IN SEARCH OF PREDICTORS OF SUGARBEET RESISTANCE DURABILITY TO *BEET NECROTIC YELLOW VEIN VIRUS*

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Abstract:

A natural trend of pathogens is to evolve into new forms that allow them to overcome host resistance. The relatively recent emergence of resistance breaking strains of *Beet necrotic yellow vein virus* (BNYVV) in the USA offers the opportunity to understand this event at the ecological and molecular levels. We have monitored the genetic response of an original wild type BNYVV strain to different host constraints. Analyses of virus populations infecting susceptible cultivars were composed of a predominant master haplotype immersed in a background of haplotypes exhibiting minimal nucleotide diversity (π = 0.0006 ± 0.0002). Surprisingly, this highly stable virus population dramatically changed its structure during passage through resistant sugarbeet cultivars. In plants carrying the *Rz1* resistant allele, most virus populations contained two master haplotypes and were more heterogeneous (π = 0.0010 ± 0.0002). Deviation from the wild type BNYVV was even more dramatic in plants carrying the stronger *Rz2* resistance gene (π = 0.0019 ± 0.0005). In this case, every single plant was infected by a different master haplotype, which gave place to virus genomes with two or more mutations. Thus, stronger restriction to BNYVV accumulation seems to promote greater virus diversification.

Introduction:

The capability of a plant virus to defeat host resistance depends on the frequency of its encounters with a potential host and its ability to generate and maintain genetic variation. BNYVV is transmitted by zoospores of *Polymyxa betae*. Initially, primary zoospores are released from soilborne resting spores called sporosori. Then, after each rain or irrigation event during the growing season, secondary zoospores are repeatedly released from intracellular zoosporangia (Rochon *et al*., 2004; Rush, 2003). This cycling production of viruliferous zoospores magnifies the BNYVV encounters with partially resistant sugarbeets.

BNYVV is a multiparticulate, single-stranded, positive sense RNA virus. RNA 1 and 2 encode the essential elements for replication, encapsidation, and cellular translocation, whereas RNA 3, 4, and 5 are translated into proteins involved in pathogenesis, vector transmission, and suppression of gene silencing (Link *et al*, 2005; Rahim *et al*., 2007; Tamada, 2002). Despite its divided genome, and the potential of mixed infections with different strains (Koenig *et al*., 1995), high genetic stability seems to be the norm between spatiotemporally separated populations (Koenig and Lennefors, 2000). p25 (RNA 3) and p26 (RNA 5) cistrons are the most variable genomic regions with strong positive selection acting on some of their amino acids (Schirmer *et al*., 2005). These genes operate synergistically to exacerbate symptoms in certain sugar beet cultivars (Klein *et al*., 2007; Link *et al*., 2005), but p25 accounts for most of the rhizomania syndrome (Tamada *et al*., 1999). Amino acids of p25 at positions 67-68 and 135 are associated with the capability of some BNYVV populations to overcome *Rz1*-mediated

resistance (Acosta-Leal and Rush, 2007). In fact, a nucleotide substitution at codon 67 is enough to overcome *Rz1*-mediated resistance (Koenig *et al*., 2009). Our working hypothesis is that generation-selection of resistant breaking mutations is affected by cellular host environment. To initially test this hypothesis, we have analyzed the genetic variability of BNYVV populations isolated from sugarbeet genotypes with different levels of resistance.

Procedures:

BNYVV-resistant sugarbeet cultivars carrying the dominant *Rz1* or *Rz2* alleles, and a susceptible (*rz1rz2*) control were soil-inoculated with a wild type BNYVV isolate (Clx, accession no. EU480492). For the second serial host passage, approximately 50% of the soil in each pot of the initial passage was used to create a replicate of the same experiment. Plant roots were harvested 12-14 weeks after planting. High fidelity amplicons derived from single-plant total RNA samples were cloned and sequenced. Same RNA preparations were used for relative viral RNA quantification by realtime RT-PCR. Genetic diversities and distances within and between populations were estimated based on the Kimura's 2-parameter model, as implemented in MEGA 3.1 (Kumar *et al*., 2004). Genetic differentiation between pairs of populations was statistically estimated by the Wright's F_{ST} index of dissimilarity as implemented in Arlequin v.2.000 (Schneider *et al*., 2000) for haplotypic data. In brief, F_{ST} measures the degree of differentiation between two putative subpopulations by the correlation between two haplotypes drawn at random from each subpopulation and comparing it against the correlation obtained when the haplotypes are taken from the same subpopulation.

