Benthamiana and its demons: PVX & PVA. Revealing the role of HCPro, in the synergistic interaction between Poty-Potexvirus

Resumen
Virus–virus-plant interactions have a tremendous importance for understanding viral pathogenesis and evolution, and consequently for the development of efficient and stable control strategies (1). In *N. benthamiana*, the co-infection with Potato virus X (PVX) and members of the genus *Potyvirus*, results in increased systemic symptoms (synergism in pathology) in compare to single infections (2-4). PVX-*Potyvirus* is one of the best-characterized synergistic infections; the co-infection produces an increase in PVX viral accumulation, but the mechanism that underlying this action is not well understood. Two hypotheses have been constructed based in our previous results: 1) that this increase in PVX viral accumulation is given by a mechanism that involves a selective increase in the translation of the potexviral mRNA, where viral and host translation factors are involved (5), or 2) that the increase in PVX viral accumulation is the consequence of the suppression of the antiviral plant defense (6). In order to study the mechanism that underlies mixed infections, we generated a PVX (*Potexvirus*) and PVA (*Potyvirus*) co-infection system in *N. benthamiana* and *N. tabacum* plants using a dual-color system of fluorescent proteins that allows us to compare differentiate, quantitate and track viral accumulation, using leaf samples. In order to answer our first hypothesis we generated a mutant virus defective in translation (PVA$^{VPgmut}$) and a VPg mutant unable to bind to eIF4E initiation factor (7). We found that these mutations in the virus reduce severely the PVA titers in the single infected plants, but in PVX-PVA$^{VPgmut}$ co-infected plants the VPg mutation does not produce any noticeable effect compare to the PVX-PVA$^{wt}$ co-infected plants. Additionally, we were able to complement partially the PVA$^{VPgmut}$ titers with the addition of *in trans* VPg$^{wt}$. The co-agroinfiltration with P0 and VPg in PVA infection performed a remarkable increase in PVA titers, as was previously characterized by (8,9), but neither the addition of P0 or
VPg produced any noticeable change in PVX titers. We therefore think that the synergistic PVX-PVA interaction is not given at translational level. We also explored the possibility that one of the best-characterized potyviral proteins, HCPro, would be producing the suppression of the antiviral plant defense. We used a PVA that has HCPro deleted (PVA\textsuperscript{ΔHCPro}) in coinfection with PVX. Here, the lack of increased PVX titers evidenced the need of HCPro for the synergistic interaction. Interestingly the addition of HCPro\textsuperscript{wt} in the PVX-PVA\textsuperscript{ΔHCPro} infection and even the addition of the HCPro\textsuperscript{wt} itself reproduce the synergistic phenotype. We used an eIF4E binding deficient mutant HCPro (ebd-HCPro) and a silencing suppression deficient mutant HCPro (ssd-HCPro) in order to further characterized the specific function of the HCPro that is related to the HCPro-dependent synergism. Our studies identified that the potyviral HCPro and specifically the silencing suppressor domain is responsible for the synergistic interaction. As it was been proposed that the role of HCPro in the silencing suppression it is associated to a local disruption in the methionine cycle (10), we explored this new role of HCPro trough the transient silencing of SAMS and SAHH in \textit{N. benthamiana}. We found that the partial silencing of SAMS and SAHH produce the same increase that the addition of HCPro or the co-infection with PVA. Taken all together, our results suggest that the PVX-PVA synergistic interaction is mediated by the suppression of the silencing antiviral machinery trough a mechanism that involves a disruption in the methionine cycle.

\textbf{Referencias}


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