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

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Introduction

Viral diseases are the main problem in the production of cucurbit plants compared to diseases caused by other agents leading to total loss and preventing the cultivation of some cucurbit crops in certain areas. Cucurbit crops are susceptible to many viruses belonging to several virus families (Kyle & Provvidenti, 1993). Almost 35 different viruses have been isolated from Cucurbitaceae and virus resistance is therefore of major agricultural importance. A member of potyvirus family, Zucchini Yellow Mosaic Virus, ZYMV, causes a serious disease in cucurbit crops (melon, watermelon, squash/pumpkin, and cucumber) worldwide (Desbiez and Lecoq, 1997). ZYMV genome contains a single long open reading frame translated into a large 340–370 kDa polyprotein that is co- and/or post-translationally cleaved to produce 8-9 products (Shukla et al., 1994). The multifunctional helper-component proteinase, HC-Pro, the second protein of the polyprotein, functions as an oligomer (Ruiz-Ferrer et al., 2005). It is a key viral factor in pathogenesis and host responses to potyvirus infection. HC-Pro differentially affects the accumulation of micro RNAs (miRNAs) and short interfering (siRNAs) (Chapman et al., 2004). Recent studies suggest a mechanism for HC-Pro involvement in symptom development of infected plants through interference with the functions of endogenous miRNAs that regulate plant development (Kasschau et al., 2003). It is important to understand the mechanisms of symptom development in response to virus infection to design new strategies to produce virus resistant plants. In plants, the HC-Pro interferes with small RNA metabolism and function, and such interference is associated with anomalous plant development. The central region in the HC-Pro is associated with suppressor activity and RNA binding. A mutation of Arg (R) to Ile (I) at position 180 in the HC-Pro of ZYMV uncouples the role of HC-Pro in symptom expression from its role in virus accumulation (Gal-On and Raccah, 2000) and silencing suppressor activity in squash plants. In addition, R180 lies in the Phe-Arg-Asn-Lys (FRNK) highly conserved amino acid motif in the potyvirus HC-Pro, and thus represents an important interface between these viruses and their hosts. The ability of this mutation to uncouple symptoms from virus accumulation creates a unique opportunity to study the etiology of symptoms. In other potyviridae such a mutation in the HC-Pro always resulted in loss of infectivity. Our goal in the present was therefore to determine how this mutation in the conserved ZYMV HC-Pro motif affects host responses to potyvirus infection in cucurbit plants. In particular, we focused on the effect of HC-ProFRNK and HC-ProFINK on small RNA accumulation.

Material and methods

In this study *Nicotiana benthamiana* plants were infiltrated with Hc-Pro FRNK/FINK. Total RNA was extracted from these plants and protein expression was studied by northern blots. Total proteins were also extracted and the Hc-Pro was studied by western blots using either Anti-HA peroxidase antibodies, or antisera for the Hc_Pro (Sambrook et al., 1989). The suppression activity of gene silencing of the Hc-Pro in these plants was checked by infiltration of plants with the sense and antisense of the green fluorescence protein (GFP) (Brigneti et al., 1998). miRNA levels in these plants were studied and compared.

Results

The Hc-Pro FRNK/FINK protein was detected in *Nicotiana benthamiana* infiltrated plants using northern blot. On the other hand the expression of protein was detected using western blots, the Hc-Pro antisera was better than the anti-HA peroxidase for the detection of the protein. Plants co-infiltrated with Hc-Pro FRNK/FINK and GFP sense construct or with sense and antisense of the GFP protein were photographed 9 days after infiltration under the UV light, no difference in the GFP fluorescence between the wild type (Hc-Pro FRNK), or the mutated one (Hc-ProFINK). miRNA accumulation was detected in these plants compared to the *N. benthamiana* wild type.

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