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Short communication

CLAVATA3-like genes are differentially expressed in grape vine (*Vitis vinifera*) tissues

Rumi Tominaga-Wada[a,*], Yuka Nukumizu[a], Takuji Wada[a], Shinichiro Sawa[b], Takuya Tetsumura[c]

[a] *Interdisciplinary Research Organization, University of Miyazaki, 1-1, Gakuen Kibanadai-Nishi, Miyazaki 889-2192, Japan*
[b] *Graduate School of Science and Technology, Kumamoto University, Kurokami, 2-39-1, Kumamoto 860-8555, Japan*
[c] *Department of Agricultural and Environmental Sciences, Faculty of Agriculture, University of Miyazaki, 1-1, Gakuen Kibanadai-Nishi, Miyazaki 889-2192, Japan*

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ABSTRACT

The CLAVATA3 (CLV3)/endosperm surrounding region [(ESR) CLE] peptides function as intercellular signaling molecules that regulate various physiological and developmental processes in diverse plant species. We identified five *CLV3*-like genes from grape vine (*Vitis vinifera* var. Pinot Noir): *VvCLE 6*, *VvCLE 25-1*, *VvCLE 25-2*, *VvCLE 43* and *VvCLE TDIF*. These *CLV3*-like genes encode short proteins containing 43–128 amino acids. Except VvCLE TDIF, grape vine CLV3-like proteins possess a consensus amino acid sequence known as the CLE domain. Phylogenic analysis suggests that the *VvCLE 6*, *VvCLE25-1*, *VvCLE25-2* and *VvCLE43* genes have evolved from a single common ancestor to the *Arabidopsis CLV3* gene. Expression analyses showed that the five grape *CLV3*-like genes are expressed in leaves, stems, roots and axillary buds with significant differences in their levels of expression. For example, while all of them were strongly expressed in axillary buds, *VvCLE6* and *VvCLE43* expression prevailed in roots, and *VvCLE25-1*, *VvCLE25-2* and *VvCLE TDIF* expression in stems. The differential expression of the five grape CLV3-like peptides suggests that they play different roles in different organs and developmental stages.

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Introduction

Peptide hormones secreted as signaling molecules are important for intercellular communication and are utilized in multicellular organisms. System in which functions in the wounding response, was the first plant peptide hormone to be identified (Pearce et al., 1991). CLAVATA3 (CLV3) was the first peptide hormone identified in *Arabidopsis* and controls the number of cells in the shoot apical meristem (SAM) (Fletcher et al., 1999). Mutations in the *CLV3* gene can induce enlargement of the SAM (Clark et al., 1995). *Arabidopsis* has 32 CLE peptides, named for the CLV3/ESR-related peptide family, including CLV3 (Betsuyaku et al., 2011). The CLE peptides are thought to function as a 12–13 amino acid peptide that regulates cellular differentiation activity in the SAM and root apical meristem as well as in vascular tissues (Cock and McCormick, 2001; Ito et al., 2006; Kondo et al., 2006; Ohyama et al., 2008, 2009). Genes homologous to *CLE* have been identified in various plant species, including the green alga *Chlamydomonas reinhardtii* (Miwa et al., 2009; Oelkers et al., 2008). Recently, Okamoto et al. (2011) identified a *CLV3*-like gene in the model legume *Lotus japonicus* and named it *LjCLV3*. The *LjCLV3* gene is responsible for maintenance of the SAM and primary and secondary inflorescence meristems in *L. japonicus* (Okamoto et al., 2011). Furthermore, CLE peptides have been discovered as controlling agents of legume nodulation in *Medicago truncatula* (Mortier et al., 2010) and *Glycine max* (Reid et al., 2011a, b, 2013). Significantly, despite a high homology to AtCLV3 the legume peptides are exclusively expressed in the root to control either nodulation responses or nitrate inhibition of nodulation.

