DIFFERENTIATING *Rz-1* AND *Rz-2* RESISTANCE REACTIONS TO *BEET NECROTIC YELLOW VEIN VIRUS* THROUGH PROTEOME ANALYSIS IN SUGARBEET

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ABSTRACT

Rhizomania, caused by *Beet necrotic yellow vein virus* (BNYVV), is one of the most economically important diseases affecting sugarbeet, and is widely distributed in most sugarbeet growing areas of the world. Control is achieved almost exclusively through planting of resistant varieties. Following the introduction of *Rz*1 varieties in the 1990s, new pathotypes that break resistance have appeared. Previous studies demonstrated that a relatively small number of differences in sugarbeet protein expression were associated with BNYVV infection as well as for resistance. Current studies examine protein differences among resistant (*Rz1* and *Rz2*) and susceptible (*rz1* and *rz2*) sugarbeet, when infected independently with the traditional (pathotype A) and *Rz1* resistance-breaking BNYVV (pathotype IV, from California's Imperial Valley). Near isogenic lines differing only for Rhizomania resistance were provided by KWS, and raised in virus-specific soils under standardized growth chamber conditions. Three independent growth chamber experiments were completed sequentially using the same growth chambers and growth parameters for all plants in all experiments to eliminate variability to the greatest extent possible. Protein was extracted from sugarbeet seedlings three weeks after planting to represent a time point early in the infection cycle. Peptides were purified and concentrated using an on-line enrichment column followed by chromatographic separation on a reverse phase nanospray column using a 90 minute linear gradient. Peptides were eluted directly into the mass spectrometer (Thermo Scientific LTQ linear ion trap). Compound lists of the resulting spectra were generated using Xcalibur 2.2 software (Thermo Scientific). Spectra obtained through mass spectrometry (MS/MS) were searched against the mouse Uniprot *Amaranthaceae* database (version 11/02/12) concatenated with reverse sequences for determination of the peptide FDR 1 (6,832 sequence entries) using both the Mascot database search engine (version 2.3) and Sorcerer™-SEQUEST®.

Search results for each independently analyzed sample were imported and combined using Scaffold software 3 (Version 3.6.4, Proteome Software, Portland, OR). Proteins containing shared peptides were grouped by Scaffold (Proteome Software, Portland, OR) to satisfy the laws of parsimony. Manual validation of MS/MS spectra was performed for all protein identifications above the probability thresholds that were based on only two unique peptides. Results identified nearly 500 proteins exhibiting variability; however, our analysis focused on only those proteins exhibiting significantly different expression between treatments. Most of the statistically significant protein differences are associated with photosynthetic pathways, supporting the effect

of BNYVV infection on foliar yellowing in the field. Others are involved in pathogen defense. Of particular interest is a Beta-1,3-Glucanase identified in BNYVV-susceptible interactions, which is a protein that has been shown to be a pathogen response protein in corn with antifungal activity. Additionally, this protein has been correlated with the ability of viruses to induce symptoms and move cell-to-cell in infected plants through degradation of callose deposits between cell walls and membranes, including in plasmodesmata. Peroxidases and a chitinase were also identified as significantly different during the susceptible interactions. Peroxidases are proteins involved in active oxygen-based stress responses in plants including responses to virus infection and systemic acquired resistance. Chitinases are involved in plant defense against fungi and could be associated with a host response to *P. betae*. Another interesting protein is glutamine synthetase, a protein exhibiting a four fold increase in BNYVV-resistant reactions compared with levels in susceptible interactions. This protein is involved in nitrogen regulation in plants. We suspect glutamine synthetase may be involved in general BNYVV infection or *P. betae* pathogenesis. Other proteins of interest have been identified, and are being evaluated.

The project is providing new information on physiological changes illustrated by protein expression variation among sugarbeet plants infected with a resistance-breaking BNYVV pathotype (BNYVV-IV), the resistance-breaking form of the virus from California's Imperial Valley, and the traditional A-pathotype (common throughout the US and world) and how this is influenced by the presence or absence of either the Rz1 or Rz2 resistance genes.