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Name of Department: Biosciences

Topic of Research: Designing and developing amiRNAs targeting cotton-infecting begomovirus

Findings

Cotton (*Gossypium* spp., family *Malvaceae*) is a crucial fibre crop that suffers severe yield losses due to Cotton leaf curl disease (CLCuD). This disease is caused by cotton-infecting begomoviruses and associated satellite molecules, and is transmitted by the whitefly (*Bemisia tabaci*). Conventional control methods are often less effective due to the rapid evolution of the viral genome. In contrast, RNAi-based strategies offer a robust approach by silencing virus genes to confer resistance.

In this study, artificial microRNAs (amiRNAs) were employed for the targeted silencing of *Cotton leaf curl Multan virus* (CLCuMuV). To this end, *in silico*-identified cotton miRNAs were modified to design amiR414e and amiR2949a, which target the overlapping C1 and C4 genes of CLCuMuV. Synthetic precursor (pre)-amiRNAs (pre-amiR414e and pre-amiR2949a) were cloned into the pBI121 vector and transformed into *Gossypium hirsutum* cv. HS6.

Notably, the amiRNA transgenes (amiR414e and amiR2949a) in transgenic (T_1 and T_2) plants of *G. hirsutum* exhibited stable Mendelian inheritance (3:1) and enhanced resistance to CLCuD. At 60 days post-inoculation (dpi), PCR analysis revealed no accumulation of CLCuMuV and absence of disease symptoms in most transgenic plants. Quantitative RT-PCR (qRT-PCR) analysis further confirmed that transgenic (T_2) plants overexpressing amiRNAs showed significantly reduced expression of the C1 gene. Moreover, amiRNA expression exhibited a negative correlation with C1 gene expression.

Overall, transgenic *G. hirsutum* cv. HS6 plants exhibited tolerance to CLCuMuV infection, underscoring the efficacy of the amiRNA-based approach in conferring broad-spectrum resistance against begomoviruses in cotton and potentially other crops.

Keywords: Cotton, RNAi, miRNA, artificial (a) miRNA, Transformation, CLCuMuV, Begomovirus