SUMMARY

Genus *Carmovirus* (family *Tombusviridae*) represents at least 19 species of plant viruses that affect numerous cultivated species, in many cases with a major economic impact. MNSV genome (gRNA) is composed of a 4.3 kb single strand RNA molecule of positive polarity, without 5' end cap structure (m7G5pppNp) and an unpolyadenylated 3’ end. MNSV genome encodes five functional proteins flanked by two untranslatable regions of 95 nt (in the 5 'end) and 280 nt (at the 3' end). The ORF closest to the 5' end is a 29 kDa protein (p29) that ends with an amber codon and code for the RNA dependent RNA polymerase (RdRp). Stop codon readthrough results in an 89 kDa protein (p89), also involved in virus replication. The two ORFs located in the central part of the genome, code for two small MPs, generally referred DGBp1 and DGBp2 (*double gene block of proteins*, DGBps). DGBp1 is an RNA binding protein required for the intercellular transport of the viral genome, while DGBp2 is a membrane-associated PD whose location in the PD is essential for cell to cell movement of the virus. Finally, ORF at the 3' end represents the coat protein (CP). In the case of MNSV, virions consist of isometric particles of approximately 30 nm in diameter, made with about 180 subunits of the coat protein.

Previous results obtained in the research group where this thesis has been performed shown that the MNSV uses the cellular secretory pathway, through its membrane protein DGBp2 (p7B) to reach the cell periphery. Knowledge about signals/motifs of membrane proteins that facilitate or permit such transport was then rather scarce. In this work we determined the residues involved in the transport of a viral transmembrane protein through the early secretory pathway (DGBp2, MNSV p7B). The residues involved are located in both the Nt (cytosolic) and Ct region (luminal) being one of the first examples in plants of a luminal ER export signal. With this information we have proposed a model in which after insertion and correct folding of the protein in the ER membrane, the luminal Ct of p7B interacts through the K_{49} residue with a transmembrane adapter associated with the actin cytoskeleton for movement and

concentration in the RE-cortical. Nt cytoplasmic seems to be necessary to associate with the COPII vesicle components.

Moreover, we have deepened in the study of the interactome of the carmovirus MPs and we have identified through a two-hybrid assay (Y2H), three cellular proteins capable of interacting with three DGBp1 from three different carmovirus (MNSV, TCV and CarMV). These cellular factors are the 60S ribosomal protein P3 (RPP3A), the g subunit of the translation initiation factor 3 (eIF3g) and the transcription factor WRKY36. These interactions were confirmed by BiFC. Furthermore, mutagenesis assays showed that binding domain of these DGBp1 is a FNF conserved domain at the very Ct end. The fact that these three proteins interact with the same host factors suggest a possible mechanism common to most if not all carmoviruses.

Viral CPs are the paradigm of protein multifunctionality. In addition to its obvious structural role, they are involved in many processes of the viral cycle, including transport of viral RNA. The unstructured Nt region of MNSV CP, as for other RNA viruses, generally is responsible for viral RNA binding so it is usually called R domain. By using substitution and deletion mutants, we have shown that this R domain (which in MNSV comprising the first 94 residues) is not involved only in the packaging and binding of the viral genome, but is also responsible of CP multifunctionality. By EMSA assays with deletion mutants we could determine that the R domain was essential for binding of RNA. It was further noted that within the R domain there was a conserved region between aa 60 to 91 region, which appears to play a role in both the genomic RNA binding and in vitro encapsidation of subgenomic RNAs. However, in packaging assays, it was observed that the R domain is essential for full genome encapsidation and that the region between residue 31 and 91 is required for both cell to cell and systemic movement. Finally, using PVX as an expression vector, we showed that MNSV CP can act as a suppressor of silencing most likely by sequestering sRNAs.

With very few exceptions, plant viruses use the phloem to move from infection sites to distal parts of the plant. In order to know the phloem proteome of infected plants and to identify in the future potential host proteins that facilitate or hinder the systemic transport of viruses, in the last chapter we conducted a comparative proteomic

analysis by 2D-DIGE between phloem of MNSV-infected and healthy melon plants. From a total of 1046 spots, 25 were detected having significant abundance changes between the two conditions. After mass spectrometric analysis, 22 spots corresponding to 19 protein, were identified (13 of which were overrepresented and 9 had decreased abundance). Many of the identified proteins were involved in cell death and control of redox homeostasis. Two of these 19 proteins were never described in phloem proteomic assays: a carboxylesterase with homology with the *N. tabacum* hsr203J and the fumarylacetoacetate hydrolase 1, both considered as negative regulators of cell death. The results suggest that defense response due to viral infection can be transferred to the phloem as a variation in level of phloem proteins that act as key regulators to maintain systemic signaling in defense against pathogens.