

## Using electron microscopy to uncover latent tobamovirus

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A combination of electron microscopy and genome sequencing is highly efficient in screening for latent virus infections independent of the virus morphology or genome. One example is hoyai tobamovirus-2 (HoTV-2 genbank accession number: MT750216.1) that has been reported from symptomatic mixed infected samples in 2011 and also as single, asymptomatic, infection in several *Hoya* species in Germany. The virus induces no symptoms in experimental host plants including *Chenopodium quinoa*, *N. tabacum* 'Xanthi nc', *N. benthamiana* and *N. tabacum* 'Samsun nn'. 14 days post inoculation, plant sap homogenates were examined using negative staining. Rod shaped virions of 300nm length were detected in newly developing leaves, confirming systemic infection. The virions' morphology and size indicated presence of a tobamovirus. The virus titer was lower when compared to tobacco mosaic virus (TMV) infected plants. For virus species identification, RNA sequencing data were scanned for virus specific sequences. The assembled virus genome could be assigned to the genus *Tobamovirus*. Phylogenetic analysis showed it is closely related to Youcai mosaic virus (YoMV). Therefore, polyclonal antisera for YoMV (DSMZ number AS-0527) was used in immuno-electron microscopy. The heterologous antiserum reacted with HoTV-2, while other tobamovirus antisera used in routine tobamovirus detection did not. The binding of antibodies along the virion was irregular when compared to TMV and its homologous antisera. Mixed infected *Hoya* plants and HoTV-2 infected *N. benthamiana* leaves were embedded to study the ultrastructure of infected plants displaying latency linking *in situ* HoTV-2 structures.