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CRISPR/Cas-based virus resistance in cotton

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Cotton leaf curl disease (CLCuD) is one of the major factors for low yield of cotton in Pakistan. Engineering resistance in cotton cultivars against CLCuD through RNA-directed Cas9 is a powerful tool to tackle the viral disease in cotton. We have targeted coding and non-coding regions of viral DNA individually with Cas9 and dCas9 and found promising potential target sites for viral interference. On basis of screening results of gRNAs tested in transient assay in *N. benthamiana*, we designed multiplex gRNA for targeting

three most promising sites simultaneously with Cas9 and dCas9. We found that targeting non-coding regions of virus is more effective for virus suppression than targeting coding regions. In transient assay, we found 70-90% decrease in accumulation of virus. Stable transformation of cotton has been optimized with DsRED and transformation with multiplex gRNAs is under progress.

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