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Mechanisms of Silencing Suppression by a Polerovirus P0 Protein

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Mechanisms of Silencing Suppression by a Polerovirus P0 Protein

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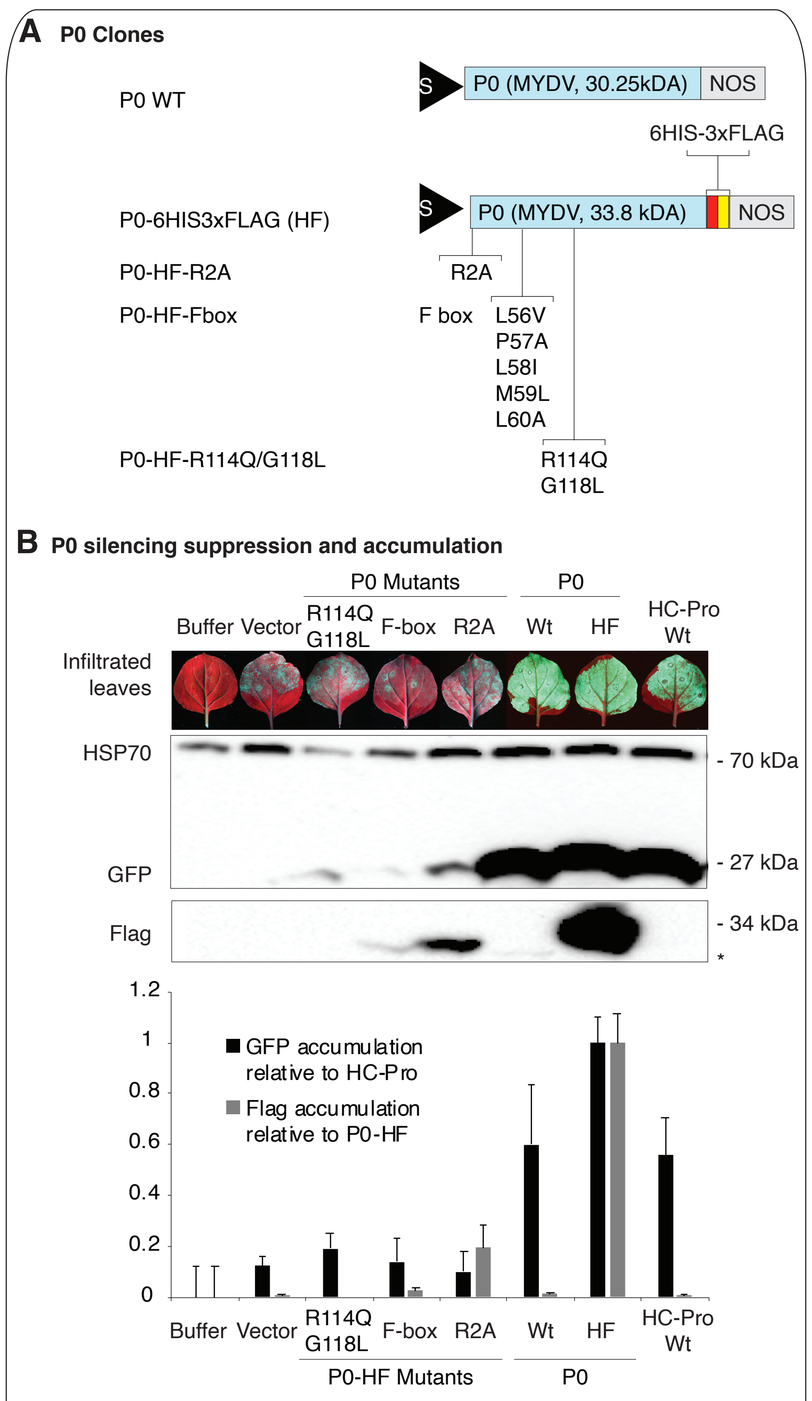
Background and Impact

Maize lethal necrosis is a detrimental disease to sub-Saharan Africa. To start to understand how to fight the disease, we must first understand the viruses that synergistically co-infect maize to cause it. RNA silencing suppressor proteins are pertinent for viral infections and are known to target different areas of the RNA silencing system in plants. Understanding these suppressors of RNA silencing will lead to a better perception of plant-virus interactions and, ultimately, help characterize maize lethal necrosis itself.

