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Occurrence, spread and pathogenicity of different *Beet necrotic yellow vein virus* (BNYVV) isolates

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Occurrence, spread and pathogenicity of different *Beet necrotic yellow vein virus* (BNYVV) isolates

Vorkommen, Verbreitung und Pathogenität verschiedener Isolate des *Beet necrotic yellow vein virus* (BNYVV)

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ABSTRACT

Rhizomania (beet necrotic yellow vein virus, BNYVV) represents an important sugar beet disease, which is transmitted by the biotrophic plasmodiophoromycete *Polymyxa betae*. As long as the disease is not controlled it can lead to yield losses up to 90%. To date yield losses due to BNYVV infestation are inhibited by cultivating resistant sugar beet genotypes, which restrict the virus replication and translocation from infected hair-roots to the taproot. The BNYVV resistance is provided in marketable sugar beet varieties by two major resistance sources (*Rz1* and *Rz2* which either occur singular or in combination). But meanwhile on sugar beet genotypes carrying one (*Rz1*) as well as two resistance genes (*Rz1+Rz2*) resistance breaks could be observed at several BNYVV A-type infected sites in the USA and in Spain. To confirm these observations a 12 weeks greenhouse resistance test with three different cultivars (two partial resistant genotypes containing either *Rz1* or *Rz1+Rz2* resistance sources as well as a susceptible genotype) under standardized conditions with naturally infested soils from 6 locations was performed. The single resistance (*Rz1*) was compromised in soils from Spain (D), France (P-type, RNA-5 containing), and the USA (IV and MN); in reference soils from Italy (R, A-type) and Germany (GG, B-type) *Rz1* resistant sugar beets were not affected. Overcoming of *Rz1+Rz2* resistance after 12 weeks could only be observed in D soil. Over and above the genomic region that encodes for the pathogenicity factor (P25) of the BNYVV RNA3 from beets grown in all soils was analysed.

Previously suggested correlation between "valine" on position 67 of P25 and a higher virulence could not be confirmed. Isolates in one of the soils as well as experiments previously published, where overcoming of resistance could be observed, contain several other aa_{67} than valine. Analyses of additional soil borne pathogens using ITS sequencing and database comparison showed the presence of three pathogens (*Rhizoctonia solani* Keskin*, Fusarium sp., Pythium sp.*). Synergism between BNYVV, *Rhizoctonia solani* Keskin and *Pythium sp.* could lead to severe virus symptoms and weight reductions particularly in the Spanish soil.

To determine if resistance breaks are correlated with the BNYVV inoculum concentration a "Most Probable Number"(MPN) - tests was conducted where same soils as in the resistance tests were examined. Thereby, D soil revealed the highest BNYVV density, the GG soil on the other hand displayed 520 times lower MPN. In order to obtain information on the aggressiveness of particular virus isolates an additional MPN with *Rz1+Rz2* genotypes was performed. Within this test D, IV, MN and P resulted again in high BNYVV densities even able to infect *Rz1+Rz2* plants after 4 weeks cultivation. These results give strong evidence that high inoculum doses are not responsible for the observed resistance breaks. To prove this conclusion another experiment with normalised

inoculum added to sterile soil was carried out. Within this test three time harvests were conducted after 4, 8 and 12 weeks. Obviously, a significant differentiation of virus isolate vs. genotype correlating to tap root weight was only observed after 12 weeks. Consistently, applying adjusted inoculum density, D, IV, MN and P produced the highest virus contents at 12 weeks. Thus, resistance breaks must be connected to high BNYVV pathogenicity and not to inoculum density.

Additional, experiments were conducted to test the influence of viruliferous *P. betae* zoospore concentrations from various origins, carrying different BNYVV-types. But due to uncertainty how many of the zoospores are actually viruliferous, the data resulted in highly different outcomes, not correlating to the results from tests in naturally infected soil.

Moreover, efforts were undertaken to shorten resistance tests and replace them with time saving artificial sugar beet leaf inoculation via co-infiltration of a BNYVV RNA3 encoding P25 infectious cDNA clone and a red fluorescing marker gene (mRFP). Although, the method itself worked very well in young sugar beet leaves, no differences concerning the sugar beet genotype could be detected. The expected variability of fluorescence intensity comparing susceptible and resistant sugar beet cultivars was not given.

CONTENT

APPENDIX

This thesis is based on following manuscripts, which will be referred to by their Roman numerals:

ABBREVIATION

A	alanine
Aa	amino acid
approx.	approximately
A-type	BNYVV isolate displaying a typical RNA composition - A-types are mainly occurring in southern, western and eastern Europe, as well as in the Northern America
BBSV	beet black scorch virus
BMYV	beet mild yellowing virus
BNYVV	beet necrotic yellow vein virus
BSBMV	beet soil-borne mosaic virus
BSBV	beet soil-borne virus
B-type	BNYVV isolate displaying a typical RNA composition - B-types are mainly occurring in central Europe (Germany, Austria, Switzerland)
BVQ	beet virus Q
BYV	beet yellows virus
C48	progenies from a cross between WB41+WB42 and C37
cDNA	copy DNA
cM	centi Morgan
CP	coat protein
DNA	deoxyribonucleic acid
dpi	days post-inoculation
dsRNA	double stranded RNA
E	glutamic acid
ELISA	enzyme linked immunosorbent assay
G	glycine
GST	glutathione-S-transferase
H	histidine
I	isoleucine
ICTV	International Committee on Taxonomy of Viruses
ITS	internal transcribed spacer
J-type	BNYVV isolate containing similar to the French P-type an additional RNA5 and differing from the P-type by the truncation of four amino acids – J-types commonly occur in Asia
kb	kilo base

kDa	kilo Dalton
L	leucine
LOD	likelihood of odds
LSD	least square difference
MP	movement protein
MPN	most probable number
mRNA	messenger RNA
N / n	number of plants / number of repetitions
NC	negative control
NES	nuclear export signal
NLS	nuclear localization signal
nt	nucleotide
ORF	open reading frame
P	protein
PC	positive control
PCR	polymerase chain reaction
pi	plant introductions
PTGS	post-transcriptional gene silencing
P-type	BNYVV isolate displaying a typical RNA composition and an additional fifth RNA - P-types are mainly occurring in a small region in F (Pithiviers), the UK and in KZ
QTL	quantitative trait loci
R	arginine
RdRp	RNA dependent RNA polymerase
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
Rz1	resistance gene against BNYVV from the “Holly” source
Rz2	resistance gene against BNYVV from the WB42 source
Rz3	resistance gene against BNYVV from the WB41 source
siRNA	short interfering RNA
SSCP	single strand confirmation polymorphism
TGB	triple gene block
TPIA	tissue print immunoassay
V	valine
var	variety
WB41	wild beet 41
WB42	wild beet 42
Y	tyrosine

FIGURES