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Hc-Pro as a tool to study silencing-suppressor-mediated induction of disease symptoms in plants.

RNA silencing is a sequence-specific gene inactivation system, which operates through diverse pathways in plants and animals. This evolutionary conserved system functions as an antiviral defense in higher plants (Voinnet, 2005). Most plant and animal viruses have evolved silencing suppressor proteins as a result of co-evolution, to counteract antiviral RNA silencing (Baulcombe, 2002; Li and Ding, 2001; Li et al., 2002; Voinnet, 2001). The plant viral suppressors have been identified in a wide range of virus families, but they share no similarities at either the nucleic acid or the protein levels, indicating differences at their mode of actions among virus families (Wang and Metzloff, 2005).

The silencing suppressor protein of the potyviridae family is the so called “helper-component proteinase” (Hc-Pro) (Urcuqui-Inchima et al., 2001). This silencing suppressor has different essential functions in the potyvirus life cycle. Beside the suppression of post-transcriptional (PTGS) and virus-induced gene silencing (VIGS) (Anandalakshmi et al., 1998), it is an indispensable helper factor for virus host-to-host transmission by aphid vectors (Pirone and Blanc, 1996). Its protease activity acts *in cis* on its own C-Terminus to release it from the precursor polyprotein (Verchot et al., 1992), and it is a general enhancer of infectivity and genome amplification. Furthermore, it is essential for cell-to-cell and systemic movement in the plant (Cronin et al., 1995; Kasschau and Carrington, 1995).

The central region of the Hc-Pro protein contains several highly conserved motifs in all potyvirus, including the FRNK box, which is associated with symptom severity during viral infection (Gal-On and Raccach, 2000). A spontaneous mutation of Isoleucine (I) for Arginine (R) at position 180 of the conserved FRNK motif in Hc-Pro of *Zucchini yellow mosaic virus* (ZYMV) potyvirus cause a drastic reduction of symptoms in the leaves of cucurbit plants (e.g. squash, cucumber), without affecting virus accumulation or silencing suppressor activity (Gal-On and Shibolet, 2005). The same mutation of Hc-Pro proteins in different members of the potyvirus family results in a loss of infectivity (Saenz et al., 2001).

Likewise, the P19 suppressor of tombusviruses, P21 of closteroviruses, P15 of pecluviruses, and γ B of hordeiviruses Hc-Pro has been shown by *in vitro* binding studies that it represent a size-selective ds-sRNA-binding-suppressor with a high affinity to 21-nt siRNA duplexes, containing 3'-2-nt overhangs (Lakatos et al., 2006; Merai et al., 2006).

Regulatory RNAs in plants comprise the miRNAs and the *trans*-acting siRNAs (tasiRNA) (Peragine et al., 2004; Reinhart et al., 2002). The miRNAs are described by endogenous genes and built hairpin loops. These primary transcripts (pri-miRNAs) are processed by proteins of the RNAi machinery (Dicer-like1/DCL1) into miRNA/miRNA* duplexes. After processing (methylation by HEN1), the single-stranded miRNAs are loaded onto the Argonaute1 (AGO1) protein and catalyzes target cleavage, whereas the passenger strand is degraded (Jones-Rhoades et al., 2006).

Previous studies indicate the dramatic effects of miRNAs on plant development, including overexpression of miRNAs, generating of miRNA resistant target genes, and mutations (*dcl1*, *hen1*, *hyl1*, *ago1*) of the key components in the miRNA biosynthesis pathway (37, Jones-Rhoades et al., 2006). Also the expression of Hc-Pro in absence of a virus in *Arabidopsis thaliana* show this developmental abnormalities, indicating that Hc-Pro acts on the components in this pathway (Dunoyer et al., 2004). RNA gel blot analyses have shown that miRNA and miRNA* are increased in infected plants, and that Hc-Pro posses smRNA-

binding ability (Lakatos et al., 2006; Merai et al, 2006). Our aim is to get an insight how the mutations in the conserved FRNK motif in the Hc-Pro of ZYMV affect host responses to potyvirus infection in cucurbit plants. The main focus will be on the effect of Hc-Pro on small RNA accumulation.

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