Beet necrotic yellow vein virus (BNYVV) P-type overcomes resistance in *Rz1* x *Rz2* sugar beet hybrids after mechanical inoculation

F. Pferdmenges[1], R. Koenig [2] and M. Varrelmann[1]

•[1] Institute of Sugar Beet Research, Department of Phytopathology, Holtenser Landstr. 77, D-37079 Göttingen, Germany

•[2]Federal Biological Research Center, Institute for Plant Virology, Microbiology and Biosafety, Messeweg 11-12, D-38104 Braunschweig, Germany

Institut für Zuckerrübenforschung

Introduction

Rhizomania in sugar beet is caused by *Beet necrotic yellow vein virus* (BNYVV, Genus *Benyvirus*) which is transmitted by zoospores of *Polymyxa betae*. BNYVV can be divided in three major subgroups by means of sequence diversity (A, B, P-type (Koenig & Lennefors, 2000) with different geographic distribution. The disease can be controlled by cultivating partial resistant sugar beet genotypes ["Rizor", Rz1 (Holly), Rz2 (WB42), Rz3 (WB41), summarized in Gidner et al., 2005] which inhibit the spread of the virus from initially infected hair-roots to the main tap root.

In addition to RNA3 encoded P25 which is responsible for virus translocation and rhizomania symptom induction, BNYVV P-type, initially identified in the Pithiviers area in France, contains a fifth RNA segment which influences the symptom expression in a synergistic fashion (Koenig *et al.,* 1997; Tamada *et al.,* 1996). Moreover, recent observations have shown that BNYVV P-type possesses enhanced pathogenicity towards Rz2 and "Rizor" sources of resistance when grown in naturally infested soil. (Richard Molard, 2002; Meulemans *et al.,* 2003; Büttner *et al.,* 2004). Influence of the virus vector on BNYVV pathogenicity and more important other soil-borne pathogens cannot be excluded. Moreover BNYVV displays highly variable inoculum concentration in soil, which is known to interfere with Rz1 resistance source (Scholten *et al.,* 1994).

Here we demonstrate the ability of BNYVV P-type in comparisons A- and B-type to overcome the resistance in a sugar beet hybrid containing both *Rz*1 and *Rz*2 after artificial mechanical inoculation.

Materials and Methods

- Greenhouse test in single plant pots; cultivation under standardized conditions for four weeks in hydroponics and eight weeks in soil after mechanical infection
- Genotypes:
  - partial resistant variety : Holly x C48 – hybrid (*Rz*1 x *Rz*2)
  - susceptible variety: susceptible parent line of above specified hybrid without any resistance source
- BNYVV isolates:
  - A-Type (Rovigo, Italy)
  - B-Type (Langendorf, Germany)
  - P-Type (Pithiviers, France)
  - Rg. (isolate „Regensburg" (R. Koenig; BBA) contains only the RNA1 and 2 segments (CP (coat protein), TGB (triple gene block), RdRp (RNA dependent RNA polymerase) and codes for vector transmisssion)
- Mechanical inoculation by means of vortex inoculation (Koenig pers. comm.) after 2x propagation on the local lesion host *Chenopodium quinoa*
- Diagnostics:
  - qualitative and quantitative ELISA with DSMZ - BNYVV polycl. antiserum (Büttner and Bürcky, 1987)
  - RNA-extraction (RNeasy; Quiagen) and RT-PCR
  - TPIA (Kaufmann et al. 1992)

Results

- BNYVV -A, -B and -P induce systemic symptoms in suceptible sugar beets after mechanical inoculation.

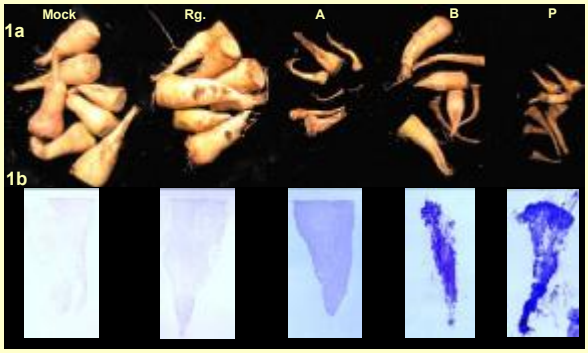


Fig. 1a: Sugar beet roots (susceptible control) after harvest displaying virus symptoms (bottle neck) and yield reduction in case of BNYVV infection. From left to right: Mock, Rg., A-type, B-type, P- type ;
Fig. 1b: Pictures of (nitrocellulose) membranes after tissue print immunoassay of selected susceptible sugar beets artificially inoculated with each virus type (same order than Fig. 1a).

