RUSH, C. M.*, LI PAETZOLD and BECKY BRYAN, Texas A&M AgriLife Research and Extension Center, 6500 Amarillo Blvd. West, Amarillo, TX 79106. **A New Method to Detect and Genotype Resistance-Breaking Strains of BNYVV from Grower's Fields.**

## ABSTRACT

Over the last 5-10 years, resistance-breaking (RB) strains of BNYVV have appeared at increasing frequency in all major sugar beet production areas in the USA. Development of a quick easy diagnostic test to determine what strains of BNYVV are present in a field would be extremely valuable to agronomists, scouts, diagnosticians and producers. Recently, a new method for identifying genetic variation in nucleic acid sequences has been developed. This method is called High Resolution Melting Point Analysis (HRM) and it is a simple, inexpensive technique that has great potential to be used to identify strains of BNYVV in grower's fields. The method is based on the melting dynamics of a PCR product and is able to differentiate PCR products with only a single nucleotide change. The objective of this study was to evaluate HRM for its ability to differentiate WT and RB strains of BNYVV.

Field soils suspected to be infested with viruliferous *Polymyxa betae* were collected. Approximately 50g soil was placed in a petri dish and just enough water was added to wet the soil. The damp soil then was incubated at 27C for 24 hours. After the initial 24 hour incubation, water was added to flood the soil and sugar beet seedlings were added, and the petri dishes were again incubated at 27 C for an additional 24 hours. During this second incubation period, it was anticipated that zoospores of *P. betae*, positive for BNYVV, would be attracted to and subsequently infect the sugar beet seedling. Sugar beet seedlings were collected and total RNA was purified, using the RNeasy Plant Mini Kit, and samples were exposed to reverse transcription for c-DNA synthesis. C-DNA from the different samples then was used as template in qPCR reactions. Samples that tested positive for BNYVV were used in HRM analysis, using primers that targeted the hypervariable region of P25 BNYVV RNA 3, and the individual melting curves were associated with WT and specific RB BNYVV genotypes.

The baiting technique to isolate BNYVV from field soil worked very well, and BNYVV was recovered from the sugar beet seedling bait plants from a majority of field soils tested. Traditional methods of baiting BNYVV from soils requires approximately 8 weeks, so recovering BNYVV by baiting viruliferous zoospores from infested soils, after just two days, represented a significant improvement. When BNYVV isolated from the sugar beet seedlings was evaluated by HRM, this method proved effective in differentiating WT and RB strains. To verify results, isolates of each strain were sent to the Gene Technology Facility in College Station, TX to sequence the entire PCR product and verify HRM results. In all samples, the full sequencing results confirmed the original strain identification determined by HRM analysis. The entire time now required to bait BNYVV from a field soil and determine whether it represents a RB or WT strain is four days, compared to approximately 10 weeks using traditional baiting and sequencing methods. HRM should be beneficial to sugar beet breeders in determining how their BNYVV reacts to specific BNYVV strains in field trials and also in studies of BNYVV population genetics.