

Curly Top Disease Resistance in Sugar Beet by Targeting *Becurtovirus* Genome with CRISPR/Cas9

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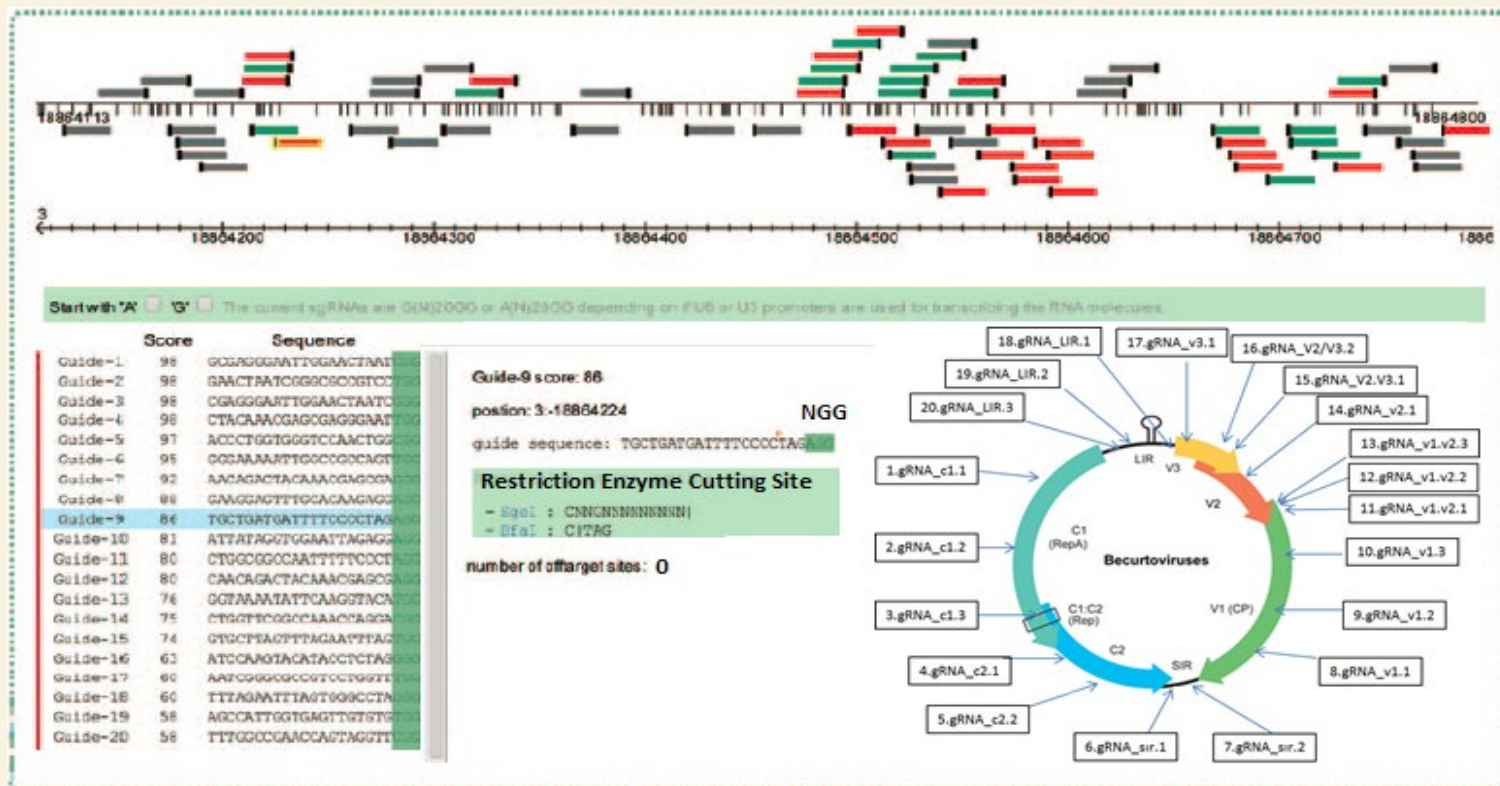
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- **CRISPR/Cas technology** had been used as a highly specific tool for generating resistant plants to DNA viruses
- **The main aim of this study** was to test the potential utilization of multiple CRISPR/Cas technology for the Becurtovirus-mediated curly top disease resistance in sugar beet

