New Disease Reports

# First report of *Grapevine yellow speckle viroid-2* infecting grapevine (*Vitis vinifera*) in Thailand

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Two viroids, *Hop stunt viroid* (HSVd) (Rakdang, 2001) and *Grapevine yellow speckle viroid-1* (GYSVd-1) (Hannok & Reanwarakorn, 2005) have been reported to infect grapevine in Thailand. From February through March 2014, 22 grapevine leaf samples (*Vitis vinifera* cvs. Black Opal seedless, Black Queen and Pok Dum) were sampled from eight vineyards in Saraburi (4 samples) and Nakhon Ratchasima (18 samples) provinces, which are the major grapevine plantation regions in Thailand. Various symptoms were found, including leaf roll with red colour, leaf malformation, vein banding and yellowing, yellow spots, and yellow flecks (speckle) (Figs. 1-2).

Total RNA from the 22 samples was extracted using a CTAB method (Doyle & Doyle, 1987) and subjected to RT-PCR for the detection of viroids known to occur in grapevine. For the detection of HSVd and *Citrus exocortis viroid* (CEVd), the methods of Shamloul *et al*. (2002) and Gross *et al*. (1982), respectively, were used. Additionally, two primer sets were designed to specifically detect the full-length genome of GYSPVd-1 [c-GYSVd1: 5′-CGAGGCTCACTCCCCCTCTGCC-3′ / h-GYSVd1: 5′-TCGTCGACGAAGGGGTGCACTCC-3′], and -2 [c-GYSVd2: 5′-GGTCCGCGGAGGCCTTCCGAGG-3′ / h-GYSVd2: 5′-TGCAGAGAAAAGAAGAAGGGCCCAG-3′] (Tangkanchanapas *et al*., 2015). The PCR products were cloned and two or three clones per sample were sequenced in one direction (First BASE Laboratories, Malaysia).

The results revealed that 3/22 and 6/22 grapevine leaf samples were infected solely with GYSVd-1 and GYSVd-2, respectively. Five of the 22 samples had mixed GYSVd-1 and -2 infections. All GYSVd-1 and GYSVd -2 positive leaf samples showed only yellow spots and yellow speckles, yet none of the other symptoms. HSVd and CEVd were not detected in any sample. The genome variants ranged in size from 353-389 nucleotides for GYSVd-1 and 362-365 nucleotides for GYSVd-2. All individual sequences were submitted to GenBank (Accession Nos. KP010005-KP010012 for GYSVd-1 and KP010013-KP010023 for GYSVd-2). A nucleotide BLAST analysis using the GYSVd-1 and GYSVd-2 sequences showed that isolates from this study shared 93-99% and 98-99% similarity with corresponding GYSVd-1 (AF059712, DQ371471, GQ995468, X87913 and X87920) and GYSVd-2 isolates (FJ490172, FJ597943 and JQ686716), respectively.

For phylogenetic analysis, maximum-likelihood method with 500 bootstrap replicates and a 50% cut-off value was used (MEGA version 7.0.21). For GYSVd-1 the Thai isolates cluster in two distinct phylogenic sub-clades indicating genetic variation, which cannot be linked to the geographic origin of sampling (Fig. 3). The Thai GYSVd-2 isolates formed a single clade indicating less variation compared to GYSVd-1. In addition, all of the GYSVd-1 and 2 infections were only found in Nakhon Ratchasima province, yet the viroids were found in all major grapevine cultivation sites in this province. However, disease incidence in all vineyards is very low (<1%). To our knowledge, this is the first report of natural GYSVd-2 infection in grapevine in Thailand.

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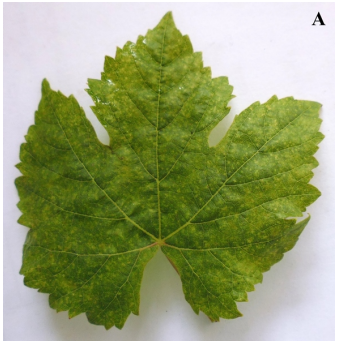


Figure 1

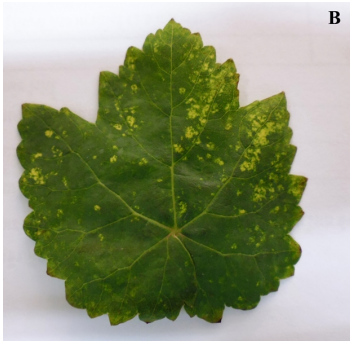


Figure 2

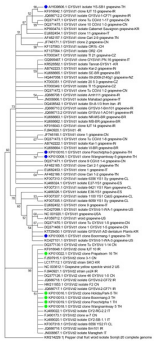

Figure 3

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