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Research article

Cloning and characterization of VIGG, a novel virus-induced grapevine protein, correlated with fruit quality

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ABSTRACT

We report here the identification and characterization of VIGG, a novel virus-induced grapevine protein. Analysis of VIGG expression in grapevine demonstrated that VIGG was constitutively expressed in leaves and stems in virus-infected grapevine, and that VIGG expression was induced by grapevine virus A (GVA) infection, but not by infection with other viruses. The virus-induced expression profile of VIGG was supported by the finding that virus-free meristem cultures prepared from virus-infected grapevines did not express VIGG. An experiment using GFP–VIGG fusion protein demonstrated that VIGG might be localized in or around the endoplasmic reticulum (ER). Treatment of grapevine cells with ER stress inducers resulted in the induction of VIGG expression. Berries from VIGG-expressing grapevines had higher organic acid and phenolic contents than those from control grapevines that did not express VIGG. Interestingly, fruit composition of a grapevine that was simultaneously infected by GVA and grapevine virus B (GVB), which did not express VIGG, was significantly different from that of GVA-infected grapevines expressing VIGG, suggesting that the effector of fruit composition alteration might be VIGG expression, but not GVA infection. Taken together, VIGG expression might suppress the decrease in organic acid content and increase phenol content in berries. Further investigation of the biological function of VIGG is expected to provide new information on the fruit quality of grapevines.

1. Introduction

Grapevine (*Vitis vinifera*) is an ancient species and is one of the most widely cultivated worldwide. Current cultivars of grapevine are susceptible to many kinds of pathogens, including fungi, bacteria, viruses, and insects. Viral infection, in particular, causes severe damage to the quality and yield of grape berries. Grapevine is a host to more than 40 viruses [1]. Therefore, techniques to identify viral species in virus-infected grapevines and to monitor virus transmission in vineyards have been developed, including ELISA with anti-virus antibodies [2,3] and real-time reverse transcription–polymerase chain reaction (RT–PCR) with virus-specific primers [4,5]. However, the management of viral infection in vineyards remains insufficient and it is not possible to completely eliminate viruses from grapevine using any currently available method. Although antiviral agents, such as tiazofurin, mycophenolic acid, and ribavirin have been investigated [6,7], no chemotherapeutic management of viral infection has been put into practical use in vineyards thus far. Another approach, namely, the genetic transformation of grapevines to confer resistance to viruses, has been considered [8-10]. Subsequent to the suggestion of Lomonossoff [11] regarding pathogen-derived resistance to plant viruses, transgenic grapevines expressing viral coat protein have been engineered [12,13]. Although transgenic grapevines showed resistance to viral infection, some risks, such as environmental safety, have been identified with the use and application of genetically modified plants [12]. It also appears that it would take a significant amount of time to achieve social acceptance of genetically modified plants.

Viral infection has led to decreases in grapevine biomass and fruit quality by decreasing sugar content, delaying ripening, and suppressing anthocyanin accumulation [14,15]. To understand physiological changes in virus-infected grapevine, molecular events associated with viral compatible diseases in grapevine have been investigated using DNA microarray technology [16,17]. The expression of genes involved in biological and physiological functions was induced by viral infection, while chloroplast genes were repressed in the plant–virus compatible interaction [16]. Various photosynthetic activities in chloroplasts were also reduced in virus-infected leaves [18,19]. Furthermore, senescence-associated genes,

Abbreviations: AZC, azetidine-2-carboxylic acid: DTT, dithiothreitol; ER, endoplasmic reticulum; GVA, grapevine virus A; RT–PCR, reverse transcription–polymerase chain reaction; TA%, titratable acidity; TM, tunicamycin; VIGG, virus-induced grapevine protein.