Figure 1. P0 is a silencing suppressor



• P0 was artificially synthesized from nucleotide sequences taken from Kenya and Rwanda. An HF tag was added to P0 for observing the stability of the protein. An inactive, yet stable mutation was made. (Figure 1A)

• Suppression of RNA silencing is shown in wild type (wt) and HF-tagged P0. ssGFP was infiltrated with P0 in wt Nicotiana benthamiana leaves. An empty vector was used as a negative control and HC-Pro as a positive control. GFP fluorescence was observed and photographed under ultraviolet light 4 days post infiltration. (Figure 1B)

• Protein was extracted and processed through western blot at 3 dpi. Heat Shock Protein 70 (HSP70) was used as a loading control. Anti-GFP probed for GFP expression while Anti-Flag probed for HF expression. Figure 1B quantifies GFP signal per treatment normalized according to HSP70 bands.

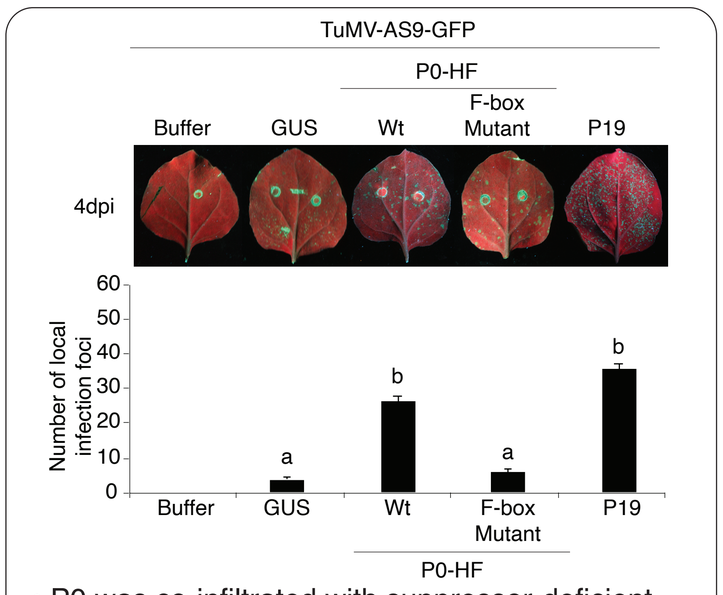
Model

• P0 is the silencing suppressor for the Polerovirus associated with maize lethal necrosis, MYDV-RMV

• P0 leads to the degradation of siRNA through Argonaute proteins

• The combination of several silencing suppressors from different viruses contribute to the detrimental nature of maize lethal necrosis because of the varying parts of RNA silencing they target

Figure 2. P0 restores pathogenicity to supressor deficent virus



• P0 was co-infiltrated with suppressor-deficient Turnip Mosaic Virus (TuMV). The spots of first infection were counted 4 days post infiltration. The number of infection foci within a 2cm by 2cm square were averaged over 4 experiments with 4 repetitions per experiment.

Goal

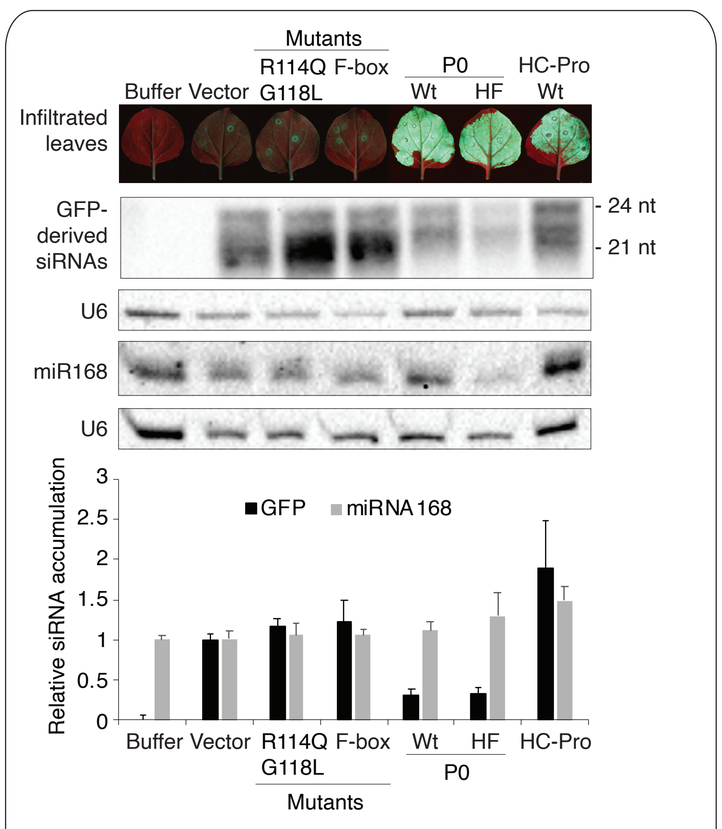
• Characterize P0 from MYDV-RMV

• Determine the silencing suppression ability of P0.

