Enhancing Plant Defense: Carbon Dots for Efficient Spray-Induced Gene Silencing

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Abstract (250 words)

Ectopic RNA application for plant defense faces challenges in tree crops, including size, diffusion, and stability of active compounds such as ribonucleoproteins and nucleic acids. While existing strategies involve expressing dsRNA in transgenic plants targeting pathogens, our research strives to develop a transient RNAi system based on Spray-Induced Gene Silencing (SIGS). This approach aims to circumvent legal barriers and public concerns associated with genetically modified organisms (GMOs). Our strategy integrates SIGS with branched polyethyleneimine-functionalized Carbon Dots (bPEI-CDs) as nanocarriers, effectively addressing unique delivery challenges in plant defense as RNA stability and uptake enhancement. We developed a cost-effective microwave-assisted protocol for the synthesis of 10 nm monodispersed bPEI-CDs. These molecules showed over 50% dsRNA protection against RNase III degradation at a 200:1 (w/w) ratio. We further demonstrated their capability to enhance cellular uptake by successfully delivering fluorolabeled-dsRNAs (Cy3-RNA) complexed with CDs into intact plant tissue-cultured cells. Notably, Cy3-RNA sprayed with CD on the surface of greenhouse grapevine leaves through a low-pressure spray application penetrated stomata cells and neighboring cells. Ultimately, the ability of dsRNA complexed with CD to trigger RNA interference was confirmed using a 21 nucleotide-dsRNA targeting eGFP . Ongoing work quantitatively compares, through qPCR, the reduced expression of GFP on eGFP-expressing transgenic microvine leaves sprayed with CD-complexed and naked siRNA. This innovative approach, leveraging CDs, aims to tackle critical barriers in dsRNA delivery, particularly by addressing cell wall-related limitations in plant. This research marks a pivotal step in optimizing RNA-based grapevine defense strategies, and propelling sustainable viticulture practices forward.

Keywords: RNA interference, Spray-Induced Gene Silencing (SIGS), Carbon dots, dsRNA delivery, Sustainable agriculture.