The presence of additional, short RNA particles has been described for a variety of RNA viruses of plants. Subviral particles can be associated with the viral genome or formed *de novo* during the infection. Two main types of subviral particles can be distinguished: defective (D RNA) and satellite RNA (satRNA); D RNAs are often derived from the helper virus genome during prolonged passages in one host, whereas satRNAs share little sequence similarity with the viral genomic RNA(s). Subviral particles can modulate helper virus accumulation and symptoms observed on infected plants, often weakening them, or even completely eliminating them. D RNAs, which interfere with helper virus accumulation and influence symptoms produced by the helper virus, are called defective interfering RNAs (DI RNAs). This unique feature makes them innovative tools to protect plants against viruses in the future. The goal of the following project is to analyze the mechanism and factors involved in the formation of subviral particles and establish their role in pathogenesis. Additional RNA molecules have been found in many representatives of the *Nepovirus* genus, which infect a wide range of economically important plants, contributing to losses in quality and yield worldwide. In the following project, two nepoviruses will be used: *Grapevine fanleaf virus* (GFLV) and *Tomato black ring virus* (TBRV), which belong to two different subgroups of the genus, A and B, respectively. SatRNAs have been described for both viral species, whereas formation of D RNAs has been a unique feature of TBRV so far. In the framework of the following project, the impact of host, virus species/isolate, amount of passages and environmental conditions on the formation and structure of D RNA particles will be established. D RNAs sequence will be obtained and compared with the helper virus genome for the presence of structure/elements, which can promote the formation of additional RNA particles. In the following project, the association of satRNAs with newly-collected TBRV isolates will be verified. Moreover, the genetic diversity and molecular evolution of nepoviruses subviral particles will be analyzed using different bioinformatics tools. Additionally, an infectious copy of satRNAs will be produced, which allows further studies on their role in pathogenesis. The impact of the subviral particles on the accumulation level of parental virus and its virulence will be analyzed. Moreover, the role of defective RNAs in TBRV transmission through seeds will also be verified. The realization of the following project will expand the knowledge about nepoviruses replication, formation of subviral particles and their role in pathogenesis and epidemiology. The knowledge about this is still very limited, whereas the detailed analysis of those mechanisms might be a step toward new, innovative tools to protect plants against viruses. DI RNA particles represent a major controlling element of virus replication The more we learn about viral pathogenesis and the interaction and competition between DI RNAs and the helper virus, the more we can focus on our research to dissect DI RNA - mediated attenuation of infection. Results of this research will be disseminated through presentations at relevant scientific meetings and published in international journals within the fields of phytopathology, virology, and evolutionary biology. The participation and scholarship for the student and Ph.D. has been planned in the project, and research will be carried out as part of a Master's of Science and Ph.D. dissertation, respectively.