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Topic of Research: Novel Clay nanosheet mediated delivery of dsRNA into *Nicotiana tabaccum* plants against CLCuD infection

Cotton leaf curl disease (CLCuD), caused by begomovirus (Family; *Geminiviridae*) associated with ssDNA satellite molecules, is a devastating disease of cotton. Begomovirus transmitted by whitefly (*Bemisia tabaci*) causes major threat to global agriculture, severely reducing crop productivity and posing challenges in crop production. Conventional strategies for managing CLCuD rely on hazardous chemical pesticides and RNAi-based transgenic plant development. However, these approaches face significant challenges, including environmental concerns, regulatory hurdles for approval, and limited public acceptance.

Findings:

This study explored the RNAi based non-transgenic strategy, also known as spray-induced gene silencing (SIGS) against CLCuD infection. V2-dsRNA designed against the begomovirus species, *Cotton leaf curl multan virus* (CLCuMuV), expressed through bacterial cell culture showed a promising alternative for crop protection. Synthesized Clay nanosheet (LDH) bounded to V2 specific double-stranded RNA (dsRNA) at a specific ratio (RNA: LDH, 1:4) called as bioclay. The bioclay spray on leaves of *Nicotiana tabaccum* provided persistence and availability of dsRNA molecules up to 37 days compared to 20 days of naked dsRNA spray. Bioclay enhanced dsRNA stability, protected it from RNase degradation, and enabled longer release than naked dsRNA. The bioclay spray has provided extended protection from CLCuMuV infection. In terms of symptom appearance, control plants developed symptoms by 30 days post virus inoculation (dpi). Plants treated with naked dsRNA showed shorter protection window, with symptoms appeared in 50% plants by 30 dpi and 80% plants by 50dpi. In contrast 78% of bioclay sprayed *N. tabaccum* plants remained asymptomatic until 75dpi. Bioclay sprayed plants also exhibited least virus accumulation at 25dpi and 50dpi, in compared to naked dsRNA sprayed and control plants. Moreover, the Bioclay-based dsRNA spray against the V2 gene of CLCuMuV on *N. tabaccum* plants demonstrated significant effectiveness in suppressing symptoms and preventing CLCuD infection compared to both naked dsRNA sprayed and control *N. tabaccum* plants.