Development of U.S. BNYVV infectious clones to study Rz1 and Rz2 resistance breaking.

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Beet necrotic yellow vein virus (BNYVV) is a *Benyvirus* consisting of four positive-sense single-stranded RNAs. BNYVV is the main agent responsible for the most devastating sugar beet viral disease worldwide, Rhizomania. Rhizomania leads to a major reduction in sugarbeet yield and sugar content, resulting in significant financial losses to the industry. To manage BNYVV, three sugar beet genotypes possessing Rz1 (the mostly widely used resistance gene), Rz2, or more recently the combined Rz1+Rz2, have been deployed. Over the last 16 years, resistance-breaking (RB) strains of the virus have been reported for Rz1, a phenomenon associated with modifications in the gene p25 encoded on RNA3. More recently, RB strains have been identified that appear to overcome Rz2- and Rz1+Rz2-mediated resistance, but the mechanism of RB remains elusive. In order to investigate the role of RNA-3 and potentially other genome regions in the RB phenomena, BNYVV infectious clones have been created for the first-time using U.S. strains of the virus.

BNYVV RNA-1 and -2 clone design and construction was based on RNA sequencing data obtained from BNYVV-infected plants grown in infested soil possessing RB isolates of virus. RNA-3 and -4 clones were directly isolated from these plants *via* RT-PCR. Our results shown that these clones, when transcribed to produce capped RNAs, are infectious on *Chenopodium quinoa* and *Beta macrocarpa* hosts. Protein p25 modifications associated with RB in U.S. will be introduced in the p25 gene and tested in sugarbeet genotypes encoding the different resistance gene complements. Through use of these clones, a better understanding of BNYVV virulence in general and the RB phenomenon specifically will be obtained. Additionally, the clones will allow us to study other viral functions, some of them may determine virus transmission and aggressiveness, leading to strategies for the inhibition of BNYVV infection in plants.

Keywords: Resistance, BNYVV, Infection, Gene.