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Rhizomania symptoms in strong Rhizomania tolerant varieties in South Minnesota.

The first *Rz1+Rz2* resistant varieties against BNYVV were introduced in the US region in 2010 to secure crop yields even as the combination of both *Rz1* and *Rz2* in first generation commercial hybrids was associated with a yield penalty in the absence of disease. *Rz1* + *Rz2* hybrid varieties having both resistant genes, *Rz1* and *Rz2*, are now recently deployed and conferred so far good tolerance to the virus without yield penalty.

In 2019, Rhizomania yellowing leaf patch symptoms were observed in *Rz1* + *Rz2* varieties in two US fields, from Kandiyohi county (Field 1) and Renville county (Field 2) in Minnesota, USA. Investigation was started to identify the potential source of these yellowing symptoms.

Material and method

The cause of the symptoms was further investigated by analyzing the soil of the patches themselves and around them for the presence of BNYVV. Soil characterization for disease incidence of the present BNYVV virus strains was carried out. The BNYVV virus was baited by planting sugar beet varieties in the soil sample in greenhouse and his incidence was determined using DAS-ELISA and virus strain typing using direct sequencing of RT-PCR amplicons.

- **Set-up:**
  Patch/non-patch comparison from two US fields + comparison with EU reference soil
- **Tools:**
  - Rhizomania bait-plant bioassay using *Rz1* + *Rz2* het and susceptible hybrid varieties;
  - Disease incidence evaluation using BNYVV DAS-ELISA on infected rootlet samples and resistance-breaking evaluation by comparison with susceptible checks;
  - BNYVV strain identification through RT-PCR & sequencing diagnostics of the RNA-2, RNA-3 and RNA-5 of the virus

Bait-plant bioassay results

Soil type	ELISA OD values	Statistic
Field 2 Non-patch	0,0	a
Field 1 Non-patch	0,0	a
Field 1 Patch	0,1	b
Field 2 Patch	0,3	c
EU Rz1 breaking SV reference	0,8	d

Patches had a detectable disease incidence and non-patches (clean) not. The intrinsic disease incidence of both patches was statistically lower compared to the strong Rhizomania pressure known from some regions in Europe.

Virus type results

Soil type	Rep	RNA-2	RNA-2 type	RNA-3	RNA-3 type	Tetrad	RNA-5
Field 1 Non-patch	1	Smear	Neg	Neg	Neg	Neg	Neg
Field 1 Non-patch	2	Pos	Neg	Neg	Neg	Neg	Neg
Field 1 Patch	1	Pos	Type A	Pos	Type A	VCHG	Neg
Field 1 Patch	2	Pos	Type A	Pos	Type A	VCHG	Neg
Field 2 Non-patch	1	Neg	Neg	Neg	Neg	Neg	Neg
Field 2 Non-patch	2	Neg	Neg	Neg	Neg	Neg	Neg
Field 2 Patch	1	Pos	Type A	Pos	Type A	VCHG	Neg
Field 2 Patch	2	Sligh pos	Type A	Pos	Type A	VCHG	Neg

In both soil patches, the type of virus was BNYVV type A for the RNA-2 & -3. The tetrad of the p25 protein was VCHG and no RNA5 was identified. BNYVV type A with VCHG tetrad is a well-known virus type with *Rz1* breaking virulence ability, but no *Rz2* breaking virulence ability so far. These observations are in line with the current knowledge.

Variety benchmarking

Soil type	Variety code	ELISA OD values	Statistic
Field 2 Non-patch	Rz1 + Rz2 reference	-0,1	a
Field 2 Non-patch	SmBSC Variety-3	0,0	a
Field 2 Non-patch	SmBSC Variety-1 - SV863	0,0	a
Field 2 Non-patch	Susceptible reference	0,0	a
Field 2 Non-patch	SmBSC Variety-2	0,1	a
Field 2 Patch	Rz1 + Rz2 reference	0,1	a
Field 2 Patch	SmBSC Variety-1 - SV863	0,2	a
Field 2 Patch	SmBSC Variety-3	0,2	a
Field 2 Patch	SmBSC Variety-2	0,3	ab
Field 2 Patch	Susceptible reference	0,9	c

Soil type	Variety code	ELISA OD values	Statistic
Field 1 Non-patch	Rz1 + Rz2 reference	-0,1	a
Field 1 Non-patch	SmBSC Variety-3	0,0	a
Field 1 Non-patch	Susceptible reference	0,1	a
Field 1 Non-patch	SmBSC Variety-1 - SV863	0,1	a
Field 1 Non-patch	SmBSC Variety-2	0,1	a
Field 1 Patch	SmBSC Variety-3	0,0	a
Field 1 Patch	Rz1 + Rz2 reference	0,0	a
Field 1 Patch	SmBSC Variety-1 - SV863	0,1	a
Field 1 Patch	SmBSC Variety-2	0,1	a
Field 1 Patch	Susceptible reference	0,3	ab

The ELISA values (~ viral content) of SV863 are in line with other *Rz1* + *Rz2* varieties. In the patches, there is a clear statistical difference between *Rz1* + *Rz2* varieties and *Rz1* + *Rz2* susceptible check indicating that *Rz2* resistance gene is not overcome.

Further investigations

Potential other causes to investigate:

- Other mutations or change elsewhere on the rhizomania genome in link with the symptoms to overcome host resistance.
- Interaction with another stressor,
  - e.g. interaction with other soilborne viruses e.g. BBSV, BSBMV;
  - e.g. *Aphanomyces*, flooding;
  - or local suitable conditions for *Polymyxa betae* multiplication causing stronger virus spreading and pressure.
- Nitrogen uptake or movement blocked by the plant due to virus presence causing a general chlorosis of foliage. Infected roots could be inefficient in water and nutrient uptake, general foliar symptoms are like water stress or nitrogen deficiency.
- Diseased plants usually occur in patches or areas of the field and not as scattered individual plants dispersed throughout the field. Because the vector (*P. betae*) thrives in moist areas, disease severity usually is greatest in depressions or compacted, poorly-drained portions of the field that tend to collect water and remain wet. Plants could be weaker in these portions of the field and show consequently more symptoms.

CONCLUSIONS

SV is dedicating resources to investigate the source of the problem, and up to now, *Rz2* resistance-breaking is not confirmed. Investigations have resulted in:

- **No new specific *Rz1* or *Rz2* resistance-breaking (RB) BNYVV strains were observed in the patches that could circumvent *Rz1* or *Rz2*-mediated resistances**
- **Only the usual BNYVV virus type A showing a VCHG tetrad resulting normally in a very low disease incidence in *Rz1* + *Rz2* heterozygous varieties was found in the patches.**
- ***Rz1* + *Rz2* genes were not broken in the bait plant test, as the difference between *Rz1* + *Rz2* and susceptible varieties remained.**
- **Disease incidence in susceptible varieties was significantly lower compared to EU reference soil.**