Association of a Circular DNA Virus in Grapevines Affected by Red Blotch Disease in California

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INTRODUCTION

In 2008, a new disease consisting of patches of red blotches along leaf margin, and red veins under the leaf surface were observed in red grape varieties in a few vineyards in Napa Valley, CA. Brix units of fruit juice of symptomatic, but not asymptomatic grapevines, were reduced (Calvi 2011). Anecdotal observations suggested that the disease was spreading in the vineyards. The name ‘grapevine red blotch (GRB)’ was proposed to distinguish this disease from leafroll disease. Absence of signs and symptoms associated with bacterial and fungal pathogens prompted investigations to determine the causal agent of GRB. RNA extracts derived from petioles obtained from symptomatic grapevines tested negative for known grapevine viruses in RT-PCR assays.

Recently, metagenomic analysis using next generation sequencing (NGS) has successfully revealed the presence of previously uncharacterized viruses (Al Rwahnih et al., 2009; Kreuze et al., 2009; Zhang et al., 2011). Herein we report on the identification of a new DNA virus in nucleic acid extracts obtained from grapevines showing GRB symptoms using NGS.

MATERIALS AND METHODS

Dormant canes were collected in fall 2010 from three symptomatic grapevines, one from each of the three commercial vineyards, and bark scrapings were obtained. Double stranded RNA was extracted without DNAase treatment, cDNA prepared and the library was amplified as described by Al Rwahnih et al. (2009). Sequence reads were generated by Eureka genomics (Hercules, CA, USA) using an Illumina Genome Analyzer IIX. After sequencing, the contigs were assembled and BLASTN and TBLASTN analysis were performed at the NCBI web site. Primers were designed to detect a new DNA virus identified in the TBLASTN analysis and PCR assays followed by agarose gel electrophoresis were conducted to detect the new virus in DNA extracts obtained from source vines and several symptomatic grapevines in fall 2011. Complete sequence of the new virus was obtained by sequencing amplified products obtained using Illustra™ TempliPhi kit (GE Healthcare Biosciences, Philadelphia, PA, USA).

RESULTS AND DISCUSSION

The TBLASTN analysis using the nucleotide sequence of contigs obtained from each of the three source vines indicated a distant homology at the amino acid level with geminiviruses. The complete sequence determined by sequencing the products obtained by rolling circle amplification of DNA from symptomatic grapevines indicated the presence of a new circular DNA virus.

PCR assays using primers specific to the new virus were able to amplify a product from DNA extracts obtained from petioles of grapevines showing red blotch symptoms. Similar results were also obtained from DNA obtained from bark scrapings of dormant canes. The new virus has been named ‘Grapevine red blotch-associated virus’. We are currently investigating the biological properties of the virus to ascertain its role in red blotch disease.
REFERENCES