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such as protease and lipase genes, which were elevated in senescent leaves [20], were expressed at elevated levels in virus-infected grapevine [17]. This relationship between viral infection and senescence may represent a strategy to recycle nutrients from infected tissues and to mobilize them for transportation to distant tissues, allowing a plant-virus compatible interaction to be established, even for long periods of time [17]. Thus, there is no doubt that the identification of all the genes or proteins that change in a compatible interaction between virus and grapevine represents one approach to answering the question "Why do viruses reduce fruit quality?". However, it is difficult to apply data derived from DNA microarray or proteomic analysis to bioinformatics evaluation in the compatible interaction. This is due to the large number of proteins that are selected as candidates for effector proteins that have the ability to change the physiological, as well as pathological, conditions of virus-infected grapevines. On the other hand, classical cloning strategies, such as the differential display method [21] and the subtractive hybridization method [22], are still useful in identifying novel virus-induced proteins. Their advantage lies in their being able to characterize the function of the proteins much more easily than the exhaustive DNA microarray technique.

In the present study, we report the molecular cloning and characterization of a novel virus-induced grapevine protein. By using the RT–PCR-based differential display method that compares the gene expression patterns in virus-infected grapevine with those in virus-free grapevine, we identified a novel and unique virus-induced grapevine protein and named it "virus-induced grapevine protein", VIGG. VIGG expression was induced by infection with grapevine virus A (GVA). VIGG was localized in or around the endoplasmic reticulum (ER) and expressed upon ER stress induction in grapevine cells. Furthermore, we demonstrated that VIGG expression in grapevines might be correlated with fruit quality.

2. Results

2.1. Cloning of VIGG from virus-infected grapevine

RT–PCR was used to determine the localization of viruses in leaf tissue of virus-infected grapevine (Virus+ in this study) infected by grapevine leafroll associated virus 3 (GLRaV-3), GVA, grapevine virus B (GVB), and Rupestris stem pitting associated virus (RSPaV). Although GVA was detected in total RNA isolated from whole leaves, RNA genomes of GLRaV-3, GVB, and RSPaV appeared to be present at significantly lower levels (Fig. 1A), suggesting that these viruses localized in the sub-tissues of leaves. When veins were excised and subjected to RT–PCR, all viruses were detected at high levels (Fig. 1A). Considering these results, cloning of the genes that were differentially expressed in response to viral infection was performed using the RT–PCR-based differential display method, with total RNA isolated from veins. Comparing the gene expression profiles between Virus+ and virus-free (Virus− in this study) grapevines, a virus-induced transcript was identified using 5′ (5′-GGC TGT GTT A-3′) and 3′ (5′-TTT TTT TTT TTG C-3′) arbitrary primers (Fig. 1B). RT–PCR analysis with transcript-specific primers showed that the transcript was expressed in Virus+ grapevine, but not in Virus− grapevine (Fig. 1C). PCR analysis of genomic DNA isolated from both grapevines demonstrated that the DNA sequence encoding the transcript existed in genomic DNA of grapevines irrespective of viral infection (Fig. 1D). In addition, VIGG gene is also present in the genomes of other *V. vinifera* cultivars, such as Pinot Noir, Cabernet Sauvignon, Merlot, Chardonnay, and Riesling (Fig. 1E). Moreover, a homologue of the transcript was identified in the EST libraries of *V. vinifera* cv. Muscat Hamburg pre-véraison berry [23]. These results suggest that the transcript was transcribed from genome of Virus+ grapevine. This transcript was

