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The variability of Beet necrotic yellow vein virus P25 pathogenicity factor previously allocated to geographically distinct isolates can be retrieved in single representative A- and B-type soils

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Introduction

Beet necrotic yellow vein virus (BNYVV) is vectored by *Polymyxa betae* and causes rhizomania in sugar beet. By cultivating partial resistant cultivars the disease can be controlled. Resistance genes like *Rz1* and *Rz2* reduce virus replication and prevent virus spread from infected hair roots into the tap root. The RNA3 encoded pathogenicity factor P25 is responsible for symptom expression and virus translocation in the root system. P25 has a highly variable amino acid composition at amino acid tetrad 67-70. The uniformly applied *Rz1* resistance is suggested to exert a positive selection on the P25 tetrad composition because isolates with the ability to overcome *Rz1* display a valine at amino acid position 67 (V67) (Schirmer et al., 2005; Acosta-Leal et al., 2008). Multiple cultivation of sugar beets with *Rz1* and/or *Rz2* as alternative resistance source followed by extensive sequence analysis should reveal if resistance breaking isolates already exist in viral population or are selected and enriched only by cultivation of resistant sugar beet genotypes.

Material and Methods

Different genotypes (susceptible, *Rz1*, *Rz1+Rz2*) were cultivated four times in natural infested soils (BNYVV Italian A- and German B-type) under defined greenhouse conditions (Fig. 1). Plants were harvested every five weeks. Representative root samples were applied for P25 RT-PCR amplification followed by sequencing of PCR fragments to deduce the variability of the P25 tetrad composition in the sample directly from the sequence electropherogram (Fig. 2).

Results

The occurrence of a tetrad composition indicative for resistance breaking ability was not observed after 20 weeks of culture duration. However high P25 tetrad variability could be observed in both standard A- and B-soils independent of the genotypes cultivated (Table 1). The entire B-type tetrad variability (AYHR and AHHR), previously described to occur in different separated geographic regions plus a new variant (ACHR) was detected in plants grown in the German B-type soil in this study. As well in the Italian soil sample, collected in the region where BNYVV A-type was initially described, three out of the 12 known A-type tetrads were detected, namely ACHG, AHHG and ALHG. Again a fourth previously unknown A-type tetrad composition ASHG was detected.

Fig. 1: Experimental setup for selection of resistance breaking BNYVV isolates by multiple cultivation of different sugar beet genotypes (susceptible, *Rz1*, *Rz1+Rz2*) under standardised greenhouse conditions

Tab. 1: Amino acid sequence variation within RNA-3 encoded P25

Position	67	68	69	70
A-type	A	C	H	G
A-type	A	L	H	G
A-type	A	H	H	G
A-type*	A	S	H	G
B-type	A	Y	H	R
B-type	A	H	H	R
B-type	A	C	H	R

*** only one PCR-product was sequenced**

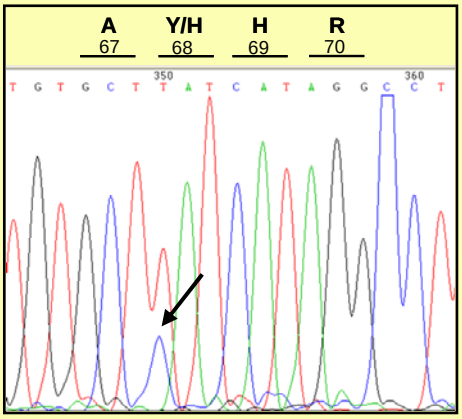

Fig. 2: Representative electropherogram of P25 tetrad of *Rz1* plants grown in B-type soil encoding sequence following direct seqencing of the RT-PCR product

Summary

- The P25 tetrad variability in a single soil sample is much higher than previously described
- No selection of a P25 mutant with V67 was observed during the experiment
- The entire B-type P25 tetrad variation was identified in the German B-type soil sample
- In addition to the ACHG P25 tetrad other known as well as a new tetrads were present in the Italian A-type soil

References: Acosta-Leal, R., Fawley, M.W., Rush, C. M. (2008) Changes in the intraisolate genetic structure of *Beet necrotic yellow vein virus* populations associated with plant resistance breakdown, Virology 376, 60–68

Schirmer, A., Link, D., Cognat, V., Moury, B., Beuve, M., Meunier, A., Bragard, C., Gilmer, D., Lemaire, O. (2005) Phylogenetic analysis of isolates of Beet necrotic yellow vein virus collected worldwide, Journal of General Virology 86, 2897-2911

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