• Discover the effect of P0 on various Argonauts (AGOs), RNA-Dependent-RNA-Polymerases (RdRp's), and dicer-like proteins (dicers).

• Find the role P0 plays within maize lethal necrosis

Figure 3. P0 degrades siRNA

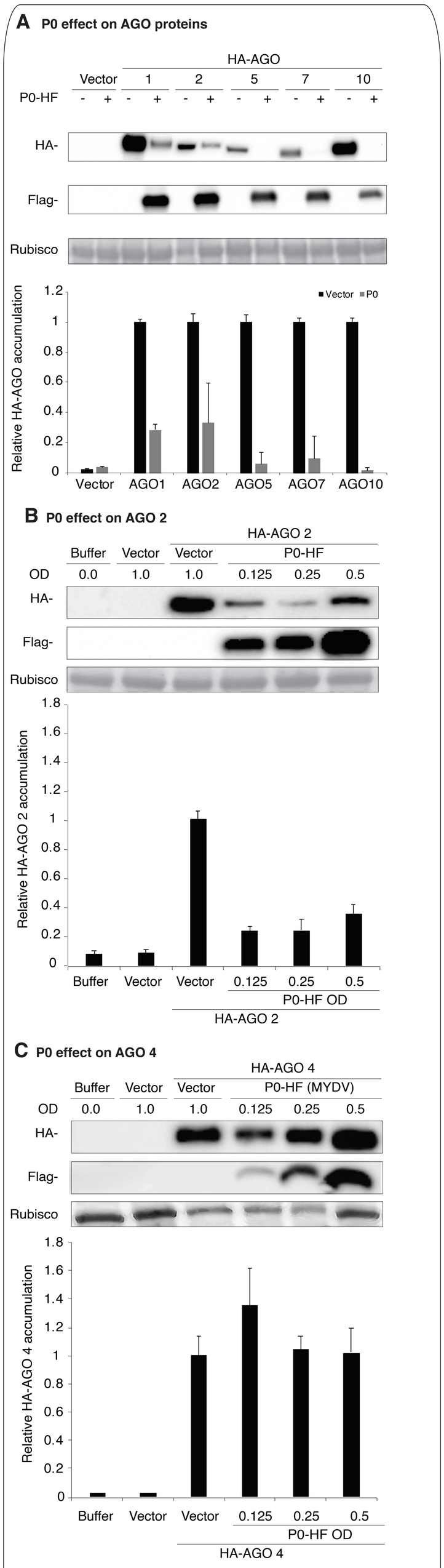


• RNA extractions were performed with the same samples as the protein. RNA was processed through a small RNA gel and northern blotting. U6 was probed for as a loading control. Anti-Dig-AP probed for GFP-derived siRNAs and miR168 expression. The graph of quantification was normalized to the loading control, Rubisco.

Key Findings

P0 from the MYDV-RMV-like polerovirus is a silencing suppressor. We created a stable, yet inactive mutation. P0 can also restore pathogenicity in two suppressor deficient viruses, further emphasizing it is an RNA silencing suppressor. We found that P0 decreases the accumulation of siRNAs which can be due to P0 affecting the stability or the biogenesis of the siRNAs. To test the stability of the siRNAs, we analyzed AGO 1, 2, 4, 5, 7, and 10 and found that P0 lowers the accumulation of all AGOs except 4. This confirms that P0 is affecting siRNA stability. Further analyses are being developed to test for P0's effect on siRNA biogenesis.

Figure 4 P0 degrades several Argonaute proteins



• HF-tagged P0 was co-infiltrated with HA-tagged AGOs 1, 2, 4, 5, 7, and 10. Protein was collected at 2 days post infiltration. Anti-HA probed for AGO expression. Figure 3A shows these results compared to vector + AGO for each AGO.

• Due to inconsistent results of AGO2 with P0 and oversaturation of AGO4 with P0, a dose response curve was performed. N. benthamiana leaves were co-infiltrated with varying concentrations of P0 and AGO2 and processed the same as the previous AGO experiment. (Figure 3B and 3C)