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PURIFICATION, CHARACTERIZATION AND cDNA CLONING  
OF TWO FILAMENTOUS VIRUSES FROM *MERINE*

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### ABSTRACT

Virus-like symptoms were evident in many of the clonally mass propagated lines of the bulbous ornamental *Nerine*, cultivated in New Zealand for cut flower and bulb production. Although filamentous virus particles were common in electron microscope investigation of *Nerine* tissue, cucumber mosaic virus was the only virus mechanically transmitted to herbaceous indicators.

Two of the filamentous viruses from *Nerine* which were not amenable to mechanical transmission were purified from field-infected *Nerine* tissue, characterized and cDNA cloned in this study. An isolate of nerine virus X (NeVX), a potexvirus, was purified from *Nerine fothergilli* 'Major' leaf tissue showing no virus-like symptoms. An isolate of a hitherto unnamed and uncharacterized potyvirus was purified from clonally mass propagated *Nerine sarniensis* hybrid leaf tissue showing severe yellow mosaic symptoms prior to senescence. Nerine virus Y (NeVY) is the name proposed for this potyvirus.

The NeVX isolate was a slightly flexuous filamentous particle with a normal length of 540nm. The molecular weight of the single coat protein subunit was 29.5kd and the size of the genomic RNA was 6.3kb. The NeVX RNA *in vitro* translation profile resembled those of the potexviruses potato virus X and daphne virus X with a major 180kd nonstructural protein band and a number of minor bands without a viral coat protein band.

Double-stranded cDNA to the 6.3kb genomic RNA was cloned into the *Pst* I site of the plasmid vector pBR322. Nick-translated pBR322 DNA containing a 1.8kb insert, representing 28.5% of the viral genome, was found to be highly specific and sensitive to NeVX isolates in dot-blot assays. The cloned cDNA probe did not hybridize to seven other potexviruses, including a ca 540nm potexvirus from *Agapanthus* which, on the basis of serological reactivity, was previously described as an agapanthus strain of NeVX. A survey using the cloned cDNA in dot-blot assays of *Nerine* species indicated that NeVX was prevalent in *Nerine* plants cultivated in different parts of the North Island of New Zealand.

Nerine virus Y was found to be a typically flexuous potyvirus with a normal length of 800nm, a single coat protein subunit with a molecular weight of 33.26kd and a genomic RNA of 10.0kb. Nerine virus Y-infected tissue had characteristic Type I potyvirus cylindrical inclusion bodies. Size fractionated viral RNA was cloned into the lambda vectors gt10 and L47AB. Two cDNA clones of size 1.54kb and 0.56kb derived from lambda gt10 and

a 9.8kb cDNA clone derived from lambda L47AB were subcloned into the plasmid vector pGEM3. In dot-blot assays cDNA from all clones hybridized to the homologous virus but not to nerine yellow stripe virus, another more common potyvirus in nerines. The 1.54kb cDNA cloned probe was found to be highly specific and sensitive and did not hybridize in dot-blot assays to four other potyviruses. A survey using the 1.54kb cloned cDNA probe in dot-blot assays indicated that NeVY was not common in *Nerine* species cultivated in different parts of the North Island of New Zealand.

The use of recombinant technology made it possible to develop highly sensitive and specific diagnostic tools for two filamentous viruses from *Nerine*. These cloned cDNA probes were found to be well suited for conducting field surveys to study the prevalence of the virus. The probes could be used in subsequent studies in screening for viral resistance and in virus elimination studies. Further, the cloned cDNAs may in the future prove useful in the characterization of the genomes of NeVX and NeVY.

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