The grapevine Pectin Methylesterases gene family and its involvement in Botrytis bunch rot control

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ABSTRACT

Plant Pectin Methylesterases (PMEs) represent a group of tissue-specific and developmentally regulated proteins. The gene family is involved in the plant cell wall (CW) remodelling process, by the control of the degree of cell wall pectin methylesterification. Pectin methylesterification also influences the susceptibility to pathogens including *Botrytis cinerea* (*Bc*), a necrotrophic fungus responsible of the *Botrytis* bunch rot (Br) in grapevine. In *Botrytis* genome, PMEs as well as other CW degrading enzymes have been identified as virulence factors. To further characterize the *PME* gene family in grapevine and in *Botrytis* bunch rot, the latest *Vitis vinifera* (*Vv*) genome assembly and annotation were revised and through sequence homology search, a total of 62 VvPME domain containing proteins were identified, 16 more than in a previous report. The *in-silico* analysis of the family by means of the *Vitis* gene expression database VESPUCCI as well as Aggregated Gene Co-expression Network approach (AggGCNs) allowed us to identify and enrich gene co-expression modules and build gene co-expression networks. Interestingly, one of the co-expression modules showed a high modulation in presence of *Bc* infection and particular attention was paid to it. To further investigate this *VvPME* gene module, their expression level in different organs and developmental stages from two grapevine cultivars with divergent *Bc* susceptibility was investigated. Furthermore, berries were artificially infected with *Bc* at mature stage to evaluate PME gene expression level and the role in the grapevine bunch rot susceptibility. The results obtained contribute to characterize the *VvPME* gene family and the role of specific members in the grapevine-*Bc* interaction and to select specific members candidate to the control of *Bc*-Br in grapevine.

BACKGROUND

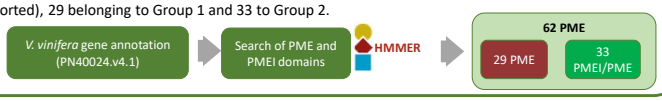
PMEs are CW modifying enzymes, responsible of the de-methylesterification of pectin, component of the primary CW, and their expression depends on tissue/organ and on developmental stage. PMEs form a large family also in grapevine (indeed 47 different isoforms were reported by Khan et al, 2019).

The role of PMEs has been associated with diverse physiological processes such as root tip elongation, leaf growth, and fruit softening (Pelloux et al, 2007). Differences in the degree of pectin methylesterification and PME activity have also been described to contribute to different virulence of the pathogen (Fan et al., 2017) as well to different susceptibility of the host plant (Raiola et al., 2011, Del Corpo et al., 2020). The overexpression of *PME1* in *Fragaria vesca*, with higher *PME* gene expression, PME activity and higher esterification degree, resulted in an increased resistance to *Bc* (Osorio et al, 2008). In *Arabidopsis thaliana*, several pathogens can alter the PME activity, and thereby induce plant immunity (Bethke et al, 2014) mediated by de-methylesterified oligogalacturonides.

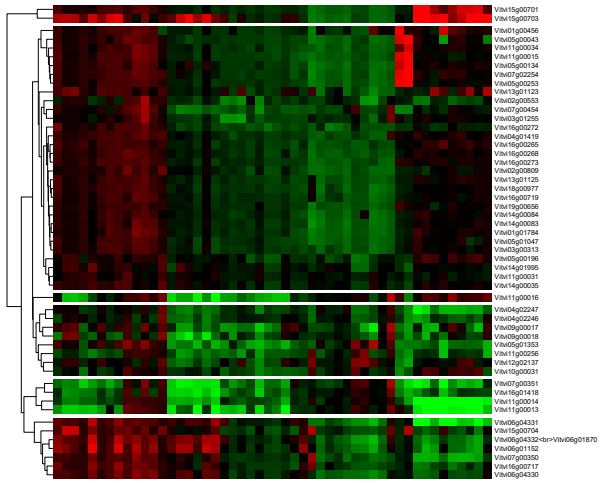
The PME structure is characterised by a **PME domain** (**Pfam01095**) and, in some cases, by a **N-Terminal PRO-region**, similar to a PMEI domain (**Pfam04043**). Depending on the presence of the PRO-region, PMEs have been classified into **Group 1** (PME domain) or **Group 2** (PME and PMEI domains) (Pelloux et al., 2007).

1. *VvPME* GENES IDENTIFICATION

The latest version of the *V. vinifera* reference genome and annotation (PN40024.v4.1; https://integrape.eu/resources/genes-genomes/) was analysed to find PME and PMEI (PME inhibitor) domain containing proteins against the Pfam (release 35) protein domain database (El-Gebali et al., 2019) using HMMER (Sonnhammer et al., 1998), with an e-value lower than $1x10^{-5}$. From a total of 41732 sequences analysed, **62 PME domain containing proteins were identified** (16 more than previously reported), 29 belonging to Group 1 and 33 to Group 2.

