

Revision of taxonomy of the virus causing Augusta disease in tulips in The Netherlands

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Augusta disease

Augusta disease is a viral disease in tulip and was observed for the first time in 1928 in The Netherlands. The disease derives its name from the tulip cultivar Augusta in which the first great outbreak was registered in 1931. The disease occurs in tulips at epidemic levels with a cycle periodicity of about 8-10 years. At the top of an epidemic the incidence goes up to 70% in some field-grown stocks and up to 20% in forced tulips.

Symptoms may vary from brown to grey, necrotic spots and/or stripes on leaves and flowers. The first necrotic symptoms appear in early spring, and later on plants often show other malformations such as curling and stunting, premature death and small or no new bulbs (Figure 1).



Figure 1: Symptoms of Augusta disease on tulip.

TNV or OMMV?

In 1949 the causative agent of Augusta disease was identified as *Tobacco necrosis virus* (TNV). Testing of infected leaf material from different tulip lots in agar gel diffusion tests and enzyme-linked immunosorbent assays (DAS-ELISA) showed that serotype D of TNV is most prevalent.

Considering recent publications of Augusta isolate sequences (GenBank accession nrs EF201605, EF201606 and EF201607) these amino acid sequences showed remarkably high homology (98%) with the new necrovirus *Olive mild mosaic virus* (OMMV) from *Olea europaea* L, (Figure 2). The amino acid sequence identity of the RNA dependent RNA polymerase (RdRp) gene of OMMV showed a close relationship with TNV-A and *Olive latent virus 1* (OLV1), and not with TNV-D. A primer set was designed to amplify the (partial) RdRp gene from TNV-A, OLV1 and OMMV.

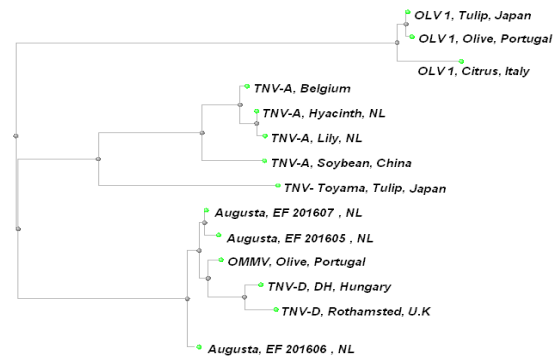


Figure 2: A phylogenetic tree generated from an alignment of the (partial) amino acid sequences of the coat protein (CP) gene of Augusta isolates (GenBank accession nrs EF202605, EF201606 and EF201607) and other viruses of the genus Necrovirus.

Results and Discussion

Viral isolates causing Augusta disease in tulip were PCR-amplified with the primer set specific for TNV-A, OLV1 and OMMV (Figure 3). The sequences of the amplified fragments were determined and showed highest homology to OMMV (90%) whereas significant homology with TNV-D was absent.

Based on both the partial coat protein and RdRp sequences, we conclude that Dutch Augusta disease isolates are to be considered as OMMV isolates and not TNV isolates. OMMV is the third necrovirus detected by sequence analysis in tulips next to TNV-Toyama and OLV 1.

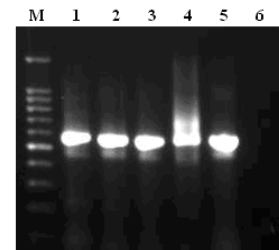


Figure 3: RT-PCR products amplified with primers specific for TNV-A, OLV1 and OMMV. Lane 1-3: Augusta isolates (the presence of viruses was confirmed by ELISA); lane 4: TNV-A isolate from bean; lane 5: TNV-A isolate from hyacinth; lane 6: water, negative control; M: 100bp Ladder (Promega).