IDENTIFICATION OF RESISTANCE-BREAKING VARIANTS OF BEET NECROTIC YELLOW VEIN VIRUS IN SUGARBEET

Vanitha Ramachandran, Eric Rivera Santiago, John Weiland, and Melvin Bolton
USDA-ARS, Edward T. Schafer Agricultural Research Center, Fargo, ND

Rhizomania is a devastating disease of sugarbeet caused by BNYVV, a multipartite RNA virus that belongs to the family *Benyvirus* (Tamada and Baba, 1973), and is transmitted by *Polymyxa betae* a soilborne parasite of sugarbeet (D'Alonzo et al., 2012). The disease significantly impacts sugarbeet yield and thus affects growers' economy. In the USA, the disease was first identified in the early 1980s and within a few years had spread to all sugarbeet production areas (Duffus, 1984; Wisler et al. 1997). To manage the disease, *Rz1* and other sources of resistance were discovered and adapted to the regional cultivars which provided considerable disease resistance. However, in a few years, resistance-breaking strains of BNYVV began to appear, starting as blinkers and later spreading to large diseased area in fields planted with *Rz1* resistance carrying cultivars (Scholten et al. 1996; Liu et al. 2005; Rush and Acosta-Leal, 2007). Research studies have indicated that the ability for BNYVV overcoming the *Rz1*-mediated resistance was mapped to BNYVV RNA 3, to a highly variable 'tetrad' amino acid of the p25 gene (Koenig et al. 2009). A recent survey on the distribution and prevalence of BNYVV strains and p25 mapping in North Dakota and Minnesota area revealed no correlation between the p25 tetrad signature and the ability to compromise *Rz1*-mediated resistance (Weiland et al., 2019).

Currently the disease is managed by host resistance; however, rhizomania is being observed in sugar beet production fields indicating the appearance of resistance-breaking variants of BNYVV. Rhizomania disease management primarily relies on resistance genes bred into commercial varieties specifically developed against BNYVV (Rush et al., 2006). There is no commercial chemistry exist to manage the disease. The disease is a major concern because of the emergence of resistance-breaking (RB) strains of BNYVV in the Red River Valley and southern Minnesota sugar beet growing areas and around the world. The objective of this study was to evaluate rhizomania suspicious soil and beet samples from Minnesota and North Dakota sugar beet production fields to identify resistance-breaking via soil-baiting assay by growing different sugar beet cultivars such as susceptible, *Rz1*, and *Rz1Rz2* for genotype comparison.

Materials and Methods

Beet and soil samples were obtained from the sugarbeet production areas of North Dakota and Minnesota courtesy of agriculturists from the American Crystal Sugar Company, Minn-Dak Farmrs Cooperative, and Southern Minnesota Beet Sugar Cooperative. Sugarbeet seeds were obtained from SESVanderhave. For healthy control, susceptible sugarbeet seeds were planted into Sunshine Mix with sand of 1:1 ratio along slow-release fertilizer with (Sungro Horticulture, MA). Plants were grown in a greenhouse under standardized conditions at 24°C/18°C day/night with 8 hours of supplemental light per day, and water was added directly as needed. Six weeks after planting in infested soil, plants were harvested and root sample consisting of 3 plants was taken from each pot. Roots were washed gently in a tray containing water taking care to retain fine root hairs, damp dried on paper towel, and stored for ELISA testing on BNYVV (Torrance et al.,1988) or stored at -80°C until used for RNA extraction.

Results and Discussion

Rhizomania symptomatic beet and corresponding soil samples were obtained from sugar beet fields of Minnesota and North Dakota. Hairy roots from beets were carefully collected and washed to remove tare attached to it. After damp drying, a portion of it was ground in ELISA extraction buffer in a volume of 600 uL and loaded 150 uL in one well of ELISA plate in three replicates. Positive and negative controls were included in each plant with diagnosis. Out of 73 beets, 23 tested positive (31%) based on ELISA analysis (Table 1). Each beet was tested in three

replicates an average was used for plotting analysis. The beets that are positive for BNYVV could be due to lack of the trait or appearance of resistance-breaking variants of BNYVV.

Table.1. Detection of BNYVV using ELISA in sugar beet obtained from field.

