BEET NECROTIC YELLOW VEIN VIRUS INFECTION RESULTS IN ALTERATION IN EXPRESSION OF METABOLIC, PHOTOSYNTHETIC, AND PUTATIVE DEFENSE PROTEINS IN SUGARBEET

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Rhizomania, caused by *Beet necrotic yellow vein virus* (BNYVV) and transmitted by the soil-borne organism, *Polymyxa betae*, is one of the most economically important diseases affecting sugarbeet, and is widely distributed in most sugarbeet growing areas of the world. Fields can remain infested with BNYVV indefinitely because *P. betae* cystosori can remain dormant for up to 25 years. Three major BNYVV pathotypes have been reported world-wide; A-type, B-type, and P-type; the A-type is distributed throughout most sugarbeet growing regions of the world and is the only form present in the US. Control of BNYVV and rhizomania disease symptoms is achieved almost exclusively through planting of resistant varieties. A number of resistance genes have been identified for control of BNYVV, with the *Rz1* resistance gene used worldwide. Although suppressed, BNYVV can still replicate at a low level in resistant plants carrying the Rz1 resistance gene. This leads to selection pressure favoring emergence of variants that can replicate and accumulate better, ultimately leading to loss of resistance (resistance break down). Within a few crop rotations following the introduction of resistant sugarbeet varieties carrying the *Rz*1 resistance gene in the 1990s, new pathotypes that overcome resistance began to appear. Little is known of the interactions between sugarbeet and BNYVV that influence epidemiology and emergence of resistance breaking strains of the virus. As other *Rz* genes and minor genes are being increasingly incorporated into breeding materials and commercial sugarbeet to enhance performance and durability of resistance, it is becoming increasingly important to develop strategies to prolong the viability of resistance sources through understanding factors that lead to emergence of variants.

In order to determine what physiological and biochemical pathways are involved in BNYVV infection of sugarbeet by traditional A-type BNYVV, and breakdown of resistance due to emergence of resistance breaking variants, studies were performed to examine differences in protein expression among various combinations of resistant and susceptible sugarbeet with traditional and resistance-breaking A-type BNYVV. Previous studies had shown that a relatively small number of differences in sugarbeet protein expression were associated with BNYVV infection as well as resistance to infection. Further studies using more advanced methods have now examined protein extracts from near isogenic lines grown in virus-specific soils under standardized growth chamber conditions using SCX

fractionation, followed by reverse phase liquid chromatography and mass spectrometry (LC-MS-MS), with subsequent separation on a reverse phase nanospray column. Peptide spectra were examined for protein identity using a Uniprot *Amaranthaceae* database, and statistically significant differences in protein expression among resistant and susceptible sugarbeet were determined. A subset of representative genes encoding differentially expressed proteins and representing all treatments were identified through reverse genetics, and molecular probes designed to the identified sequences were used to examine RNA levels for validation of protein expression data. All transcript (RNA) levels examined reflected similar expression patterns to the results of protein expression analysis. Proteins exhibiting significantly different expression between BNYVV infection of resistant and susceptible near isogenic sugarbeet lines were associated with several protein classes.

Initial analysis involved proteomic profiling to compare protein expression differences that occur in resistant sugar beet (containing the *Rz1* gene) during interactions with BNYVV pathotype A (BNYVV-A) and the resistant breaking strain from the Imperial Valley (BNYVV-IV). These results were compared with expression in susceptible sugarbeet and to a limited scale, sugarbeet carrying a secondary resistance source, the *Rz2* gene. Proteomic profiling identified 203 proteins with confidence that were differentially expressed (statistically significant differences) as a result of infection by BNYVV. The differentially expressed proteins were associated with photosynthesis and energy production, plant metabolism or secondary metabolism, and responses to stimuli (including plant defense responses). Changes in expression of photosynthetic proteins likely reflects the pale yellow observed on leaves of infected sugarbeet. Proteins associated with primary and secondary metabolism may be involved in general effects on plant function during virus infection; whereas secondary metabolic pathways are often associated with defense against pathogens. Some such proteins include peroxidases, and other proteins that have also been identified for pathogen defense in other host plant systems. Proteins determined to have significantly different expression among treatments will help us identify pathways altered during BNYVV infection of resistant and susceptible sugarbeet. Subsequent studies will examine how alterations to some of these pathways may facilitate development of alternative strategies for control of BNYVV. This information can help us understand the changes that occur in sugarbeet that lead not only to infection and rhizomania disease development, but also to identify the mode by which resistance genes protect sugarbeet from the virus.