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Characterization of a complex of necroviruses involved in a necrotic disease of corn salad (*Valerianella locusta*)

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Since 2008, a novel disease of unknown origin has been observed in corn salad (*Valerianella locusta*) in the Nantes area (west of France). The characteristic symptoms of the disease are collar and vein leaf necrosis with necrotic spots on leaves, leading in some cases to plant stunting or death. The symptoms have been observed in greenhouses throughout the year but also under field conditions, with an impact reaching 2-3% of the plants within one plot. Moreover, disease symptoms are observed throughout the year. In order to understand the etiology of the disease, mechanical transmission was attempted on healthy plants, including corn salad and *Nicotiana benthamiana*. Necrotic lesions and vein necrosis were observed on the inoculated plants, indicating a biotic, transmissible and likely viral origin of the disease. Double stranded RNA (dsRNA) and small interfering RNA (siRNA) were purified from symptomatic *N. benthamiana*, and analyzed by deep sequencing (454 pyrosequencing and Illumina sequencing, respectively). Mapping and de novo assembly of reads, followed by Blast analysis, allowed the identification of several necroviruses and a related satellite unknown so far on corn salad. Complete (or near complete) genome sequences were assembled for each virus. Besides Tobacco necrosis virus A (TNV-A), Olive mild mosaic virus (OMMV), and Tobacco necrosis virus D (TNV-D), which belong respectively to the genus Alphanecrovirus and Betanecrovirus, a putative new viral species in the genus Alphanecrovirus, for which the name of Tobacco necrosis virus X (TNV-X) is proposed, was also identified and sequenced. Moreover, Satellite tobacco necrosis virus (STNV) was found in association with either TNV-A or OMMV or in mixed infections of both viruses. The contribution of each virus to the disease was evaluated by inoculating corn salad plants with isolated or purified viruses. All four viruses induced necrotic symptoms reminiscent of the field disease. OMMV was found to induce the most severe necrosis, in comparison to TNV-D which induced only some systemic necrosis, TNV-A and TNV-X infections being considered as intermediate severity. Most of the necroviruses are naturally transmitted by the fungi *Oplidium sp.* The presence of *Oplidium sp.* was indeed detected in a significant number of infected plots, indicating a potential for necroviruses spread in field conditions. Taken together the results obtained indicate that the new necrotic disease of corn salad is caused by several necroviruses. It is likely that the current cultural practices, with its repeated monoculture cropping is a major contributor to the emergence of this novel disease.

Mots-clés : Etiology, Virus disease, Necrovirus, NGS, diagnostic.



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