Plant Pathology Seminar Series

"Development and Evaluation of ELISA and qRT-PCR for Identification of *Squash vein yellowing virus*"

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Squash vein yellowing virus (SqVYV) is the cause of viral watermelon vine decline, a devastating disease in Florida (Susan et al, 2011), causing $60–$70 million monetary losses in 2004 (Huber 2006). It belongs to the genus *Ipomoviris* (Adkins et al., 2007; Webb et al., 2012) and is transmitted semipersistently by sweetpotato whitefly (*Bemisa tabaci* (Gennadius) biotype B) (Webb et al., 2006, 2012).

Since symptoms of one virus disease might be different on different hosts and symptoms of different virus diseases might be similar, it is difficult to identify a virus disease just by visual diagnoses. Many diagnostic methods have been developed, like RT-PCR, tissue-blot nucleic acid hybridization assays, ELISA and qRT-PCR (Turechek et al., 2010; Webster et al., 2017). RT-PCR is impractical for large-scale testing of many samples because of the high costs (Turechek et al., 2013). Tissue blot can be used to test large numbers of samples but it is less sensitive and specific than RT-PCR (Webster et al., 2013). ELISA is sensitive and cost-effective for large number of samples and qRT-PCR is very sensitive for targeted applications, but neither ELISA nor qRT-PCR is available for SqVYV. Webster et al. in a 2017 study developed and applied ELISA and qRT-PCR for diagnosing and detecting SqVYV (Webster et al., 2017). Both methods were capable of detecting SqVYV in a wide range of cucurbit hosts. ELISA was able to detect the virus in infected host tissue diluted to at least 2,560 fold, which was sufficient for detection in symptomatic squash and watermelon plants (Webster et al., 2017). The qRT-PCR method was capable of reliably detecting as few as 3.4 copies of a cloned fragment of SqVYV genomic RNA with an average cycle threshold value of 36.4. The sensitivities and specificities for each detection method were estimated by latent class analysis for a set of inoculated squash and watermelon plants at two sampling scales, individual plants representing the upper scale and samples from the plant representing the lower scale (Webster et al., 2017). The number of samples per plant varied from 1 to 8, and a plant was diagnosed positive if any of its samples tested positive. qRT-PCR showed high sensitivities (≥0.99) at both sampling scales for squash and watermelon, whereas the sensitivities for ELISA ranged from 0.58-0.76. The specificities for both tests were very similar (≥0.94) with ELISA sometimes outperforming qRT-PCR (Webster et al., 2017). The ELISA test was proven to be a cost-effective, high-throughput system for screening large numbers of samples, and the qRT-PCR a highly sensitive method for more targeted surveys for diagnosis of SqVYV.

4:10 pm | Monday, April 3 | Johnson Hall 343

Plant Pathology 515, Spring 2017

College of
Agricultural, Human, & Natural Resource Sciences
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