EVALUATION OF RHIZOMANIA RESISTANCE-BREAKING IN MINNESOTA AND NORTH DAKOTA SUGARBEET FIELDS

Vanitharani Ramachandran, Hyun Cho, and Melvin Bolton

Sugarbeet Research Unit, USDA-ARS, Edward T. Schafer Agricultural Research Center, Fargo, ND

Rhizomania is an economically important disease of sugarbeet that impacts crop productivity and grower profitability. The disease is caused by beet necrotic yellow vein virus (BNYVV), an RNA virus belonging to the family *Benyviridae* (Tamada and Baba, 1973). BNYVV is transmitted by *Polymyxa betae*, a soilborne obligate parasite of sugarbeet roots. In the United States, rhizomania was first detected in the early 1980 and rapidly spread to all sugarbeet production regions (Duffus, 1984; Wisler et al. 1997). Disease management has relied on genetic resistance such as *Rz1,* and other sources incorporated into commercial cultivars. However, in a few years, *Rz1*-mediated resistance was compromised by the emergence of resistance-breaking strains of BNYVV. Rhizomania symptoms appeared initially as blinkers and later widespread to large areas in fields planted with *Rz1* resistant cultivars (Scholten et al. 1996; Weiland et al., 2019). Although rhizomania has been managed by host resistance, symptoms are being observed in sugarbeet production fields, suggesting the appearance of resistance-breaking (RB) variants of BNYVV. Identification of these RB-variants of BNYVV is critical for developing future disease management strategies. Next-generation high-throughput sequencing offers a powerful approach to obtain comprehensive sequence information for both known and unknown viruses. In this study, rhizomania suspicious sugarbeet fields were identified, and soil samples were collected for evaluation. Soil-baiting assays were conducted using susceptible, *Rz1*, and *Rz1* plus *Rz2* seeds under laboratory conditions to assess resistance-breaking potential. Virus detection was performed using BNYVV-specific ELISA. Subsequently, HTS was applied to characterize nucleotide sequence changes in BNYVV and compare RB variants with non-resistance-breaking strains of BNYVV. Identifying these nucleotides and associated amino acid changes will allow the characterization of RB variants of BNYVV and provide insights for breeding and management strategies.

Methodology

A survey of rhizomania incidence was conducted in coordination with agriculturists and cooperatives across Minnesota and North Dakota including American Crystal Sugar Company, Minn-Dak Farmers' Cooperative, and Southern Minnesota Beet Sugar Cooperative. Soil samples were collected from around the roots of sugarbeet plants that are suspicious of rhizomania, and resistance-breaking potential was assessed using soil-baiting assays with different sugarbeet genotypes, which were kindly provided by the seed company, SESVanderHave. Soil-baiting assay was carried in a greenhouse under standardized conditions at 24°C day and 18°C night with eight 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 samples consisting of three to four plants

were 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 of BNYVV or stored at -80°C until used for RNA extraction and library construction to accomplish high-throughput sequencing. Roots from soil-bait plants were carefully collected and washed gently 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. Each ELISA plate was included with positive and negative controls to confirm the assay reagents in the diagnosis.

Results and Discussion

Rhizomania disease prevalence was monitored across sugarbeet growing regions of Minnesota and North Dakota in collaboration with the cooperatives and industry agriculturists. Symptoms of rhizomania were observed in multiple fields, and soil samples were collected from these locations for further analysis. Resistance-breaking was assessed in soil-baiting assays by growing sugarbeet varieties including susceptible type, Rz1, and Rz1Rz2. Detection of BNYVV in the root tissue of bait plants by ELISA confirmed the presence of rhizomania. When BNYVV was detected only in susceptible variety and not in Rz1 or Rz1Rz2, indicating that the soil contained rhizomania but did not overcome the resistance conferred by these genes. In contrast, detection of BNYVV in both resistant varieties as well as the susceptible variety indicates the presence of resistance-breaking strains of the virus in the soil. Out of 24 soil samples tested, BNYVV was detected in 18 samples. Among these, 16 samples were positive in the Rz1 variety, and 3 samples were positive in Rz1Rz2, indicating the presence of BNYVV strains capable of overcoming host resistance (Table 1). The ELISA was performed in triplicate, and the average values were used for analysis. Upon completion of the analysis, rhizomania resistance-breaking evaluation results were shared with cooperatives to guide informed decisions on crop rotation, cultural practices, and varietal selection. Overall, assessing resistance-breaking in field soil samples provides critical information for growers, enabling effective disease management strategies.

Table 1. Evaluation of rhizomania resistance breaking and ELISA detection of BNYVV. In the table symbols: ++, +, +/-, and - refers to highly positive, moderately positive, slightly positive, and negative for BNYVV, respectively.

Samples	Location	Susc.	Rz1	Rz1+Rz2
2025_Soil-L1	North Dakota	-	-	-
2025_Soil-L2	North Dakota	-	-	-
2025_Soil-L3	North Dakota	++	+	-
2025_Soil-L4	North Dakota	-	-	-
2025_Soil-L5	North Dakota	-	-	-
2025_Soil-L6	North Dakota	++	+	-
2025_Soil-L7	North Dakota	++	++	-
2025_Soil-L8	North Dakota	+	+	-
2025_Soil-L9	North Dakota	++	+/-	-
2025_Soil-L10	North Dakota	++	+	+/-
2025_Soil-L11	North Dakota	++	++	+/-
2025_Soil-L12	North Dakota	+	+	-
2025_Soil-L13	North Dakota	++	++	-
2025_Soil-L14	Minnesota	-	-	-
2025_Soil-L15	Minnesota	++	++	+/-
2025_Soil-L16	Minnesota	++	++	+/-
2025_Soil-L17	Minnesota	-	-	-
2025_Soil-L18	Minnesota	++	++	-
2025_Soil-L19	Minnesota	++	++	-
2025_Soil-L20	Minnesota	+/-	-	-
2025_Soil-L21	Minnesota	++	++	++
2025_Soil-L22	Minnesota	++	+	+
2025_Soil-L23	Minnesota	++	++	+/-
2025_Soil-L24	Minnesota	++	++	++

References

1. 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 sugar beet caused by beet necrotic yellow vein virus. Plant Dis. 68:251.
2. 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.
3. Tamada, T., and Baba, T. (1973) *Beet necrotic yellow vein virus* from Rhizomania affected sugar beet in Japan. Ann. Phytopathol. Soc. Jpn. 39, 325–332.
4. 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
5. 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 sugar beet in Minnesota. Plant Dis. 81:229