Common grape vine (*Vitis vinifera*) belongs to the most important fruit crops, cultivated for over 6000 years (McGovern et al., 1997). The grape vine genome has been sequenced from a highly homozygous (Jaillon et al., 2007) as well as a heterozygous line derived from var. Pinot Noir (Velasco et al., 2007). In this study, we identified five grape *CLV3*-like genes (*VvCLE 6*, *VvCLE 25-1*, *VvCLE 25-2*, *VvCLE 43* and *VvCLE TDIF*) as potential candidates for *Arabidopsis CLV3* orthologs using a publicly available BLASTX search (Jaillon et al., 2007; Velasco et al., 2007). These results suggested the existence of peptide hormones in the important commercial fruit crop and the possibility of commercial use of peptide hormones in agriculture. Expression analyses of the *CLV3*-like genes indicated that three of the five genes (*VvCLE 25-1*, *VvCLE 25-2* and *VvCLE TDIF*) were predominantly expressed in stems and axillary buds. On the other hand, two of the five genes (*VvCLE 6* and *VvCLE 43*) were strongly expressed in roots and axillary buds. The dissimilar patterns of expression suggest that members of this peptide

Abbreviations: CLE, CLAVATA 3/endosperm surrounding region; CLV3, CLAVATA3; NAA, 1-naphthaleneacetic acid; MS medium, Murashige–Skoog medium; PCR, polymerase chain reaction; SAM, shoot apical meristem.

* Corresponding author. Tel.: +81 985 58 7864; fax: +81 985 58 7864.
E-mail address: rtominaga@cc.miyazaki-u.ac.jp (R. Tominaga-Wada).

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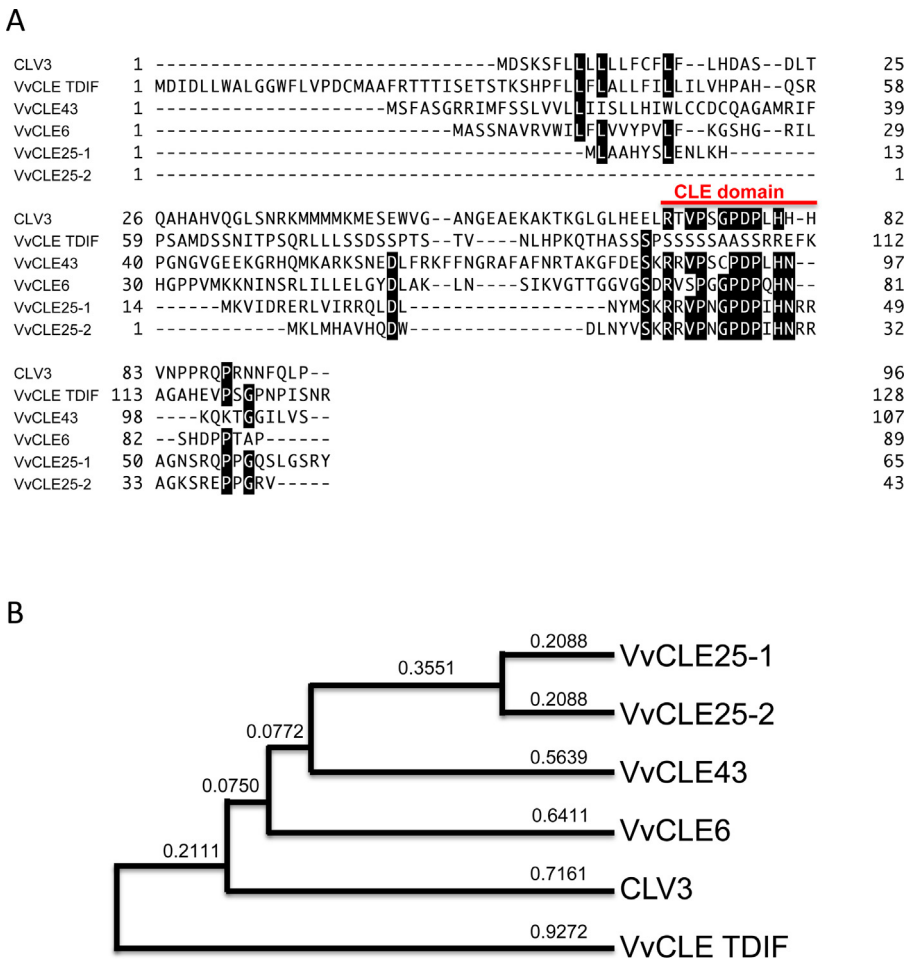

