EVALUATING RHIZOMANIA RESISTANCE-BREAKING STRAINS OF *BEET NECROTIC YELLOW VEIN VIRUS* USING HIGH-THROUGHPUT SEQUENCING

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Rhizomania, caused by *Beet necrotic yellow vein virus* (BNYVV), is an economically important disease of sugarbeet that impacts sugarbeet productivity and growers' economy. BNYVV is 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). 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). The disease is managed through resistance genes, *Rz1* and other sources of resistance, that were introduced to the commercial cultivars. In a few years, the *Rz1*-mediated resistance has been compromised with the appearance of resistance-breaking strains of BNYVV. The appearance of rhizomania disease started 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). Further research 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).

Rhizomania disease is managed by host resistance introduced into commercial cultivars used for sugar beet production. On the other hand, rhizomania disease is being observed in sugar beet production fields indicating the appearance of resistance-breaking variants of BNYVV. Identification of the resistance-breaking strains of BNYVV is important for developing new disease management strategies for the future. Next-generation high-throughput sequencing (HTS) is a powerful technology that can provide the sequence information of known and unknown viruses. To evaluate the rhizomania-resistance breaking, soil samples from rhizomania suspicious sugarbeet production fields will be obtained, and viruses in the soil will be recovered using soil-baiting assay. Rhizomania resistance-breaking will be evaluated by growing seeds of different sugarbeet varieties that includes susceptible, *Rz1*, and *Rz1* plus *Rz2* in the field soil under laboratory conditions. Then, by applying, HTS analysis to the sugarbeet grown in rhizomania-infested soil samples, the sequence information of BNYVV present in the roots of sugarbeet plants grown in rhizomania-infested soil will be identified. Identification of the nucleotide changes and the associated amino acids will allow the characterization of the resistance-breaking strains of BNYVV.

Materials and Methods

Survey of rhizomania disease was conducted in coordination with agriculturists and cooperatives of Minnesota and North Dakota sugarbeet growing areas: American Crystal Sugar Company, Minn-Dak Farmers' Cooperative, and Southern Minnesota Beet Sugar Cooperative. Soil samples from around the roots of sugarbeet plants those are suspicious for rhizomania disease were collected from multiple fields on North Dakota and Minnesota. The sugarbeet seeds with different genotypes were kindly provided by the seed company, SESVanderhave. The soil-baiting assay was carried out using 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 and library construction to accomplish high-throughput sequencing. We have an established bioinformatic pipeline to carry out the downstream sequencing data analysis.

Results and Discussion

A survey was conducted for rhizomania disease prevalence in the sugarbeet growing area of Minnesota, North Dakota, and South Dakota in cooperation with agriculturists. Sugar beet samples with rhizomania symptoms and the corresponding soil samples were obtained from multiple sugar beet fields of Minnesota, North Dakota, and South Dakota. Hairy roots from beet samples 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. Out of 143 beet samples, 85 tested positive (59%) based on ELISA analysis (Table 1). Each beet sample was tested in three replicates and an average was used for plotting analysis. The beet samples 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 fields. In the table symbol ++ refers to highly positive for BNYVV, + symbol stands for moderately positive for BNYVV, and – symbol denotes negative for BNYVV in the beet samples.

Sample	Location	Beet	++	+	-
1	SD	10	0	0	10
2	ND	7	4	2	1
3	ND	7	5	1	1
4	ND	7	0	0	7
5	ND	4	3	0	1
6	ND	5	3	1	1
7	ND	8	5	2	1
8	ND	7	4	3	0
9	MN	9	1	5	3
10	MN	9	0	0	9
11	MN	5	1	1	3
12	MN	4	0	1	3
13	MN	9	3	3	3
14	ND	9	1	5	3
15	MN	6	2	3	1
16	MN	5	3	1	1
17	MN	10	5	1	4
18	MN	7	4	3	0
19	MN	6	4	0	2

20	MN	9	4	1	4
		143	52	33	58
			Rhizomania positive	85	58

The obtained rhizomania suspicious soil samples were used in soil-baiting assay to recover the BNYVV from rhizomania-infested soil by growing different sugar beet cultivars representing susceptible, *Rz1*, and *Rz1Rz2* sugar beet genotypes. BNYVV detection in the roots of bait plants was accomplished using ELISA. For each sugar beet cultivar three replicates were used for ELISA analysis, and the average value was used for plotting. Positive and negative controls were included in each ELISA plate diagnosis. Twenty-five soil samples were obtained from various locations of Minnesota, North Dakota, and South Dakota sugarbeet production fields. Soil-baiting analysis is ongoing and expected to be completed end of February 2024. Once, completed the results will be communicated to the cooperatives depending on the locations from where the samples were obtained. Based on the ELISA analysis and sugarbeet genotype comparison, a subset of the samples will be subjected to next generation high-throughput sequencing analysis to identify the molecular changes at the nucleotide levels on the BNYVV genome to understand rhizomania resistance breaking variants of BNYVV. In summary, evaluation of rhizomania resistance breaking in field soil samples will provide important information to growers to make informed decisions on disease management strategies.