LIU, HSING-YEH*, JOHN L. SEARS, AND ROBERT T. LEWELLEN, USDA-ARS, 1636 E. Alisal St. Salinas, CA 93905. **Biological and molecular analyses of *Beet necrotic yellow vein virus* isolates that overcome host resistance genes.**

ABSTRACT

Rhizomania is an important virus disease of sugar beet. The disease is caused by *Beet necrotic yellow vein virus* (BNYVV) and vectored by the plasmodiophorid *Polymyxa betae*. The disease can only be controlled effectively by the use of resistant cultivars. During the 2002-2003 beet growing season, several sugar beet fields with cultivars partially resistant to BNYVV grown in the Imperial Valley of California were observed with severe rhizomania symptoms, suggesting that resistance conditioned by *Rz1* allele had been compromised. Soil testing with sugar beet baiting plants followed by enzyme-linked immunosorbent assay (ELISA) was used to diagnose virus infection. Resistant varieties grown in BNYVV-infested soil from Salinas, CA were ELISA negative. In contrast, when grown in BNYVV-infested soil collected from the Imperial Valley, CA all resistant varieties became infected and tested positive by ELISA. Based on host reaction, eight distinct BNYVV isolates have been identified from Imperial Valley soil (IV-BNYVV) by single local lesion isolation. These isolates do not contain RNA-5 as determined by reverse transcription-polymerase chain reaction (RT-PCR) assays. Single-strand conformation polymorphism banding patterns for the IV-BNYVV isolates were identical to A-type and different from P-type. Sequence alignments of PCR products from BNYVV RNA-1 near the 3' end of IV-BNYVV isolates revealed that both IV-BNYVV and Salinas BNYVV isolates were similar to A-type and different from B-type.

The pathogenicity of IV-BNYVV isolates was studied. Each IV-BNYVV isolate was mechanically inoculated to systemic host *Beta macrocarpa,* which was planted in sterilized soil. After showing systemic infection, virus-free *Polymyxa betae* were incorporated into the soil. One month later, the infected roots and soil were used for inoculum. New 280 ml styrofoam cups with holes punched in the bottom for drainage were placed in sterilized plastic saucers. Cups were filled with infested soil from each isolate (one part of inoculum with nine parts of sterilized soil). A plastic divider was inserted into each pot dividing each pot into four sections. The sugar beet varieties used were rhizomania-resistant varieties: Beta 4430R (*Rz1rz1*), KWS Angelina (*Rz1rz1+Rz2rz2*) and breeding line 1927-4H5 (*Rz1rz1*+Wild beet resistance) and rhizomania-susceptible variety Beta 6600 (*rz1rz1rz1*). Approximately 30 sugar beet seeds of each variety were layered on top of each section and were covered with sand to a depth of about 1 cm. Four replicates of each isolate were randomly placed on greenhouse benches. Each cup was about 30 cm apart to avoid contamination by splashing between cups. Greenhouses were maintained between 24-30 C. Roots from each section of these cups were harvested and tested for B NYVV by ELISA after 6 weeks post emergence of seedlings. Resistant varieties grown in Salinas BNYVV isolate-infested soil remained resistant. In contrast, when grown in Imperial Valley BNYVV isolates-infested soil, all resistant varieties tested positive for BNYVV in ELISA tests. From the pathogenicity test, results suggested that eight IV-BNYVV isolates could transmit by its vector, *Polymyxa betae*, and infect all three resistant cultivars tested.

Each IV-BNYVV isolate was purified. Viral RNA extracted from purified virion preparations using the RNeasy Mini Kit (QIAGEN Inc., Valencia, CA) according to the manufacturer's

instructions, was denatured by heating at 95 C for 10 min and annealed with a specific antisense oligonucleotide primer. First strand cDNA and PCR procedures were conducted. The PCR products were sliced and gel purified using QIAqueck Gel Extraction Kit (QIAGEN Inc., Valencia, CA) according to the manufacturer's instructions. The eluted DNAs were sequenced by a commercial company and sequences were analyzed. Sequence alignments of coat protein gene and P-25 protein (encoded by BNYVV-RNA-3, involved in symptom expression) of IV-BNYVV isolates revealed only minor amino acid changes compared to the existing A-type of Salinas BNYVV isolates. Our results suggest that the resistance-breaking BNYVV isolates from Imperial Valley likely evolved from existing A-type isolates.