Structural and functional characterization of *Grapevine fanleaf virus* coat protein determinants involved in its transmission by *Xiphinema index*

Transmission is a key step in the virus life cycle. *Grapevine fanleaf virus* (GFLV) and *Arabis mosaic virus* (ArMV), the two major causal agents of grapevine fanleaf disease, are specifically transmitted by *Xiphinema index* and *X. diversicaudatum*, respectively. Both viruses (genus *Nepovirus)* belong to the class of icosahedral viruses with pseudo *T = 3* symmetry. Previous experiments demonstrated the specificity of transmission to be solely determined by the coat protein (CP). In order to characterize structural domain(s) and residues within the CP that confer GFLV transmission, a multidisciplinary approach combining reverse genetic, X-ray crystallography and cryo-electron microscopy (cryo-EM) was performed. For the genetic approach, several GFLV-ArMV chimeric CP were generated and tested for transmission by nematodes. This allowed the identification of one region consisting of 11 residues in GFLV transmission. In addition, the characterization of a spontaneous GFLV variant poorly transmitted by *X. index* termed GFLV-TD, revealed the importance of Gly297Asp mutation in transmission. GFLV and GFLV-TD crystal structures were solved at 3 and 2.7 À, respectively. Structural comparisons revealed that the near complete loss of GFLV-TD transmission of resulted from the single occurrence of a negatively charged side chain highly exposed at the capsid surface. These results suggest the virus-vector binding site to consist of a negatively charged pocket formed by specific loops of the CP B domain. Finally, ArMV capsid structure was obtained by cryoEM 3D reconstruction. GFLV and ArMV structural differences mainly locate in the CP A domain. Minor differences were also detected in the CP B domain, on the pocket protrusions. Altogether, this work enabled to obtain the three-dimensional structure of two viruses responsible of fanleaf disease and the functional and structural characterisation of two viral transmission determinants. It opens new research prospects aiming at better understanding the molecular mechanism governing *Nepovirus* transmission by nematodes.