SUMMARY

Pelargonium line pattern virus (PLPV) is one of the most frequent viral agents in geranium. It's an icosahedral virus, with a monopartite, positive-sense, single-stranded RNA genome, lacking a cap structure at the 5´ end and poly(A) tail at its 3´ end. Its genomic RNA (gRNA) contains five genes that encode two proteins involved in replication, two proteins involved in movement, (MP1 and MP2), and a coat protein (CP) that also acts as viral suppressor of RNA silencing. The first two genes are translated from the gRNA whereas the remaining three are translated from the single subgenomic RNA (sgRNA) that the virus produces during infection. The PLPV belongs to the family *Tombusviridae* and presents unique characteristics such as: i) production of a sole sgRNA that is structural and functionally tricistronic, ii) presence of a non-AUG start codon (CUG or GUG) initiating the MP2 ORF, iii) absence of AUG codons in any frame between the AUG initiation codons of MP1 and CP genes, and iv) sequence-based phylogenetic clustering of all encoded proteins in separate clades from those of other family members.

In this doctoral thesis we have tried, on the one hand, to further study the regulation of PLPV gene expression and, on the other hand, to obtain information on degradation

processes to which its genome is subjected, particularly on those related to the mechanism of RNA silencing.

The first objective in this work was to determine the mechanism and the viral RNA elements involved in the generation of the sgRNA of PLPV. An RNA-RNA interaction involving distant segments of the gRNA of plus polarity has been identified. Such long-range interaction seems to act in *cis* and specifically mediates the production of the negative strand of the sgRNA, a type of molecule easily detectable in infected tissue. These results together with the possibility of uncoupling the synthesis of negative and positive strands of the sgRNA and with the observation of sequence similarity between the 5´ ends of the gRNA and the sgRNA (with promoter function in their complementary strands), suggest that PLPV follows a model of premature termination for the formation of its unique sgRNA.

The second objective of this work was to identify the elements of the viral genome that are involved in its translation through a cap-independent mechanism. Transfection of *N. benthamiana* protoplasts with PLPV-based reporter constructs has allowed us to corroborate the functionality of the CITE in the context of both the gRNA and the sgRNA. Additionally, it has been shown that this element establishes a long-distance

RNA-RNA interaction with the 5´-region of the corresponding RNA which is essential for its activity. Moreover, data that support the relevance of the CITE during the infectious cycle of the virus have been obtained. Finally, in order to gain information on the role of PLPV as inducer and target of RNA silencing that the plant triggers as a defense against the virus, we have characterized the viral small RNAs (vsRNAs) present in PLPV-infected *N. benthamiana* plants. High-throughput sequencing, computational analysis and molecular hybridization assays have shown that: i) vsRNAs of the PLPV accumulate in extraordinarily high proportions in infected tissue, ii) 21 and 22 nt vsRNAs are the most frequent ones, iii) the vsRNAs of positive and negative polarities are in similar proportions, and, iv) there is variability in the vsRNAs 5´-proximal nucleotide. These results have provided clues on the viral molecules that must act as substrates for the formation of the vsRNAs and, also, about the components of the silencing machinery of the host that are likely involved in the response against the virus.