# GENERATION AND DISPERSION OF RESISTANCE BREAKING VARIANTS OF *BEET NECROTIC YELLOW VEIN VIRUS* IN THE FIELD

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## Abstract:

Around 10 years after the massive deployment of commercial sugarbeet varieties encoding the *Rz1* allele, which confers partial dominant resistance to *Beet necrotic yellow vein virus* (BNYVV) infection, the emergence of resistant breaking (RB) variants of BNYVV was verified in the Imperial Valley of California. Preliminary data suggest that breakdown of *Rz1*-mediated resistance is also occurring in other production regions of North America. Genetic sequencing of the viral RNA 3, encoding the pathogenic determinant p25 gene, revealed a strong correlation between its amino acid motifs and type of plant virus interaction (i.e., in this case, compatible = disease and incompatible = asymptomatic infection) in the 'Imperial Valley' pathosystem. Thus, most plants from yellow spots in the field and with severe symptoms of rhizomania were infected by virus haplotypes encoding the $V_{67}L_{68}E_{135}$ p25 motif. By contrast, most asymptomatic plants outside the yellow spots were infected by virus haplotypes encoding the avirulent $A_{67}L_{68}D_{135}$ or less frequently by the nationwide wild type $A_{67}C_{68}D_{135}$ motifs. This specific evolutionary trajectory of BNYVV from wild type to RB genotypes apparently was favored under the Imperial Valley conditions. An alternative evolutionary trajectory was found in Minnesota, where wild type $A_{67}C_{68}D_{135}$ evolved directly into an RB variant encoding $V_{67}C_{68}D_{135}$. These observations demonstrate that the replacement of alanine by valine at position 67 of p25 is critical to overcome *Rz1*-mediated sugarbeet resistance under natural conditions.

## Introduction:

Most known *R*-genes, conferring resistance to virus infections, have lasted more that 25 years without the generation of successful RB variants in the field (Garcia-Arenal and McDonald, 2003). However, there are some exceptions and the *Rz1*-BNYVV interaction is one of them. Some of the host conditions that facilitate generation-selection of viral RB genotypes have been uncovered (Acosta-Leal et al., 2008; Acosta-Leal and Xiong, 2008). In general, host genotypes that allow virus accumulation above a certain threshold apparently are prone to develop RB infections. The resistance mechanisms governed by *Rz1* are phenotypically expressed by restricting virus accumulation in taproots and suppressing rhizomania development (Tamada et al., 1999). At the biochemical level, resistance is associated with differential expression of genes involved in pathogenesis and hormone-mediated plant development (Burketová et al., 2003; Larson et al., 2008). Reverse genetics experiments indicate that a viral determinant for resistance breakdown is the presence of valine at position 67 of p25 (Koenig et al., 2009). This amino acid change was previously associated with rhizomania in field samples from California Imperial Valley (CIV, Acosta-Leal and Rush, 2007). The objective of this work was to determine the genetic changes of BNYVV associated with dispersion of the disease in Rz1-cultivars in two different pathosystems, CIV and Minnesota.

## Procedures:

BNYVV titer was estimated in asymptomatic and symptomatic (i.e., greens and yellows, respectively) *Rz1*-plants collected from three locations of Minnesota and four fields from CIV during 2005-7. Realtime RT-PCR, targeting the RNA2 CP region, was used for this purpose. Also, same samples were proceed to estimate the titer of specific wild type $A_{67}C_{68}$ motif of p25 (RNA 3) using allele specific TaqMan probes (Acosta-Leal and Rush, 2007).

## Results & Discussion:

Roots from yellow plants contained greater CP titer that green plants (Fig. 1). However, this difference was not statistically significant with those samples collected from MN-2005 (Fig. 1B). Coincidently, only in these samples another benyvirus, *Beet soilborne mosaic virus* (BSBMV), was detected in high incidence. It was 100 and 60 percent in green and yellow *Rz1*-plants, respectively. Also the BSBMV titer was greater in asymptomatic that symptomatic *Rz1*-plants.

