Genetic variability of BNYVV and BSBMV and quantification of Cercospora Leaf Spot with remote sensing

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Beet Necrotic Yellow Vein Virus (BNYVV) and Beet Soil Borne Mosaic Virus (BSBMV) are closely related viruses found throughout the growing regions of Minnesota and North Dakota. BNYVV is the casual agent of rhizomania and results in a severe reduction of extractable sucrose and yield. Both viruses are vectored by the same fungus, *Polymyxa beta*, and occupy similar ecological niches. This has raised questions of virus recombination, resulting in a new strain that shares features from both parent strains. None of the US germplasm is resistant to BSBMV and recombination between BSBMV and BNYVV could result in a virus with the damage of BNYVV and the ability to infect like BSBMV. With the increase in acres planted to beet varieties resistant to BNYVV, the possibility of selection for new more resistant strains of this virus has increased. This is compounded by the fact that nearly all of the US commercial beet varieties resistant to BNYVV owe their resistance to the Holly gene. In 2002, a strain of BNYVV was found in California's Imperial Valley on a beet variety that should have been resistant to BNYVV. USDA in Salinas CA confirmed virulence of this isolate on resistant sugar beets, putting nearly all of the US germplasm at risk. By understanding the variability that exists in natural populations of these viruses, we can evaluate the risk to Minnesota and North Dakota sugar beet growers.

Varieties and control methods for Cercospora leaf spot (CLS) are evaluated at many locations throughout the sugar beet growing regions of Minnesota and North Dakota. Evaluation of the severity of CLS is handled by different personnel at each site. Visual evaluations are a subjective measure and their accuracy depends on the skill of the evaluator. Since the human eye measures relative differences, the quality of visual assessments is also affected by the range of symptoms present within a trial. We are evaluating remote sensing technologies for their ability to measure CLS severity based on absolute measures of leaf reflectance. This approach has the potential to minimize the level of subjectivity of CLS severity measures. This would allow relatively unskilled labor to evaluate plots on a more frequent basis than is currently possible and comparison of data from different studies more realistic.

Results

Objective 1. Evaluate the potential of hand-held radiometers for measuring Cercospora leaf spot severity ratings and compare the accuracy and repeatability of results to visual ratings. This project was conducted in cooperation with Mohammed Kahn at his Drayton CLS trial and Mark Bredehoeft at his Willmar CLS trial. The Drayton site was evaluated on the 4th of September with Visual assessments by Mohamed Kahn and Charlie Rush. The Willmar site was evaluated on the 5th of September and the 18th of September. On the 5th of September, visual assessments were made by Mark Bredehoeft and Charlie Rush. On the 18th of September, visual assessments were made by Mark Bredehoeft and Karl Steddom. Canopy reflectance was measured with a CropScan multi-spectral radiometer with three readings per plot. At each site, canopy reflectance and visual assessments were repeated twice except for the Willmar evaluation on the 18th, which was only evaluated once. Spectral data from the CropScan radiometer was used to calculate 11 different vegetative indices prior to statistical analysis. CLS was severe at the Willmar site but mild at the Drayton site. Correlations between the spectral data and vegetative indices with visual assessments showed the Renormalized Difference Vegetative Index (RDVI) gave the highest correlations overall with visual disease estimates at both locations and all repetitions. Correlations between visual assessments and RDVI were generally very high with most of the correlation coefficients around -0.80. The three radiometer readings per plot could have bee replaced with only a single reading from anywhere in the plot, reducing the time for the radiometer to nearly the same as visual assessments. Neither the visual estimates nor any spectral indices gave good correlations with beet quality, tonnage, or recoverable sugar per acre. This is not surprising since yield integrates all agronomic factors from the whole life of the beet, where our evaluations were only a single point in time. Multiple evaluations throughout periods of disease pressure will allow calculation of the area under the disease progress curve, which should have a better correlation with yield. RDVI had a slightly better correlation with sugar per acre than visual assessments. Analysis is still underway on this data and a manuscript will be prepared shortly.

Objective 2. Quantify intraspecific genotypic variation among isolates of BNYVV and BSBMV. Soil samples were taken from fields in Minnesota, North Dakota, Colorado, Wyoming, Nebraska, and Texas which had previously been identified with BSBMV and BNYVV. Samples were baited with sugar beet seedlings from the soil samples in the green house. ELISA analysis was performed to verify the presence of the viruses. Viral RNA was isolated from beet roots infected with the virus. PCR amplification with BSBMV and BNYVV specific primers was used to identify genetic diversity.

Variation has been identified by gel electrophoresis of PCR products amplified with the virus-specific primers. The BSBMV isolates utilized for the variation analyses were Willmar1, Willmar2, Willmar3, Fargo1, Fargo2, Colorado1, Colorado2, Texas1, Texas2, Texas3, and Texas4. BSBMV RNA 2 primers designed to amplify positions 704 through 2229, which includes the BSBMV 75KDa read-through protein, revealed size variation between the published BSBMV sequence (Texas1) and Fargo2, Colorado2, and Texas2 isolates. The size variation indicated a deletion of approximately 400 bp. Primers designed to amplify positions 123 through 741, which includes the BSBMV 21 KDa protein, and primers designed to amplify positions 2103 through 3313, which includes the BSBMV 42 KDa protein, did not detect variation.