Location	Beet infected/tested	Soil samples
Minnesota	23 / 63	5 locations
North Dakota	0 / 10	2 locations
Total number of samples	23 / 73	7 locations

Recovery of BNYVV from rhizomania-infested soil samples was accomplished using soil-baiting assay by growing different sugar beet cultivars representing susceptible, *Rz1*, and *Rz1Rz2* sugar beet genotypes. BNYVV was detected in the roots of bait plants using ELISA. For each sugar beet cultivars were planted in three replicates and three technical replicates were used for ELISA analysis, and the average value was used for plotting. Positive and negative controls were included in each plant with diagnosis. Soil samples were obtained from rhizomania suspicious fields from seven different locations. Out of seven locations, only one location appeared positive for rhizomania in susceptible cultivar, whereas soil from another location tested slightly positive for BNYVV in susceptible, Rz1, and Rz1Rz1 cultivars (Table 2). As the next step, total RNA was extracted, constructed RNA-Seq libraries and sequenced using Next-Generation sequencing. Data analysis revealed the presence of reads matching to BNYVV, and other soil-borne viruses indicating mixed infections. The role of co-existing viruses in the mixed infection on disease complexity needs to be investigated.

Table.2. Rhizomania evaluation in field soil samples from Minnesota and North Dakota. Detection of BNYVV in the roots of bait plants using ELISA. Symbols ++ refers to intensely positive, + refers to moderately positive, +/- refers to slightly positive, and – refers to negative for BNYVV.

Soil samples	Susceptible	Rz1	Rz1+Rz2
Location-1	-	-	-
Location-2	+/-	+/-	+/-
Location-3	+	-	-
Location-4	-	-	-
Location-5	-	-	-
Location-6	-	-	-

References

1. Tamada, T. and Baba, T. (1973) *Beet necrotic yellow vein virus* from Rhizomania affected sugarbeet in Japan. Ann. Phytopathol. Soc. Jpn. 39, 325–332
2. D'Alonzo, M, Delbianco, A., Lanzoni, C., Autonell, CR., Gilmer, D and Ratti, C. (2012) Beet soil-borne mosaic virus RNA-4 encodes a 32 kDa protein involved in symptom expression and in virus transmission through Polymyxz betae. Virol. 423, 187-94.
3. Duffus, J. E., Whitney, E. D., Larson, R. C., Liu, H. Y., and Lewellen, R. T. (1984). First report in Western Hemisphere of rhizomania of sugarbeet caused by beet necrotic yellow vein virus. Plant Dis. 68:251.
4. Wisler, G. C., Widner, J. N., Duffus, J. E., Liu, H. Y., and Sears, J. L. (1997). A new report of rhizomania and other furoviruses infecting sugarbeet in Minnesota. Plant Dis. 81:229.
5. Scholten, O. E., Jansen, R. C., Paul Keizer, L. C., Bock, T. S. M., and Lange, W. (1996). Major genes for resistance to beet necrotic yellow vein virus (BNYVV) in Beta vulgaris. Euphytica 91:331-339.

6. Liu, H.-Y., Sears, J. L., and Lewellen, R. T. (2005). Occurrence of resistance breaking beet necrotic yellow vein virus of sugarbeet. Plant Dis. 89:464-468.
7. Rush C. M., and Acosta-Leal R. (2007). Emergence of a resistance-breaking strain of beet necrotic yellow vein virus. Phytopath. 97: S170.
8. Koenig, R., Loss, S., Specht, J., Varrelmann, M., Luddecke, P., and Deml, G. (2009). A single U/C nucleotide substitution changing alanine to valine in the beet necrotic yellow vein virus P25 protein promotes increased virus accumulation in roots of mechanically inoculated, partially resistant sugarbeet seedlings. J. Gen. Virol. 90:759-763.
9. Weiland, J.W., Bornemann, K., Neubauer, J.D., Khan, M.F.R and Bolton, M.D. (2019). Prevalence and Distribution of Beet Necrotic Yellow Vein Virus Strains in North Dakota and Minnesota. Plant Dis. 103:2083-2089.
10. Rush, C. M., Liu, H. Y., Lewellen, R. T., and Acosta-Leal, R. (2006). The continuing saga of rhizomania of sugar beets in the United States. Plant Dis. 90: 4-15.
11. Torrance, L., Pead, M. T., and Buxton, G. (1988). Production and some characteristics of monoclonal antibodies against beet necrotic yellow vein virus. Ann. Appl. Biol. 113, 519–530.