Tackling the grapevine Pectate Lyase gene family and its role in the berry texture determination

B. ROJAS[1,2,*], J. LAGRÈZE[1,2], B. FARNETI[2], D. PORRO[3], M. MALNOY[2], C. MOSER[2], L. DALLA COSTA[2] and G. MALACARNE[2]

[1] Center Agriculture Food Environment (C3A), University of Trento/Fondazione Edmund Mach, San Michele all'Adige (Trento), Italy
[2] Research and Innovation Center, Edmund Mach Foundation, San Michele all'Adige (Trento), Italy
[3] Center of Technology Transfer, Edmund Mach Foundation, San Michele all'Adige

INTRODUCTION

Grapevine (*Vitis vinifera* L.) is one of the most commercially valuable fruit trees worldwide. Table grapes represent an important economic sector, where consumers highly appreciate the **berry firmness trait**. Although several studies have addressed the key role of **the cell wall in fruit firmness**, the main players among cell wall degrading enzymes during fruit ripening are still unclear. **This work characterizes the grapevine *Pectate Lyase* (*VvPL*) gene family which catalyzes the eliminative cleavage of de-esterified pectin during the berry development**. Using the latest grapevine genome assembly and annotation (Canaguier et al., 2017), **17 members of the family containing the PL domain were identified.**

Fig 1. Schematic mechanism of the Pectate lyase role in the cell wall solubilization. After the demethylesterification of the homogalacturonan (HG) by the Pectinmethylesterase (PME), Pectate lyases can breakdown the HG into galacturonic acid units.

AIMS AND OBJECTIVES

I. Identification of grapevine *PL* gene family and selection of candidate genes for functional characterization

II. Characterization of berry texture in table grape genotypes

III. Gene expression analysis of *VvPLs* in berries with contrasting firmness

IV. Functional analysis of selected *VvPL* genes by genome editing.

RESULTS

I. Identification of grapevine *PL* gene family and selection of candidate genes for functional characterization

Vitis vinifera gene annotation (Vcost.V3)

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Search of Pfam 00544 in HMMER

⬇

17 Isoforms Pectate Lyases

VvPL05 and VvPL16 are the most induced *VvPL* genes in Cabernet Sauvignon at the beginning of veraison (0 DFV). This result comes from the investigation of expression Atlas from Fasoli et al. (2018) by VitViz platform (http://vitviz.tomsbiolab.com/).

RESULTS

II. Characterization of berry texture in table grape genotypes

The study of firmness during the berry ripening indicates a contrasting phenotype between a soft and a firm variety in table grape. At five phenological stages, 60 berries were sampled, and their firmness was assessed with a durometer in the field. The mean is presented, and the statistical differences were determined through t-test ($p < 0.5$).

III. Gene expression analysis of *VvPLs* in berries with contrasting firmness

VvPL05 and VvPL16 are highly expressed in the soft variety at middle ripening. Flesh and skin were analyzed independently in a soft and a firm table grape variety at three phenological stages: veraison (V), middle ripening (MR) and full ripening (FR). The Normalized relative expression (NRE) of each gene was calculated using the method by Hellemans et al. (2007) and adopting *VvEF1a* and *VvGAPDH* genes as normalizers. An ANOVA One-Way Post-test Tukey ($p < 0.5$) was performed to assess the significance of the comparisons. Vertical bars indicate the mean. N=3

IV. Functional analysis of selected *VvPL* genes by genome editing.

| NOS-T | Pro-Ubq10At | Cas9 | NOS-T | U6At | sgRNA | scaffold |

Embryogenic calli in culture media → Embryogenic callus after transformation → Somatic embryos development → Organogenesis

gRNA3_VvPL05

Line	Mutation
VvPL05_1	Insertion of a T
VvPL05_2	Mixed mutation
VvPL05_3	Mixed mutation

gRNA3_VvPL14

Line	Mutation
VvPL14_1	Deletion of 8 pb
VvPL14_2	Mixed mutation
VvPL14_3	Mixed mutation

gRNA4_VvPL16

Line	Mutation
VvPL16_1	Insertion of a G
VvPL16_2	Insertion of a G
VvPL16_3	Mixed mutation
VvPL16_4	Mixed mutation
VvPL16_5	Mixed mutation
VvPL16_6	Mixed mutation

'Sugraone' transformation for the knock-out of *VvPL05*, *VvPL14* and *VvPL16* through the CRISPR/Cas9 system has achieved three edited lines for *VvPL05*, three for *VvPL14*, and six for *VvPL16* as indicated by the Sanger sequencing of the target site.

PERSPECTIVES

- Cell wall characterization of berries with contrasting firmness through Immunohistochemistry and FT-IR analyses.
- Characterization of *VvPLs* knock-out lines

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