Fig. 1. Amino acid sequences and phylogenic tree of CLV3-like proteins. (A) Sequence alignment of CLV3, VvCLE TDIF (vvi: 100853965), VvCLE43 (vvi: 100249076), VvCLE6 (vvi: 100242106), VvCLE25-1 (vvi: 100854096) and VvCLE25-2 (vvi: 100853484). Shaded letters indicate identical residues. The CLE domain is indicated by a red line. (B) Phylogenic tree based on deduced amino acid sequences of CLV3-like proteins (CLV3, VvCLE TDIF, VvCLE43, VvCLE6, VvCLE25-1 and VvCLE25-2) aligned with a multiple alignment program (Genetyx ver. 16.0.2, Genetyx, Tokyo, Japan). The dendrogram was created using clustering with the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Branch length indicates relative evolutionary distances. Numbers above branches are genetic distances based on 10,000 bootstrap replicates. Distances are shown as the p-distance.

hormone family function differently in growth and development of grape vine.

Materials and methods

Primers

All primer sequences used in this study are listed in Table 1.

Table 1
Primer sequences used in this study.

Primer name	Sequence (5′–3′)
VvCLE6-F	5′-GCTCAAATGCTGTACGGGTGTG-3′
VvCLE6-R	5′-CCCATGGGAGCCCTTGAATA-3′
VvCLE25-1-F	5′-CAAATGGACCCGATCCCATA-3′
VvCLE25-1-R	5′-CTGCCTAAGCTTTGACCAGGAG-3′
VvCLE25-2-F	5′-GCATGCTGTTCATCAGGATTGG-3′
VvCLE25-2-R	5′-CCTGTTGTGAATGGGATCAGGTC-3′
VvCLE43-F	5′-TGAGGATTTGTTCCGCAAGTTC-3′
VvCLE43-R	5′-GAGGATCTGGGCAACTGGGTA-3′
VvCLE TDIF-F	5′-GGAGCAGCTGGTTCATGGATA-3′
VvCLE TDIF-R	5′-TGTTCTAAATGCTGCCATGCAA-3′
VvUb2-F	5′-TCCAGGACAAGGAAGGGATTC-3′
VvUb2-R	5′-GCCATCCTCAAGCTGCTTTC-3′

Plant material and growth conditions

Common grape vine (*Vitis vinifera* L.) var. Pinot Noir was grown in sterilized bottles, containing MS medium (Murashige and Skoog, 1962) with 0.5 μM NAA, 3.0% (w/v) sucrose and 1.5% (w/v) agar, in growth chambers (SANYO, MLR-350). Growth conditions were 16-h days and 8-h nights at 25 °C under white light (80 μmol m^{-2} s^{-1}). Plants were subcultured onto fresh medium every two months through nodal segments.

Real-time reverse transcription PCR analysis

Total RNA from leaves, stems, roots and axillary buds was extracted with MagDEA RNA 100 (GC) (PSS, Chiba, Japan) using a Magtraction System 12 GC (PSS, Chiba, Japan). To remove contaminating genomic DNA, RNA samples were treated with DNase I (Ambion, Austin, TX, USA) according to the Magtraction System protocol. Plant tissue (100 mg) was homogenized using a TissueLyser II (Qiagen, Valencia, CA, USA) with 100 μL of RLT buffer (Qiagen, Valencia, CA, USA). Sample supernatants were applied to the instrument, and RNA was eluted with 50 mL of sterile distilled water.

First-strand cDNA was synthesized from 1 μg total RNA in a 20 μL reaction mixture using the Prime Script RT Master Mix (Perfect Real Time) (Takara, Tokyo, Japan). Real-time PCR was performed using a Chromo4 Real-Time IQ5 PCR Detection System

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