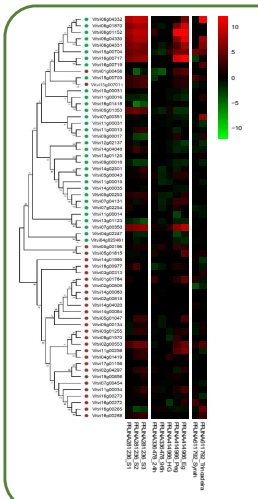


2. VESPUCCI CO-EXPRESSION ANALYSIS



VESPUCCI *VvPMEs* co-expression. The *VvPME* genes identified in the grapevine genome were analysed in the database VESPUCCI (Moretto et al, 2022; http://vespucci.colombos.fmach.it/), to evaluate their co-expression profile in different organs/tissues and developmental stages and upon infection with pathogens. **One co-expression modules comprehends seven genes**, is highly **expressed** in ripe fruit (EL-38, pericarp, mesocarp and endocarp) and **upon infection with *Bc***.

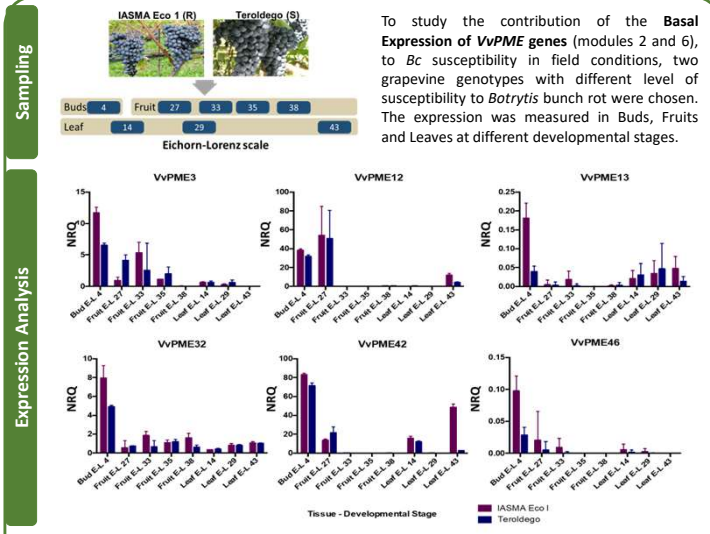
3. PHYLOGENY AND EXPRESSION



A phylogenetic tree was generated with the 63 aminoacid sequences containing a PME domain, aligned with MUSCLE and using the Maximum likelihood method (Bootstrap 1000 replicates). Group 1 (red circle) and 2 (green circle) PMEs are differentiated in the tree and cluster in two distinct groups.

The expression level of the 63 *VvPME* genes in four grapevine *Bc* infection RNAseq experiments was analysed (PRJNA281236, PRJNA336478, PRJNA414966, PRJNA611792). The heatmap (Fold Change(log_2) Treatment vs Control) shows an induction of the genes *Vitvi06g04332* (*VvPME8*), *Vitvi06g01870* (*VvPME9*), *Vitvi06g01152* (*VvPME10*), *Vitvi06g4330*, *Vitvi06g04331* (*VvPME11*), *Vitvi15g00704* (*VvPME12*), *Vitvi16g00717* (*VvPME13*), *Vitvi07g00350* (*VvPME1*). All these genes belong to the same co-expression module (Panel 2. VESPUCCI CO-EXPRESSION ANALYSIS). Other upregulated genes in *Bc* infection experiments are *Vitvi02g00553* (*VvPME42*) and *Vitvi11g00256* (*VvPME37*).

4. BASAL EXPRESSION OF *VvPME* GENES



To study the contribution of the **Basal Expression of *VvPME* genes** (modules 2 and 6), to *Bc* susceptibility in field conditions, two grapevine genotypes with different level of susceptibility to *Botrytis* bunch rot were chosen. The expression was measured in Buds, Fruits and Leaves at different developmental stages.

The expression of selected *VvPME* genes was measured by RT-qPCR. The most significant results for *VvPME3* (*Vitvi11g00014*), *VvPME12*, *VvPME13*, *VvPME32* (*Vitvi14g01995*), *VvPME42*, *VvPME46* are here shown. *VvPME12*, *VvPME13* and *VvPME42 and VvPME46*, highly upregulated in *Bc* infection (Panel 3) are not expressed in the fruit in the stages from hard green berry till harvest, while they are expressed in bud, fruit setting (EL27) and in the leaf. Interestingly, all the genes tested show lower expression levels in Buds (E-L 4) in the *Bc* susceptible cultivar Teroldego than in Eco I. Differential expression was also observed for *VvPME12* in senescent leaves (E-L 43), for *VvPME32* in green fruit and ripe stages (E-L 33 and E-L 38) as well as in senescent leaves for *VvPME42*.

The amplification of *VvPME10* (*Vitvi06g01152*), the putative orthologue of *AtPME17*, and *VvPME8*, *VvPME9*, *VvPME11*, highly expressed during *Bc* infection (Panel 3), is not shown because it was technically challenging due to the high sequence similarity of these *PME* genes.

CONCLUSIONS AND PERSPECTIVES

The genome-wide characterization of *VvPME* gene family allowed the identification of members putatively involved in *Bc*-grapevine interaction in fruit and other organs. The gene expression analysis in infected tissues of genotypes with divergent susceptibility to *Bc* together with functional analysis will provide new targets for *Bc* control by genetic improvement or new breeding technologies (NBTs).

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