METHODOLOGY

1. Designing of the gRNAs to target the Becurtovirus genome

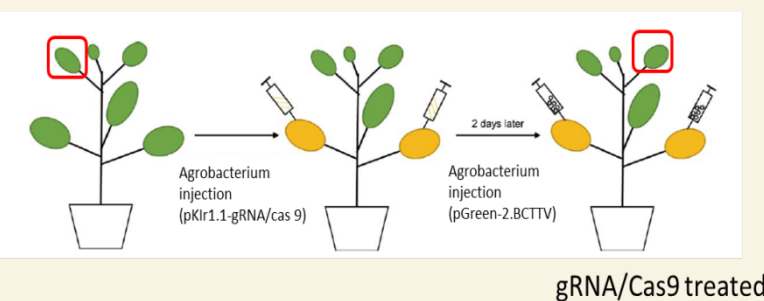


2. Transferring each gRNA into Cas9 containing Agrobacterium plasmid

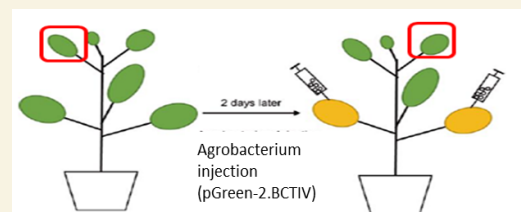


In the study, 20 gRNAs were designed to target the genic and intergenic regions of Becurtovirus genome. All the gRNAs were inserted into Cas9 containing agrobacterium plasmid and agroinoculated into sugar beet leaves for transient expression. After viral agroinoculation of the leaves, loss of restriction enzyme cutting site on the the gRNA targeted viral genome was detected with PCR and confirmed by sequencing. Viral accumulation was detected by qPCR and the most effective four gRNAs inhibiting the viral replication were inserted into a multiple CRISPR plasmid to completely degrade the viral genome.

3. Agroinoculation of gRNA/Cas9 construct into Becurtovirus agroinfected sugar beets

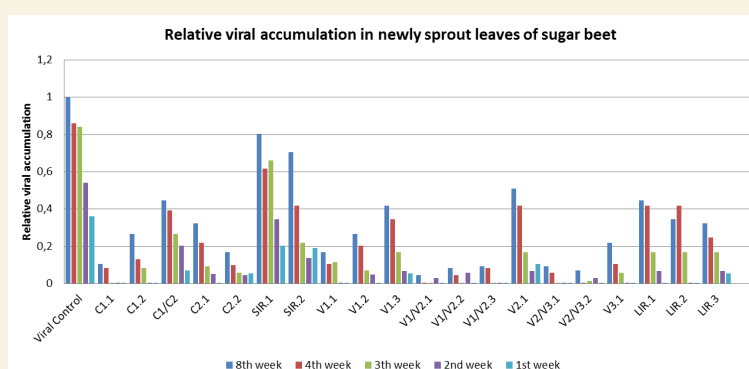


gRNA/Cas9 treated

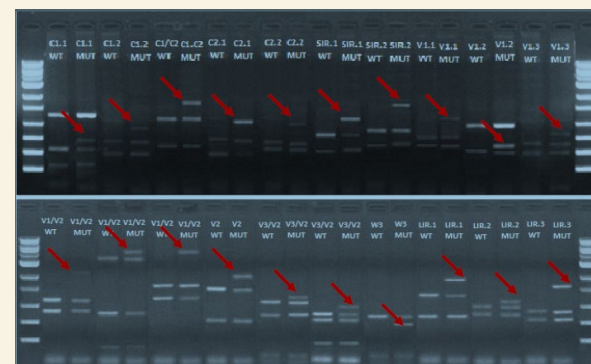


Positive viral control

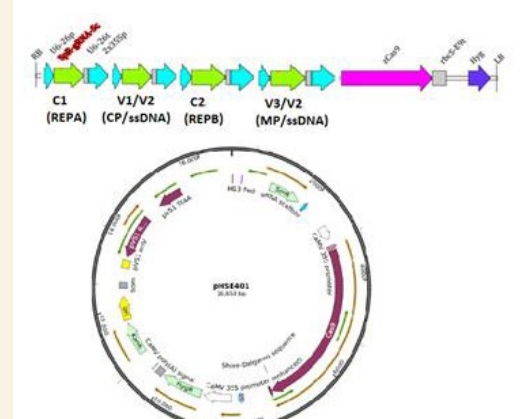
5. Relative qPCR assay to detect viral replication and its mobility in sugar beet



