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# HVA22a and HVA22c, two candidate proteins involved in *potyvirus* replication and movement

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*Potyvirus* is one of the largest genera of plant viruses responsible for serious diseases in vegetable and fruit crops worldwide. The completion of the viral cycle results from a complex interplay between virus- and plant-encoded factors also called susceptibility factors. In this scheme, absence or non-adequacy of a single susceptibility factor leads to full or partial resistance to viruses. In this context, this work aims at identifying plant factors involved in the potyvirus cell-to-cell movement, a key step of the viral cycle. Indeed, to invade the whole plant after replication, plant viruses move intracellularly to reach the plasmodesmata (PD), the symplasmic tunnels between plant cells that are the gateway for this movement, cross them to enter in the neighboring cells, and enter into sieve elements.

The potyviral species *Turnip mosaic virus* (TuMV) represents one of the rare examples of plant viruses that utilizes the host endomembrane system to produce membranous vesicles mobile between cells, reminding of animal viruses that utilize membrane-derived vesicles for exit from infected cells and entry into healthy cells. This is the 6K2 protein of TuMV, a small transmembrane protein that is involved in this rearrangement of the endoplasmic reticulum (ER) for the generation of those viral vesicles important not only for replication but also for intracellular and intercellular movement. Viral RNA and proteins, together with host proteins essential for potyvirus replication are recruited in these small vesicles, which are transported through PD to neighboring plant cells to achieve the systematic infection.

In *Arabidopsis thaliana*, AtHVA22a and c (***H****ordeum* ***v****ulgare* **a**bscisic acid responsive gene 22) belong to a multigenic family of transmembrane proteins, homologous of reticulons and DP1/Yop1 family, which are responsible for the constriction of ER tubules and that also play a role in role in positive strand RNA virus replication in animals and plants. Moreover, semi-quantitative proteomics analysis of PD fractions purified from *A. thaliana* suspension cells showed that AtHVA22c and AtHVA22a are highly enriched in PD proteome.

In our study, we showed that TuMV-6K2 interacts with AtHVA22a and AtHAV22c, by split-ubiquitin yeast two hybrid assay (SuY2H) in yeast and further confirmed those interactions in planta by Bimolecular Fluorescence Complementation (BiFC). Overexpression of AtHVA22a increases TuMV propagation in *Nicotiana benthamiana*. However, lack of AtHVA22c expression has a negative effect of TuMV propagation in *A. thaliana*. Furthermore, both AtHVA22a/c are partially localized at the viral replication complex (VRCs) during TuMV infection and have partial localization with 6K2-induced vesicles. The interactions between AtHAV22a/c and 6K2 observed in BiFC are also localized at the VRCs during viral infection.

Altogether, our results indicate that HVA22a and HVA22c, are candidate proteins potentially involved in replication and cell to cell movement of TuMV.