Diseases Caused by Viruses

First Report of Rose Leaf Rosette-Associated Virus Infecting Rose (*Rosa* spp.) in California, U.S.A.

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Rose leaf rosette-associated virus is a member of genus *Closterovirus*, family *Closteroviridae*. Rose leaf rosette-associated virus (RLRaV) was first discovered in China in 2015 from a mixed infected wild rose (*Rosa multiflora* Thunb.) showing small leaf rosettes on branches, die-back, and severe decline symptoms (He et al. 2015). In 2013, a rose plant (cv. Roses Are Red) was introduced to the Foundation Plant Services (University of California, Davis) rose collection. The plant originated from a private rose breeder collection located in California. In 2019, total nucleic acids (TNA) were isolated from leaf tissues of one asymptomatic plant (Roses Are Red plant) using a MagMax Plant RNA Isolation Kit (Thermo Fisher Scientific, U.S.A.). Extracted TNA were screened by reverse-transcription quantitative PCR for six common viruses infecting roses, including prunus necrotic ringspot virus, apple mosaic virus, rose spring dwarf associated virus, rose yellow vein virus, rose rosette virus, and blackberry chlorotic ringspot virus; however, the results were negative. Therefore, the sample was subjected to high-throughput sequencing (HTS). Briefly, TNA was depleted of rRNA and advanced for cDNA library preparation using the TruSeq Stranded Total RNA kit (Illumina, U.S.A.). HTS was performed on an Illumina Next-Seq 500 platform. The raw reads were trimmed, de novo assembled, and

subsequently annotated using the tBLASTx algorithm (Al Rwahnih et al. 2018). HTS generated 23.6 million 75-nucleotide (nt) single-end raw data reads. De novo assembly generated a contig (16,528 nts) resembling the RLRaV reference sequence (KJ748003) with 74% identity at the nucleotide level. Putative coat protein and heat shock protein 70-like protein were identified based on >90% identity with RLRaV genes. To confirm HTS results, reverse-transcription PCR was performed using two primer sets, (i) Clo-F4916 (5'-GGTGTCCAACGC TATCGTG-3') and Clo-R5215 (5'-TGTCCTCAAACCGCCTACAT-3') targeting nucleotide sequences of putative polyprotein 1a, and (ii) Clo-F10006 (5'-GATTCCGCGGACGAATTAAT-3') and Clo-R10311 (5'-GGTAACCGAAAGGTAAAGTATTC-3') targeting nucleotide sequences of putative protein p25. The RLRaV amplicons with expected size of 300 nt were confirmed using bidirectional Sanger sequencing. The near-complete sequence of the new RLRaV isolate was deposited in GenBank under accession number MW056181. In addition, HTS analysis showed that RLRaV was in mixed infection with two mycoviruses (rose cryptic virus with 8,267 mapped reads and rose partitiviruses with 7,283 mapped reads). To our knowledge, this is the first report of RLRaV affecting roses in California. Further research is needed to determine the prevalence of RLRaV in California as well as evaluation of RLRaV's effect on rose performance.

References:

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