A greater CP titer in yellow plants was associated with lower titer of RNA 3 encoding wild type $A_{67}C_{68}$ p25-motif (Fig. 2). Most drastically, $A_{67}C_{68}$ p25-motif was completely undetected in yellow plants from MN-2007 (Fig. 1C). These observations suggested that most of those heavily infected *Rz1*-plants were carrying a different, and consequently, undetected p25-motif. Allelic discrimination realtime RT-PCR assays indicated that this putative different p25-motif did not correspond to the resistance breaking $V_{67}L_{68}$ p25-motif commonly found in CIV. Sequencing of high-fidelity amplicons derived from green and yellow Rz1-plants from MN revealed that only yellow plants were infected by a newly found RB variant encoding a $V_{67}C_{68}$ p25-motif. The genetic change behind this amino acid shift was a nucleotide transition from C to U at codon. Thus, same amino acid substitution at position 67 account for resistance breakdown in CIV and MN.

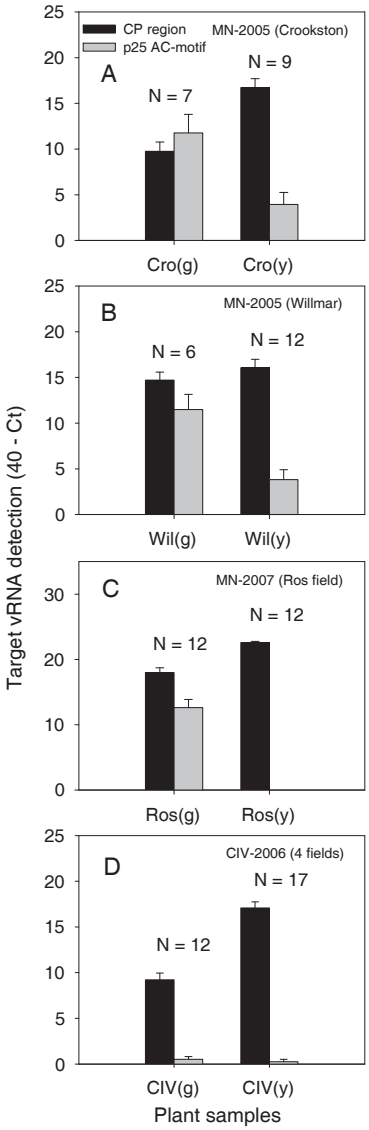


**Fig. 1**. Relationship between CP (RNA 2) titer and wild type $A_{67}C_{68}$ p25 (RNA 3) titer in *Rz1*-plants collected in three locations from Minnesota and one from California.

Phylogenetic analysis of field isolates of BNYVV collected from a) susceptible symptomatic, b) *Rz1*-resistant symptomatic and c) *Rz1*-resistant asymptomatic sugarbeet plants revealed that a wild type viral RNA3 was broadly distributed in North America infecting susceptible plants (Acosta-Leal et al., 2008). This wild type BNYVV genotype encodes the $A_{67}C_{68}D_{135}$ amino acid motif at p25. On the other hand, viral isolates taken from asymptomatic *Rz1*-plants are spitted in two evolutionary lineages: the MN-lineage represented by isolates with RNA3 sequences identical to wild type BNYVV and the CIV-

lineage characterized by avirulent isolates encoding an $A_{67}L_{68}D_{135}$ p25-motif. Both avirulent groups of isolates can accumulate above a moderate threshold in *Rz1*-plants, which suggests that the genetic differences between them are not critical for their surviving in this restrictive host environment. Moreover, this limited virus accumulation in both cases was apparently good enough for the generation-selection of RB variants where a single nucleotide substitution in the hypervariable tetrad of p25 was required. This explains why Rz1-mediated resistance has been exceptionally easy to overcome by BNYVV.

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