Diseases Caused by Viruses

First Report of *Squash vein yellowing virus* Naturally Infecting Butternut Squash (*Cucurbita moschata*) in Texas

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Virus diseases are major constraints to the production of cucurbits in the Texas Lower Rio Grande Valley. In September 2020, a ~8.1-ha butternut squash (*Cucurbita moschata*) field in Hidalgo County, Texas, was observed with virus-like symptoms of vein yellowing, leaf curl, mosaic, and foliar chlorosis. The proportion of plants with virus-like symptoms in this field was estimated at 30%, and seven samples (symptomatic = 5; nonsymptomatic = 2) were collected randomly for virus diagnosis. Initially, equimolar mixtures of total nucleic acid extracts (Dellaporta et al. 1983) from two symptomatic samples from this field and extracts from 12 additional symptomatic samples from six other fields across south and central Texas were used to generate one composite sample for diagnosis by high-throughput sequencing (HTS). The TruSeq Stranded Total RNA with Ribo-Zero Plant Kit (Illumina) was used to construct a cDNA library from the composite sample, which was then sequenced on the Illumina NextSeq 500 platform. More than 26 million single-end HTS reads (75 nt each) were obtained, and their bioinformatic analyses (Al Rwahnih et al. 2018) revealed several virus-like contigs belonging to different species (data not shown). Among them, six contigs that ranged in length from 429 to 3,834 nt shared 96 to 100% identities with isolates of squash vein yellowing virus (SqVYV), genus *Ipomovirus*, family *Potyviridae*. To confirm the HTS results, total nucleic acid extracts from the cucurbit samples from all seven fields ($n = 46$) were used for cDNA synthesis with random hexamers and the PrimeScript first strand cDNA Synthesis Kit (Takara Bio). A 1- μ l aliquot of cDNA was used in 12.5- μ l PCR reaction

volumes with PrimeSTAR GXL DNA Polymerase (Takara Bio) and two pairs of SqVYV-specific primers designed based on the HTS-derived contigs. The primer pairs SqVYV-v4762 (5'-CTGGATTCTGCTGGAAGATCA)/SqVYV-c5512 (5'-CCACCATTAAAGGCCATCAAAAC) and SqVYV-v8478 (5'-TTTCTGGGCAAACAAACATGG)/SqVYV-c9715 (5'-TTCAGCGACGTCAAGTGAG) targeted ~0.75- and ~1.2-kb fragments of the cylindrical inclusion (CI) and the complete coat protein (CP) gene sequences of SqVYV, respectively. The expected DNA band sizes were obtained only from the five symptomatic butternut squash samples from the Hidalgo Co. field. Two amplicons per primer pair from two samples were cloned into pJET1.2/Blunt vector (Life Technologies) and bidirectionally Sanger sequenced, generating 753-nt partial CI-specific sequences (MW584341 to 342) and 1,238 nt that encompassed the complete CP (MW584343 to 344) of SqVYV. In pairwise comparisons, the partial CI sequences shared 100% nt/aa identity with each other and 98 to 99% nt/aa identity with corresponding sequences of SqVYV isolate IL (KT721735). The CP cistron of Texas isolates shared 100% nt/aa identity with each other and 90 to 98% nt (97 to 100% aa) identities with corresponding sequences of several SqVYV isolates in GenBank, with isolates IL (KT721735) and Florida (EU259611) being at the high and low spectrum of nt/aa identity values, respectively. This is the first report of SqVYV in Texas, naturally occurring in butternut squash. SqVYV was first discovered in Florida (Adkins et al. 2007) and subsequently reported from a few other states in the United States (Adkins et al. 2013; Batuman et al. 2015; Egel and Adkins 2007), Puerto Rico (Acevedo et al. 2013), and locations around the world. The finding shows an expansion of the geographical range of SqVYV and adds to the repertoire of cucurbit-infecting viruses in Texas. Further studies are needed to determine the prevalence of SqVYV in Texas cucurbit fields and an assessment of their genetic diversity.

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