Mediated RNA Virus Interference in N. benthamiana Using Novel CRISPR-CasRx

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Plant diseases cause an estimated of 10-15% reduction on global crop yields annually and 47% of this loss is caused by viruses. CRISPR-Cas systems endow bacterial and archaeal species with adaptive immunity mechanisms to fend off invading phages and foreign genetic elements. In this project, the ability of a newly discovered member of Cas13 variants, known as CasRx, was interrogated for robust interference against RNA viruses in planta. The RNA targeting ability of the CRISPR-CasRx system was tested against the modified Tobacco Mosaic Virus (TMV) in transient assays, where the coat protein was replaced with a green fluorescent protein (GFP) as a signal of the virus's activity. Different settings were experimented in targeting TMV in N. benthamiana via Agrobacterium by targeting the virus in two separate cellular localizations; the nucleus and cytoplasm of the plant. Results show that CasRx mediate high and robust interference activities against modified TMV-RNA virus in transient assays, with no significance in the different localization targets. Importantly, CasRx exhibits strong specificity against the target virus in comparison to a non-specific crRNA. Overall, CasRx is a robust Cas13 variant for RNA virus interference applications in planta and studying key questions relating to virus biology. Furthermore, the data gives an insight on future applications, including how permeant built-in plant immunity can be engineered by generating CasRx-crRNA overexpressing lines against TMV and other viruses. Also possible is the potential optimization of CRISPR/CasRx expression in different plant species and single and multiple targeting of viruses in the same plant.

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