Characterization of a Viral Suppressor of RNA Silencing Encoded by CLRDV-AL

Bailee Kendra Price[1,], Mary Funmilayo Akinyuwa [2], and Sung-Hwan Kang [3]*

[1] Undergraduate Student, College of Sciences and Mathematics, Auburn University
[2] Graduate Student, Department of Entomology and Plant Pathology, Auburn University
[3] Assistant Professor, Department of Entomology and Plant Pathology, Auburn University

Cotton leafroll dwarf virus (CLRDV) is an infectious agent of Cotton Blue Disease. CLRDV is a member of the genus Polerovirus (Delfosse et al., 2014). CLRDV has six open reading frames (ORFs). Among them, ORF 0 encodes P0, which was identified as a viral suppressor of RNA silencing (VSR). CLRDV strains that were prevalent and caused severe yield loss in the cotton industry in African and South American countries had noteworthy differences among their VSRs, the P0 proteins (Agrofoglio et al., 2019). VSRs allow the virus to infect the host by disrupting the RNA silencing process, a major conserved mechanism, in which dsRNA is cleaved into siRNA by Dicer (Baulcombe, 2004; Wassenegger, 2002).

A new CLRDV strain has been isolated from Alabama and the rest of the United States Cotton Belt. To understand the pathogenicity of the Alabama strain of CLRDV (CLRDV-AL), our project aimed to characterize a CLRDV-AL encoded VSR, P0 protein, by expressing various recombinant P0 proteins via *Agrobacterium*-mediated infiltration to examine them for silencing suppression potency, intracellular localization, and self-interaction.

Sequence alignment comparing CLRDV-AL to the previously isolated strains from South American countries indeed showed some mutations within the domain known for VSR function (Agrofoglio et al., 2019).

To verify the P0 protein's ability to suppress the RNA silencing, P0 was concurrently expressed next to the VSRs from other viruses along with a green fluorescent protein (GFP) in *Nicotiana benthamiana* 16c plants, a transgenic line that constitutively expresses GFP (Fig. 1). This assay demonstrated that P0 protein of CLRDV-AL is not a potent silencing suppressor compared to other VSRs such as P19 and HC-Pro known for their strong silencing suppression.

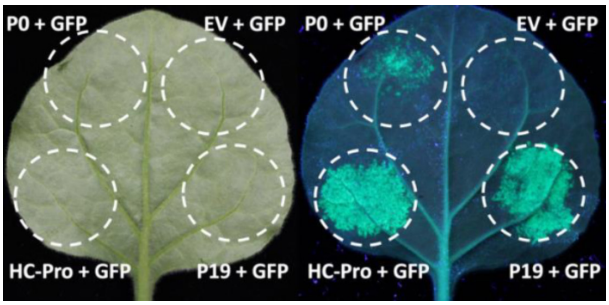

Fig. 1. GFP silencing and its suppression in GFP-transgenic N. benthamiana plants.

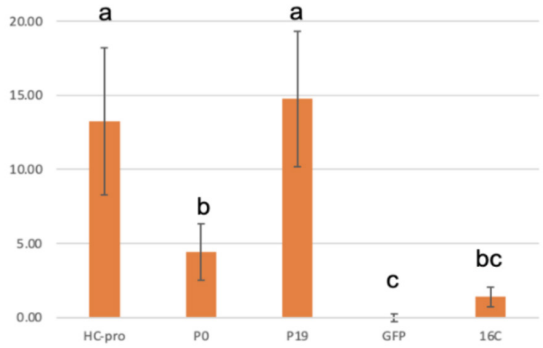

Fig. 2. The P0 protein of CLRDV-AL is a weak VSR.

Infiltrated leaf tissue showing suppression of GFP silencing was examined using a fluorescence microscope. The fluorescence intensity was calculated from more than 15 images per treatment using Region of Interest (ROI) on ImageJ, and the relative intensity was analyzed by a one-way ANOVA test in R (Fig. 2; significant difference, $p < 0.01$, was denoted by letters).

As some VSRs are known to form a dimer that interacts with small-interfering RNAs, the self-interaction of P0 proteins was examined using the yeast-two-hybrid

*Corresponding author: bkp0019@auburn.edu

(YTH) assay (Fig. 3). No growth of the co-transformed yeast cells on the selective media implied no homo-dimerization of CLRDV-AL P0 proteins.

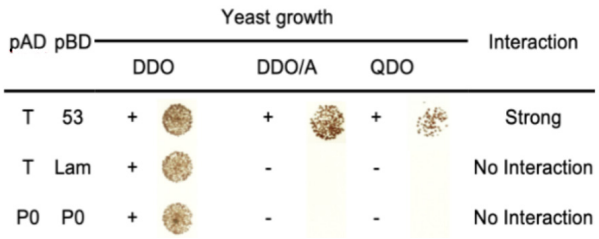

Fig. 3. Self-interaction of CLRDV-AL P0 protein. A pair of pAD-T and pBD-53 is a positive control. A pair of pAD-T and pBD-Lam is a negative control.

The intracellular localization of P0 investigated by expressing GFP-tagged P0 proteins in the plant cells using a fluorescence microscope revealed the possibility of P0 protein being a membrane-bound protein (data not shown). Hence, considering YTH is not a suitable method for the membrane proteins, the homo-dimerization of P0 proteins is still in question.

Our study showed that VSR encoded by CLRDV-AL has interesting characteristics that differ from well-known VSRs. More studies may be needed to identify the cause of such differences further.

Statement of Research Advisor

Bailee was an inaugural member of the AU plant virology lab, and she continued her research as an undergraduate research fellow. Bailee led and conducted experiments in this project, from constructing various clones to testing them in plants and yeasts. A graduate research assistant, Mary Akinyuwa, assisted her with acquiring and analyzing the data. This research demonstrated peculiar characteristics of the protein encoded by newly isolated Polerovirus.

- Sung-Hwan Kang, Entomology and Plant Pathology, College of Agriculture

References

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Authors Biography

Bailee K. Price is a senior-year student pursuing a B.S. in Biomedical Sciences at Auburn University. She was a research assistant and, subsequently, a research fellow in the Plant Virology laboratory. She has played key research roles in the alignment of sequences, cloning of constructs, agroinfiltration, and YTH assays.

Mary F. Akinyuwa is a Ph.D. student at Auburn University. Her research focuses on the molecular characterization of viral proteins. She earned M.Sc. in Plant Health from the European Erasmus Mundus program. She has contributed to the study by assisting with microscopy, YTH assay, and analyses of fluorescence images.

Sung-Hwan Kang, Ph.D., is an assistant professor of plant virology at Auburn University, where he began working in 2020. He earned his Ph.D. in Microbiology and Molecular Biology at the University of Nebraska. His research interest includes the molecular mechanism of host infection by viral pathogens in plants.