Tab. 1: Infection rate [%] as determined in ELISA (Fig. 4) after mechanical inoculation between virus types [A, B, P, Rg. and Mock] and genotypes [RZ1 x Rz2 = resistant and suscep. = susceptible]

Virus type	A	A	B	B	P	P	Mock	Mock	Rg	Rg
Genotype	*Rz1* x *Rz2*	suscep.	*Rz1* x *Rz2*	suscep.	*Rz1* x *Rz2*	suscep.	*Rz1* x *Rz2*	suscep.	*Rz1* x *Rz2*	suscep.
Infection rate	0	42,9 %	0	31,6 %	88,9 %	100 %	0	0	0	0

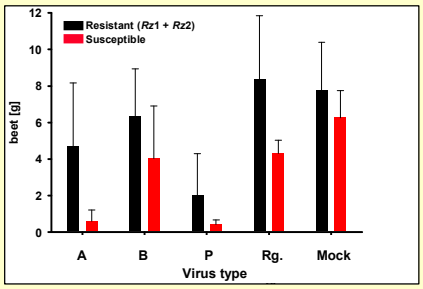


Fig. 3: Mean weight values after artificial inoculation of resistant (black) and susceptible (red) varieties with different virus isolates (A, B, P, isolate Rg. (only RNA1+2) and mock); Error bars show the standard division

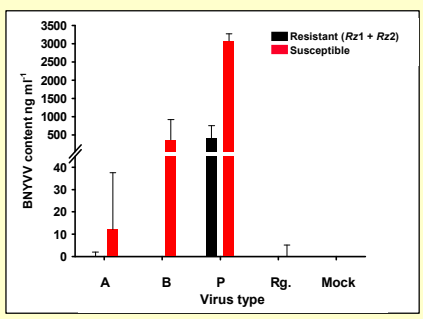


Fig. 4: ELISA readings of sugar beet hair roots after artificial inoculation with different virus isolates (A, B, P, isolate Rg. and mock) and two different genotypes: resistant (black) and susceptible (red). Error bars show the standard deviation

This study is kindly supported by: Gesellschaft zur Förderung der privaten Pflanzenzüchtung.

- Only BNYVV -P induces systemic symptoms in *Rz1* x *Rz2* sugar beet hybrids after mechanical inoculation.

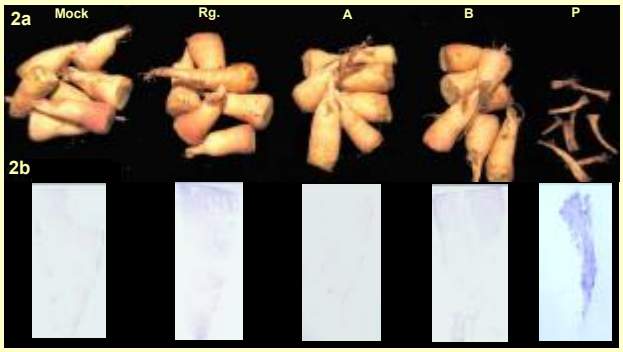
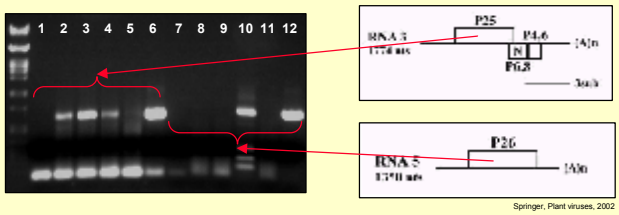


Fig. 2a: sugar beet roots [resistant variety (Rz1 x Rz2)] after harvest displaying virus symptoms (bottle neck) and yield reduction in case of BNYVV infection. From left to right: Mock, Rg., A-type, B-type, P- type ;
Fig. 2b: pictures of (nitrocellulose) membranes after tissue print immuno assay of selected resistant sugar beets artificially inoculated with each virus type (same order than Fig. 2a).



Springer, Plant viruses, 2002

Fig. 5: RT-PCR amplification of P25- and P26-ORF resp. using gene specific primers; RNA-samples (RNeasy, Qiagen) were randomly taken from hair-roots for all virus types (BNYVV-A, -B and -P) applied, to prove the presence of RNA3 and RNA5 encoding the viral pathogenicity factors respectively. 1-6 shows P25 (1= water control; 2 = A; 3 = B; 4 = P; 5 = Rg.; 6 = positve plasmid control); 7-12 shows P26 (1 = water control; 2 = A; 3 = B; 4 = P; 5 = Rg.; 6 = positve plasmid control).

- Organisation of viral functions localised to BNYVV RNA3 + 5
- RNA3:
  P25: pathogenicity factor; translocation in the root system, rhizomania symptom develpment, yield losses
- RNA5 (only present in P-types):
  P26: influence on symptomatology P26 aa-homology synergism to P25

Conclusions

- By means of artificial mechanical inoculation with high inoculum dose BNYVV A- and B-type are not able to infect the resistant hybrid (*Rz*1 x *Rz*2) systemically as demonstrated by TPIA when compared to the susceptible control (Fig. 2b and Fig. 4).
- High yield losses (Fig. 1a, 2a, and 3) and high BNYVV content in the hair roots (Fig. 4) and systemic BNYVV spread in the tap root (Fig. 1b and 2b) in the susceptible and the resistant genotype after artificial inoculation with the P-type.
- Lower yield losses in the susceptible variety after artificial inoculation with A- and B- compared to P- Type (Fig. 1a, and 3); hardly any influence after infecting the resistant variety with A and B (Fig 2a and 3).