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EXPLORING THE SEQUENCE SPECIFICITY OF THE GENE SILENCING MECHANISM FOR THE FULL EXPLOITATION OF PATHOGEN-DERIVED RESISTANCE TO GEMINIVIRUSES

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Tomato yellow leaf curl is one of the most devastating diseases of the cultivated tomato caused by a close-related group of geminiviruses: the *Tomato yellow leaf curl* (TYLCV). Several laboratories worldwide have tried to introduce resistance to TYLCV species by transforming plants with viral-derived sequences. Nevertheless, only partial resistance has been obtained, suggesting that TYLCV should possess some special features that make it able to overcome pathogen-derived resistances.

We have previously shown that: a) transgenic expression of a truncated form of TYLCV-Sardinia (TYLCSV) replication-associated protein (Rep210) confers resistance to the homologous virus (*1, 2, 3, 4*) but not to a Spanish isolate of TYLCSV (TYLCSV-ES1) (*3, 4*); b) virus-specific small RNAs accumulated in TYLCSV-infected wt plants *(4)*; c) TYLCSV is able to overcome with time Rep210-mediated resistance by silencing the transgene and to spread in silenced transgenic plants (*4*); d) TYLCSV is able to infect silenced Rep-derived transgenic plants accumulating, before inoculation, virus specific siRNAs suggesting that TYLCSV can accommodate at some extent RNAi-mediated targeting of an essential viral gene (5); e) the susceptibility to TYLCSV-ES1 isolate could be the result of an early activation of the virus-induced silencing (*4*).

The above data prompted us to develop a new strategy (*6*) to escape the virus-induced gene silencing (VIGS) of transgenic viral sequences for enhancing pathogen-derived resistance to geminiviruses.

We have explored the sequence specificity of the RNA silencing mechanism to build a synthetic Rep210 transgene to be an inefficient target of the TYLCSV-induced gene silencing. Silent point mutations were introduced in the synthetic Rep210 transgene, distributed along the sequence in such a way that the continuous homology between the mutated sequence and the corresponding viral gene sequence was below or equal to 5 nucleotides. Transgenic plants expressing the synthetic Rep210 transgene showed an enhanced and broad resistance to viral infection. This general antisilencing strategy could also be applied to ameliorate cosuppression phenomena when overexpression of a plant gene is required.

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