

EVALUATION OF THE ROLES OF CUCUMBER MOSAIC VIRUS 2b PROTEIN AND CO-INFECTING SATELLITE RNAs IN THE DIFFERENTIAL EXPRESSION OF MICRORNA-REGULATED GENES IN TOMATO

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Solanum lycopersicum, Cucumber mosaic virus, transcription factors, microRNA, gene expression profiling

Cucumber mosaic virus (CMV) encodes for a multifunctional protein, the 2b protein, that operates as the viral suppressor of RNA silencing in host plants. In arabidopsis, it was shown that the 2b protein can interfere with the microRNA (miRNA)-mediated regulation of gene expression of endogenous factors controlling plant development and leaf architecture, and that 2b proteins encoded by CMV strains belonging to two different subgroups were differently able to display such interference. We synthesized a chimeric infectious clone of CMV RNA2, pFny(LS2b), in which a 3'-terminal portion of RNA2 including the 2b coding sequence of CMV-Fny (an aggressive subgroup IA strain) was replaced by the analogous fragment of CMV-LS (a mild subgroup II strain). The clone was used to obtain a recombinant CMV strain, denoted CMV-Fny(LS2b).

CMV-Fny-induced symptoms in tomato consisted in severe mosaic, leaf shoestring and growth reduction, whereas those induced by either CMV-LS or CMV-Fny(LS2b), were limited to mild leaf narrowing, suggesting that most virus-induced developmental defects are due to the activity of CMV-Fny 2b protein.

Gene expression profiling by quantitative RT-PCR analysis of some transcription factors (e.g. ARF8, NAC1, lanceolate, phantastica) and other genes involved in the short RNA processing (e.g. AGO1, AGO4), most of which are known to be regulated by miRNA-guided mRNA cleavage, showed that CMV-Fny infections induced the modulation of most transcript levels, whereas there was no evidence for this in the case of both CMV-LS and CMV-Fny(LS2b) infections.

In another set of mRNA quantification assays, the same group of genes resulted not modulated when CMV-Fny was inoculated on transgenic tomato plants expressing an ameliorative satellite-RNA (satRNA) variant, codetermining an asymptomatic phenotype.

Altogether, these results suggest that the pathogenic properties of CMV-Fny on tomato are due to the ability of its 2b protein to interfering with developmental processes regulated by miRNA, that CMV-LS 2b protein has limited effects on such regulation, and that the suppression of symptoms mediated by ameliorative satRNAs may be driven by the substantial down-regulation of CMV RNA and gene products, including the 2b protein, in infected cells.