All of the read-through PCR products were purified from the electrophoresis gel and sequenced to verify the deletions sites. As was predicted from the gel electrophoresis, the sequence results confirmed deletions in Fargo2, Colorado2, and Texas2 isolates. The Colorado2 isolate has a 460 bp deletion between positions 1458 to 1918, which results in a +1 nucleotide frame shift. The Fargo2 isolate has a 411 bp in-frame deletion from positions 1458 to 1869. The Texas2 isolate has a 363 bp in-frame deletion from positions 1498 to 1861. In addition, the percent similarity of the isolates with deletions is comparable to the percent similarity of the other isolates sequenced.

The 75KDa read-through protein is one of the six open reading frames (ORF's) of BSBMV and BNYVV RNA2. The read-through protein is a complex which contains the coat protein and 495 additional amino acids, and results from the translational suppression of the coat protein termination codon. It has been shown to be essential for transmission of BNYVV virus by *Polymyxa betae.* Deletions from Fargo2, Colorado2, and Texas2 isolates removed a significant part of the read-through gene, but do not appear to have any effect on serological detection or transmission by the fungus *Polymyxa betae*.

The remaining ORF's of BSBMV RNA-1, RNA-2, RNA-3, and RNA-4 will be analyzed for genotypic variations. The same strategy will be used for BNYVV isolates. Isolates in which variation is detected by PCR and gel electrophoresis will be sequenced to determine the amount of variability, and prevalence of observed genotypes in the BSBMV and BNYVV viruses.

Objective 3. Relate virus genotype to incidence and severity of infection in resistant and susceptible sugar beet cultivars. Sugar beet root and soil samples were collected and rated for disease severity from different fields. Cultivars in these fields were part of BNYVV resistance trials in the American Crystal and Southern Minnesota regions. Root samples were tested for BNYVV and BSBMV infection by ELISA (Table 1). The results reveal that variety trials Beta 4818, HM 2411, Crystal 999, and HM 7169 contained the major percentage of BNYVV infection. It is interesting to note the high percentage of BSBMV recovered from some of the cultivars.

Table 1. Percentages of BNYVV and BSBMV virus infection from resistance trial cultivars.

Cultivar	# of Samples	% BNYVV	% BSBMV
HM 7169	7	57.1	0
Beta 4848	16	37.5	0
HM 2411	15	33.6	6.6
Crystal 999	15	33.3	13.3
Crystal R932	3	33.3	100
Holly 46519	7	28.6	0
VH 46177	11	20	0
Beta 4811	17	17.6	0
Beta 4930	5	0	20
HM RH5	4	0	0
Beta 4600	4	0	50
Crystal 952	1	0	100
Beta 3945	1	0	100
VH 46140	1	0	100
VH 46109	1	0	100
HM 7073	1	0	100

Soil samples have been baited with sugar beet seedlings in the green house. ELISA will be performed on those samples to determine virus presence. RNA extraction, PCR, gel electrophoresis, and sequencing will be used as described in objective 2 to identify specific viral genotypes that predominately infect resistant or susceptible cultivars.

Objective 4. Measure disease tolerance among BNYVV tolerance cultivars by quantitative PCR. In order to determine whether sugar beet cultivars perform well as a result of genetic resistance or regional adaptability, fluorescent real-time PCR will be used to quantify viral titer in susceptible and disease tolerant cultivars. An ABI Prism 7000 Sequence Detection System has been purchased and a technician with a Masters in Plant Pathology and 4 years of real-time PCR experience from Michigan State University has been hired to meet the project objectives. BNYVV and BSBMV specific real-time assays have been designed and await arrival of the ABI7000 for development. Virus-specific primers and probes were designed with ABI Primer Express software.

Susceptible and resistant beet cultivars will be infested with BNYVV and ELISA analysis will be performed to determine viral infection. RNA extracted from the infected roots will be analyzed by fluorescent real-time PCR to quantify viral titer in the roots of the sugar beet seedlings. Quantitative results of viral tolerance will be obtained.

Past Objectives: Two manuscripts based on research funded by this board have been accepted for publication in Phytopathology, the main journal for the field of Plant Pathology.

Spatial association and distribution of beet necrotic yellow vein virus and beet soilborne mosaic virus in sugar beet fields. F. Workneh, E. Villanueva, K. Steddom, and C.M. Rush.

Remote detection and differentiation of rhizomania and nitrogen deficiency in sugar beets. K. Steddom, G. Heidel, D. Jones, and C.M. Rush.

In addition, Dr. Rush has written a review of BNYVV and BSBMV that will be published in the Annual Review of Phytopathology.

Ecology and epidemiology of *Bennyviruses* and plasmodiophorid vectors. C.M. Rush