Sequence variability of *Grapevine Rupestris stem pitting-associated virus* in Washington state vineyards.

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Introduction

Washington state ranks second, after California, in wine grape acreage and production in the USA. Among the several viruses documented worldwide on grapevines (Martelli, 2000), *Grapevine leafroll-associated virus*-1, -2, -3, -4, -5, and -9 and *Grapevine Rupestris stem pitting-associated virus* (GRSPaV) have so far been documented in WA vineyards (Martin et al., 2005; Naidu et al., 2006). GRSPaV (family *Flexiviridae*, genus *Foveavirus*) is closely associated with the Rupestris stem pitting (RSP) disease of the Rugose Wood complex and prevalent in many viticulture regions (Minafra and Boscia, 2003). Although GRSPaV has been removed from the list of quarantine pests in WA, it is still a controlled pest in the state certification program. Since wine grape cultivars are largely grown in WA as own-rooted vines, GRSPaV may not show obvious symptoms in these cultivars. Thus, own-rooted vines may carry the virus as symptomless infections. Therefore, an understanding of the prevalence of GRSPaV in WA vineyards is valuable in maintaining the sanitary status of WA vineyards.

Several molecular variants of GRSPaV have been documented in different viticulture regions (Meng et al., 2006; Nolasco et al., 2006). However, information on the variability of GRSPaV in WA vineyards is not available. Therefore, we have initiated a comprehensive study to document the prevalence of molecular variants of GRSPaV in wine grape cultivars grown in the state.

Materials and Methods

A total of twenty nine isolates were collected from twelve different wine grape cultivars. The coat protein (CP) gene was amplified by a one-tube RT-PCR method (Rowhani et al., 2000) using the primers RSP52 (5'-TGAAGGCTTTAGGGGTTAG-3') and RSP53 (5'-CTTAACCCAGCCTTGAAAT-3'). The amplicons (905 base pairs) were cloned into pCR2.1 vector (Invitrogen Corp, Carlsbad, CA). Two to three independent clones per isolate were sequenced in both orientations and a consensus sequence was obtained for each isolate. Additional clones were sequenced when there were sequence differences between clones obtained from the same isolate. In such cases, sequences from individual clones were included for further analysis. Thus, a total of thirty four sequences obtained from twenty nine isolates were analyzed in this study. Nucleotide sequences were aligned and compared using Vector NTI Advance10 software (Invitrogen). Multiple sequence alignments were performed using Clustal W [(BioEdit version7.0.5.3 (Ibis Therapeutics, Carlsbad, CA)] and phylogenetic analysis was performed by the neighbor-joining method using PAUP Version4.0b10 (Sinauer Associates, Inc., Sunderland, MA). Corresponding CP sequences of other GRSPaV isolates available in GenBank (accession numbers AF026278, AF057136, AY881626, AY881627, AY927670, AY927673, AY927674 and AY927684) were included in these analyses.

Results and Discussion

In pair-wise comparisons, CP sequences showed nucleotide identities from 80 to 99 percent, indicating molecular variability among the twenty nine GRSPaV isolates obtained from WA vineyards. In addition, GRSPaV isolates derived from the same cultivar in four different vineyards showed nucleotide sequence identities ranging from 80 to 98 percent. Furthermore, CP sequences of GRSPaV isolates obtained from individual grapevines in five different cultivars showed sequence variability ranging from 82 to 96 percent, demonstrating the quasi-species nature of GRSPaV. Phylogenetic analysis of these sequences together with corresponding CP sequences from the GenBank revealed that GRSPaV isolates from WA are scattered in all four lineages described earlier by Meng et al. (2006) and Nolasco et al. (2006). Ten of the thirty four sequences from WA clustered with GRSPaV-1 lineage, six with GRSPaV-SG1 lineage, three with GRSPaV-BS lineage and fifteen with GRSPaV-VS lineage. Thus, of the thirty four GRSPaV CP sequences obtained so far, the majority of them belonged to GRSPaV-VS lineage.

This study provided evidence for the presence of divergent variants of GRSPaV in Washington State vineyards. Antibodies are not available for the detection of different GRSPaV isolates; therefore results obtained from this study provide information for the development of better diagnostic tools that would detect all the strains of GRSPaV in certification and 'clean' planting programs.

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