4. RE-PCR assays and sequencing to detect mutations on the viral genome

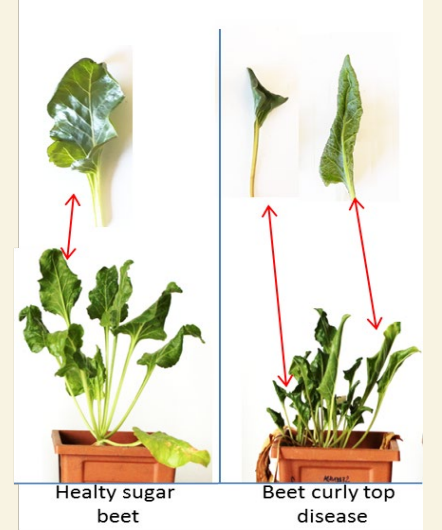


6. Designing multiple CRISPR system to completely degrade Becurtovirus genome

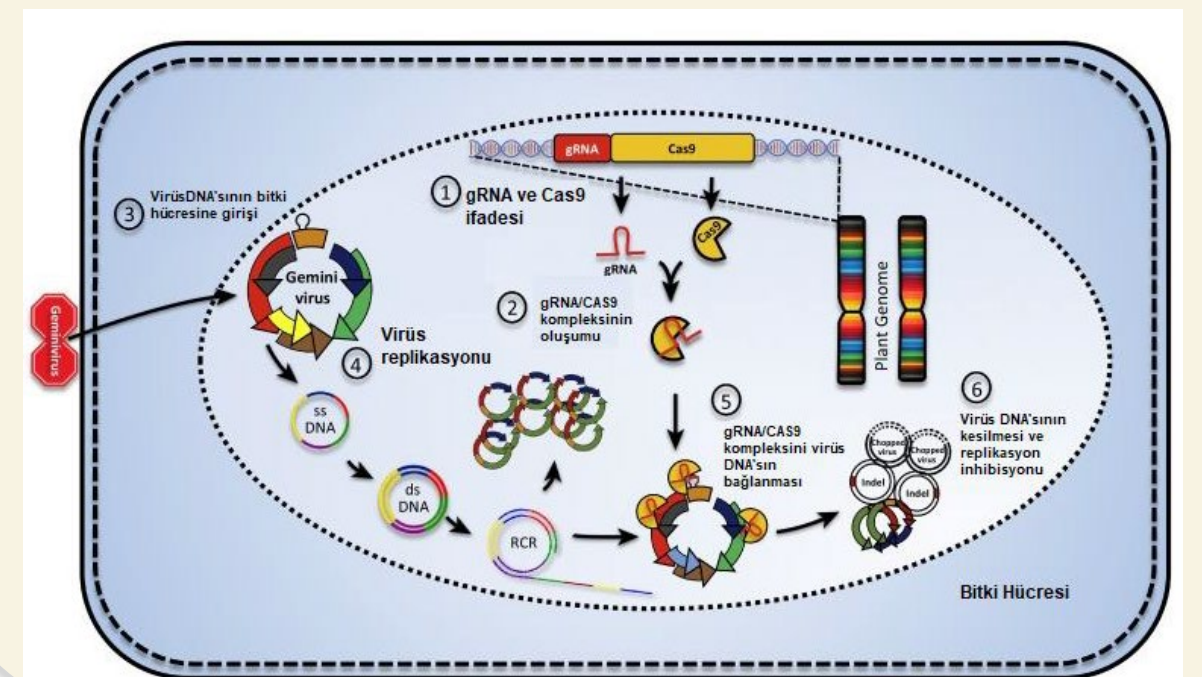


BACKGROUND

Beet curly top disease (BCTD) is a pathogenic viral infection for sugar beet (*Beta vulgaris*) that causes yield limitation in arid and semi-arid regions of the world. BCTD has become more prevalent in sugar beet fields with the effect of global warming in the Mediterranean Basin and the Middle East (e.g., Iran and Turkey). Beet curly top Iran virus (BCTIV, Becurtovirus) have been reported to be the main causative viral agents for the BCTD in this region. Disease incidence was reported to be more frequent in Turkey in last decades and caused %50 yield reductions in sugar beet fields during dry seasons.



CRISPR/Cas technology is a simple and efficient gene targeting technology. Any gene or genomes can be directly targeted and mutated easily by just designing a gRNA/Cas9 construct. This gene targeting technology was also utilized against several DNA viruses (geminiviruses) such as BCTV (Curtovirus, Ji et al. 2015), BeYDV (Mastervirus, Baltes et al. 2015), TYLCV (Begomovirus, Ali et al. 2015), and CLCuMuV (Begomovirus, Yin et al. 2019). CRISPR/Cas-mediated viral resistance was reported to be highly effective on inhibiting the viral accumulation in plant cells and could be utilized to increase viral disease resistance in plant species.



OUTCOMES

In the study, 20 gRNA/Cas9 constructs targeting replication, capsid, mobility and ssDNA regulatory genes and intergenic (LIR-SIR) regions in the genomes of Becurtovirus were designed and first transiently expressed in sugar beet with agrobacterium vector. It was determined by PCR and qPCR tests that gRNA/Cas9 constructs stopped the replication and spread of the virus in the range of 20% to 80% in all sugar beet lines. In transient expression experiments, 4 gRNAs that inhibit virus replication and movement at the highest rate and target the virus's REPA, REPB, capsid and movement genes were combined into a single Cas9-containing vector and agroinoculated into sugar beet with transient transformation. The results revealed that this quaternary system completely stopped the virus replication and relieved the symptoms of the beet curly top disease on sugar beets and provided 100% resistance. All these results indicated the excellent potential of multiple CRISPR system on the viral resistance for agricultural plants.



Becurtovirus agroinfected sugar beets



Becurtovirus agroinfected sugar beets treated with multiple CRISPR

ACKNOWLEDGEMENTS

This study was supported by The Scientific and Technological Research Council of Turkey (TUBİTAK) with project number TOVAG_2170232