Results & Discussion:

BNYVV content was estimated by relative quantification realtime RT-PCR in total RNA samples extracted from lateral or tap roots of individual plants. Viral titers in lateral roots from the first host passage were statistically different ($P < 0.01$) only between *Rz2*-plants (3.37 ± SE = 0.42) versus *Rz1*-plants (4.67 ± 0.42) or the susceptible control (4.80 ± 0.22), but not between *Rz1*-plants and the susceptible control. In the following serial host passage, tap and lateral rootlets were separated during sampling, and virus titer was determined only in tap rootlets, which are better indicators of the resistance level (Tamada *et al*., 1999). This procedure revealed that virus content in tap rootlets was directly proportional to the strength of resistance. Thus, *Rz2*-plants exhibited the lowest virus content (0.56 ± 0.27), followed by *Rz1*-plants (3.26 ± 0.28), and then the susceptible control (4.52 ± 0.16, Fig. 1).

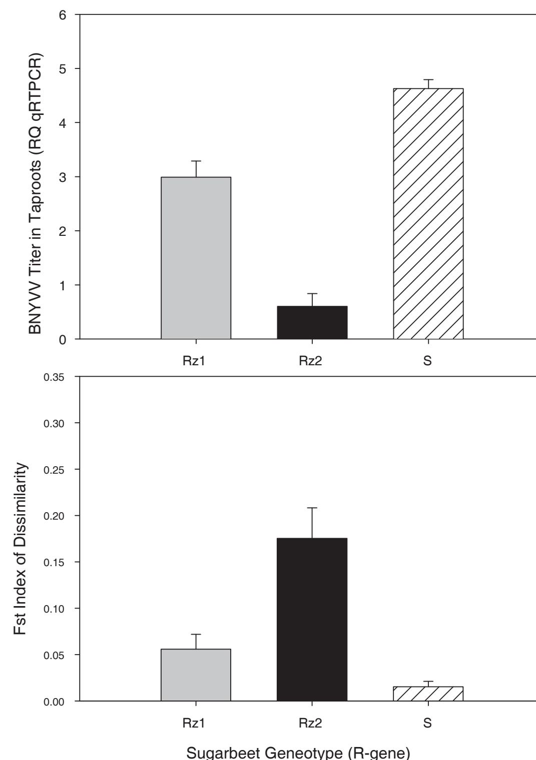

Fig. 1. Relationship between titer of BNYVV RNA in tap roots and the index (Fst) of genetic dissimilarity between single-plant virus populations infecting two resistant (*Rz1* and *Rz2*) and a susceptible (S) sugarbeet genotypes.

Nucleotide diversity (π) of virus populations cloned from S, *Rz1*-, and *Rz2*-plants from second host passage was 0.00063 ± 0.0002, 0.0010 ± 0.0002, and 0.00188 ± 0.0004, respectively. Pairwise comparisons between these three virus populations by the Fst index of dissimilarity revealed that also their genetic composition was statistically different ($P < 0.01$, Fig. 1).

By comparing the genetic composition of a parental wild type BNYVV population with the progenies generated in susceptible and resistant plants, we found that the genetic virus diversity was inversely proportional to virus content in a specific host genotype. Thus, whereas the same wild type haplotype predominated in all of the analyzed susceptible plants, which is consistent with the high genetic stability of BNYVV observed in the field (Acosta-Leal *et al*., 2008; Koenig and Lennefors, 2000), most resistant plants were infected by different master haplotypes. Selection analyses suggest that these haplotypes drifted at random from a population subjected to purifying selection. These findings have significant implications to our understanding of BNYVV evolution. Moreover, the relationship between virus content and genetic diversity of BNYVV in a resistant genotype might be an indicator of the resistance durability.

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