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Viral Suppressors of RNAi: Assay and Functional analysis

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Abstract

RNAi is a complex surveillance and regulatory process inherent to all eukaryotic cells, which mediates small RNA dependent repression of the target gene expression in a sequence specific manner. It serves as an important tool in plant defence against virus attack by activating virus induced gene silencing (VIGS) to repress viral proliferation. As a counter defense mechanism, members of different viruses encode proteins known as RNA silencing suppressors (RSS) that suppress RNA silencing at different stages of the pathways. The expression of RSS is essential for virus infection and virulence. The virus-encoded RSS can therefore serve as important biological tools to dissect the detailed RNA silencing pathways. The RSS are also being explored as tools to ameliorate the negative influence of RNA silencing on transgene expression in plant transformation technology with application in biofarming sectors to boost transgene expression. This has generated a need to screen the viral protein for suppressor functions and understand the suppressor interaction with the host plant to screen for molecules that compromise the plant RNAi machinery with least affect on plant phenotype.

The present work was, initiated with an aim to develop a new assay for suppressor identification. The assay is based on the reversal of siRNA mediated silencing of GUS reporter gene expression. The efficiency of this assay was tested by comparing with the established assays of reversal of GFP fluorescence using well characterized RSS, FHVB2. The assay can be used to detect suppressor activity of any viral protein.

In depth functional analysis is also required to understand the mechanism of action of RSS on the host RNAi pathways. Though a lot of information exists on the RSS mediated inhibition of siRNA biogenesis and function, the effect of RSS on the miRNA-mediated pathways is not explored. Thus, FHVB2 over-expressing transgenics were raised to functionally analyze the effect of FHVB2 on host plant miRNAs. The work was initiated in this direction by identifying the host factors interacting with FHVB2 using phage display and subtractive hybridization.

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