LIU, HSING-YEH* and ROBERT T. LEWELLEN, USDA, Agricultural Research Service, 1636 East Alisal Street, Salinas, CA 93905. **Distribution and differentiation of resistance-breaking isolates of *Beet necrotic yellow vein virus* in the United States.**

ABSTRACT

Rhizomania causes major reductions in root quality and yield, making it one of the most economically important diseases of sugar beet worldwide. This disease is caused by *Beet necrotic yellow vein virus* (BNYVV) and is vectored by the plasmodiophorid *Polymyxa betae* Keskin. In the United States, the disease was first identified in California in 1984, but it now occurs in every major sugar beet production region in the country. Most sugar beet production areas are dependent upon partially resistant sugar beet cultivars to control this devastating disease. During 2003 and 2004 in the Imperial Valley of California, partially resistant sugar beet cultivars with *Rz1* allele seemed to be compromised. Resistance-breaking BNYVV (RB-BNYVV) isolates have been identified from these plants. In this research, a survey for the RB-BNYVV isolates in the sugar beet growing regions in the United States was conducted in 2004-2005 and the coat protein and P-25 protein of RB and NRB-BNYVV isolates were sequenced and analyzed.

The soil samples from rhizomania infested sugar beet fields throughout the United States were surveyed in 2004-2005. Pots were filled with infested soil from each soil sample (one part of soil with nine parts of sterilized sand). After pots were filled with appropriate soil samples, they were drenched with fungicides metalaxyl (Apron 25 W) at 0.2 g/liter and PCNB (Terraclor 75 W) at 0.25 g/liter to control damping-off and root rot caused by *Pythium* spp. and *Rhizoctonia* spp. Approximately 100 sugar beet seeds were placed on top of each pot and covered with sterilized sand to a depth of approximately 1 cm. The sugar beet varieties used were rhizomania-resistant varieties: 'Beta 4430R' (*Rz1rz1*), 'Beta G017R' (*Rz2rz2*), and 'KWS Angelina' (*Rz1rz1+Rz2rz2)* and rhizomania-susceptible triploid variety 'Beta 6600' (*rz1rz1rz1*). Each soil sample has four subsamples for each variety. Pots were arranged on greenhouse benches in a randomized complete block design with three replications for each soil sample. Greenhouses were maintained between 24-30°C. Six weeks post emergence the roots from each pot were harvested and tested for BNYVV by enzyme-linked immunosorbent assay (ELISA).

Our soil survey indicated that the resistance-breaking isolates not only existed in the Imperial Valley and San Joaquin Valley of California but also in Colorado, Idaho, Michigan, Minnesota, Nebraska, and Oregon. Out of all the soil samples tested, 92.5% of 'Beta 6600' (*rz1rz1rz1*), 77.5% of 'Beta 4430R' (*Rz1rz1*), 45.0% of 'Beta G017R' (*Rz2rz2*), and 15.0% of 'KWS Angelina' (*Rz1rz1+Rz2rz2*) tested positive in ELISA for BNYVV. 'Angelina' with two alleles for resistance (*Rz1* and *Rz2*) had a lower incidence level than either 'Beta 4430R' or 'Beta G017R' with the single allele *Rz1* or *Rz2* for resistance.

The coat protein gene from RNA-2 and P-25 protein (encoded by RNA-3, involved in symptom expression) of BNYVV isolates were sequenced. Analyses of the deduced amino acid sequences of coat protein and P-25 protein of RB-BNYVV isolates revealed the high percentage of identity with NRB-BNYVV isolates (99.9% and >98.0% respectively). The coat protein sequence of RB-BNYVV isolates and NRB-BNYVV isolates are 99.9% identity, indicating that

the resistance-breaking determinant may not be on the coat protein gene. The P-25 protein gene of BNYVV RNA-3 facilitates the multiplication and spread of the virus in root tissue and may have a major role in the production of rhizomania symptoms. Tamada et al., 1999 reported that P-25 protein deletion mutants of BNYVV did not cause rhizomania disease in sugar beets. Single amino acid changes in the P-25 protein of BNYVV RNA-3 determine resistance responses of *Beta vulgaris* spp. *maritima*. Nucleotide sequences for the RNA-3 encoded P-25 protein of RB and NRB-BNYVV isolates were determined and deduced amino acid sequences were compared. The P-25 proteins in all isolates consist of 219 amino acid residues and there was a maximum of 10 amino acid differences. The variable amino acids in P-25 proteins were located at 67 and 68 residues. In the United States, the two amino acids found in the NRB-BNYVV isolates were conserved (AC). The RB-BNYVV isolates were variable including AF, AL, SY, VC, VL, as well as AC. In order to confirm that the RB-BNYVV isolates in these two amino acids in the residues of 67 and 68 in P-25 protein were AC, re-isolation and sequencing were repeated and the results remained the same. Therefore, we cannot depend on the change of these two amino acids to differentiate RB and NRB isolates of BNYVV.

The large-scale cultivation of partially resistant cultivars with the sugar beet resistance genes *Rz1* and *Rz2* may impose selection pressure and lead to partial or total breakdown of resistance. Consequently, the durability of beet cultivars which are resistant to BNYVV should be reassessed, not only to the original A-pathotype but also to those RB-BNYVV isolates. Additional sources of resistance with different genetic determinants should also be sought to increase the stability and durability of the resistance. Rational thought needs to be given to their individual and combined deployment to help conserve the efficacy of individual resistance genes.