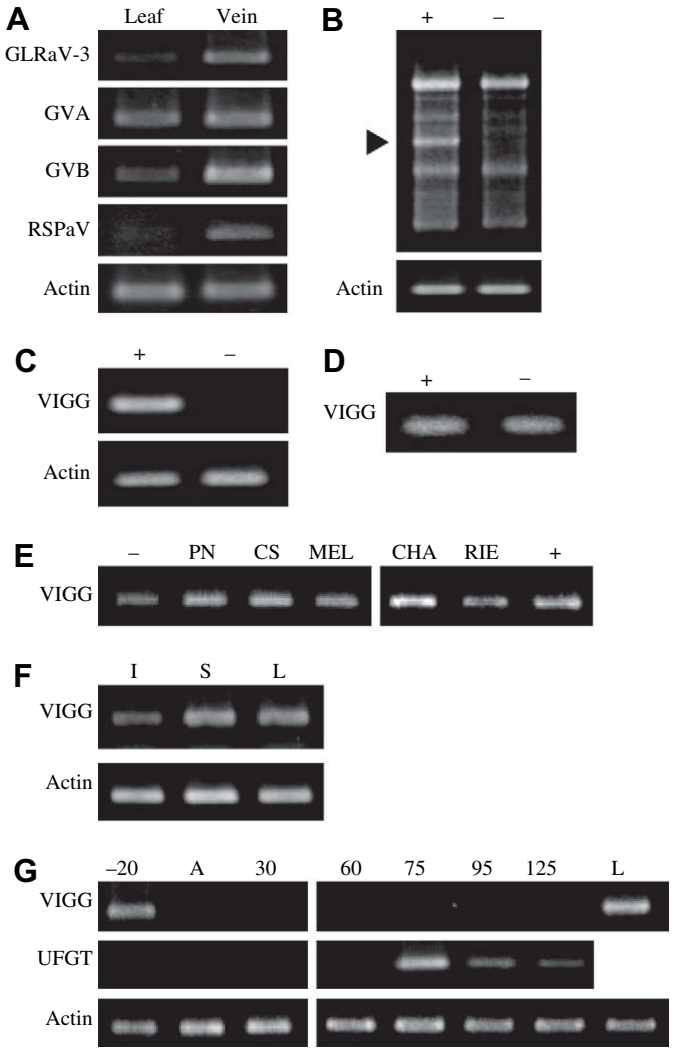

Fig. 1. Cloning of VIGG from virus-infected grapevine. (A) Localization of viruses in leaf tissues. GLRaV-3, GVA, GVB, and RSPaV were mainly detected in veins. (B) RT–PCR-based differential display analysis. Total RNA was isolated from Virus+ and Virus− grapevines and subjected to RT–PCR-based differential display analysis using 5′(5′-GGC TGT GTT A-3′) and 3′(5′-TTT TTT TTT TTG C-3′) arbitrary primers. Arrowhead indicates the position of a VIGG transcript. (C) RT–PCR analysis. Total RNA was isolated from Virus+ and Virus− grapevines and subjected to RT–PCR using VIGG-specific primers. (D) Genomic PCR analysis. Genomic DNA was isolated from Virus+ and Virus− grapevines and subjected to PCR using VIGG primers. (E) Genomic PCR analysis. Genomic DNA was isolated from Pinot Noir (PN), Cabernet Sauvignon (CS), Merlot (MEL), Chardonnay (CHA), and Riesling (RIE) and subjected to PCR using VIGG primers. (F) Tissue distribution of VIGG in virus-infected grapevine. Total RNA was isolated from inflorescence (I), stem (S), and leaf (L) in Virus+ grapevine and subjected to RT–PCR analysis. (G) Timing of VIGG expression during berry development. Total RNA was isolated from berries at the indicated stages (anthesis was on June 13, 2007) and subjected to RT–PCR analysis. Numbers on top indicate days before or after anthesis (A). Leaf (L) was used as control for VIGG expression. UFGT primers (UFGT) were used as control for berry development. β-Actin primers (Actin) were used as internal control for RT–PCR. +, Virus+ grapevine; −, Virus− grapevine.

expressed in inflorescences and stems of VIGG+ grapevine, as well as in leaves (Fig. 1F). UDP glucose-flavonoid 3-*o*-glucosyl transferase (UFGT) transcript, whish is induced during berry development, was detected 75 days after anthesis, while VIGG transcript was detected in berries 20 days prior to anthesis, but not after anthesis (Fig. 1G).

We also completed the identification of the full length of the transcript using 5′ RACE, and determined a 1280 bp transcript and a 336 bp open reading frame (ORF) encoding a novel protein