The Occurrence of *Grapevine Fanleaf Virus* in Washington State Vineyards

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Grapevine degeneration in grapevines caused by *Grapevine fanleaf virus* (GFLV) has been documented in many viticultural regions worldwide (Andret-Link et al. 2004). It is one of the major economically important virus diseases affecting the longevity of grapevines and reducing the fruit yield and fruit quality. Infected grapevines show a range of foliar symptoms consisting of leaf deformation, yellow mosaic, vein banding, ring and line patterns and flecks. GFLV cause yield reductions as high as 80% depending on the cultivar and severity of infection (Martelli and Savino 1990). Plant-to-plant spread of GFLV is known to occur by dagger nematode (*Xiphinema index*) and hence the infected grapevines appear in patches in the field. Long distance spread of the virus, however, occurs by transfer of infected propagation material. GFLV (genus: *Nepovirus*, family *Comoviridae*) has a bipartite genome consisting of two single-stranded, positive sense RNAs called RNA-1 and RNA-2 (Pink et al., 1988). Genetic diversity of GFLV genome has been studied in different countries (Arani et al. 2001; Vigne et al., 2004; Pompe-Noak et al., 2007), thereby establishing quasi-species nature of the virus.

GFLV has been documented in grapevines in different viticultural regions of USA (Qiu et al., 2007; Arani et al., 2001; Milkus and Goodman, 1999). However, the status of GFLV in the Pacific Northwest vineyards, consisting of Idaho, Oregon, and Washington and accounting for the second largest grape-growing region in the USA, is currently not known.

Materials and Methods

During our reconnaissance studies in 2007, dormant wood cuttings were collected randomly from Chardonnay grapevines in two geographically separate vineyards in Eastern Washington State. A total of 26 samples from one Chardonnay block and 31 from another Chardonnay block were tested separately for GFLV by one tube-one step reverse transcription-polymerase chain reaction (RT-PCR) method using virus-specific primers. A forward primer (5'-ACCGGATTGACGTGGGTGAT, corresponding to nucleotides [nt] 2231-2250) and reverse primer (5'-CCAAAGTTGGTTTCCCAAGA, complementary to nt 2533-2552) of GFLV-F13 isolate (GenBank accession number: X16907) were used in RT-PCR assays for amplification of a 322 nucleotide fragment specific to the coat protein (CP) of GFLV (Rowhani et al., 1993). The amplicons were cloned into pCR2.1 vector (Invitrogen Corp, Carlsbad, CA). Three independent clones per amplicon were sequenced from both orientations and a consensus sequence derived for each amplicon using Vector NT1 Advance10 software (Invitrogen). Multiple alignments were performed using Clustal W (BioEdit version7.0.5.3, Ibis Therapeutics, Carlsbad, CA) and phylogenetic analysis was carried out using MEGA4 (Tamura et al., 2007). Corresponding sequences of GFLV isolates in Genebank (accession numbers: DQ922668, AY017338, DQ362921, X16907, U11768, X60775, AF304013, AF304014, AF304015) were included in these studies. A selected number of GFLV-positive samples were tested by enzyme-linked immunosorbent assay (ELISA) with GFLV-specific antibodies.

Results and Discussion

In RT-PCR, a 322 nt DNA fragment specific to GFLV CP was amplified from two out of thirty one grapevines in one Chardonnay block and six of the twenty six grapevines in the second Chardonnay block. ELISA results further confirmed the presence of GFLV in samples that were positive in RT-PCR. Pair wise comparison of sequences derived from the eight grapevines showed 99-100% nucleotide sequence identity among themselves, indicating that GFLV isolates from the two vineyards may be

identical. A comparison of GFLV sequences obtained in this study with corresponding sequences in the GeneBank showed 87-92% identity at the nucleotide and amino acid sequence level. These results indicate that GFLV isolates from Washington State vineyards are distinct strains of the virus.

GFLV-positive samples from the first Chardonnay block tested positive for *Grapevine leafroll-associated virus* (GLRaV)-3 and those from the second Chardonnay block tested positive for GLRaV-1, GLRaV-3, and *Grapevine virus A.* These results indicate mixed infection of GFLV with other grapevine viruses. In addition, presence of GFLV as mixed infection with different viruses in the two Chardonnay blocks may suggest independent origin of planting material in these blocks. To our knowledge, this is the first report of GFLV in grapevines in the Pacific Northwest region of USA. Consequently, this study adds to existing knowledge on the distribution of GFLV in grapevines in the USA. Further investigations are being carried out on the distribution, symptoms, molecular variability and nematode vector transmission of GFLV.

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