, particularly in the later stages. All members are transmitted through soil by free-living nematodes, principally of *Longidorus* or *Xiphinema* species, feeding on roots. They are also transmitted and disseminated through seed and pollen of both crop species and weeds. It is the combination of a mode of transmission (nematode) and virus morphology (polyhedral) that provides the name of the genus.

They have a worldwide distribution, although, as a result of their transmission via nematodes, many individual members of the genus have a rather restricted geographical range. This may be wider if seed or vegetatively propagated material has been more widely distributed. Diseases occur in many important crop species, including soybean in the United States (tobacco ringspot nepovirus; TobRSV) and raspberries in the UK (raspberry ringspot nepovirus; RRV). Arguably, the most important is the disease of grapevine caused by grapevine fanleaf nepovirus (GVFV).

Nepoviruses have bipartite, single-stranded RNA genomes of messenger sense [ss(+)RNA], each species independently packaged into icosahedral particles of T = 1 symmetry (2) approx 28 nm in diameter; each particle contains 60 copies of a single capsid protein, with an approximate M_r of 55 kDa. The larger of the two genomic RNAs, RNA-1 has M_r approx 2.8×10^6 (ref. 3;