

Accepted Manuscript

Changes in the secretome of *Vitis vinifera* cv. Monastrell cell cultures treated with cyclodextrins and methyl jasmonate

S. Belchí-Navarro, L. Almagro, R. Bru-Martínez, M.A. Pedreño



PII: S0981-9428(18)30494-7

DOI: <https://doi.org/10.1016/j.plaphy.2018.11.007>

Reference: PLAPHY 5489

To appear in: *Plant Physiology and Biochemistry*

Received Date: 9 August 2018

Revised Date: 8 October 2018

Accepted Date: 7 November 2018

Please cite this article as: S. Belchí-Navarro, L. Almagro, R. Bru-Martínez, M.A. Pedreño, Changes in the secretome of *Vitis vinifera* cv. Monastrell cell cultures treated with cyclodextrins and methyl jasmonate, *Plant Physiology et Biochemistry* (2018), doi: <https://doi.org/10.1016/j.plaphy.2018.11.007>.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Changes in the secretome of *Vitis vinifera* cv. Monastrell cell cultures treated with cyclodextrins and methyl jasmonate

S. Belchí-Navarro^a, L. Almagro^{a*}, R. Bru-Martínez^b, MA Pedreño^a

^aDepartment of Plant Biology, Faculty of Biology, University of Murcia, Campus de Espinardo, E-30100 Murcia, Spain

^bPlant Proteomics and Functional Genomics Group, Department of Agrochemistry and Biochemistry, Faculty of Science, University of Alicante and Instituto de Investigación Sanitaria y Biomédica de Alicante ISABIAL-FISABIO, Alicante, Spain

***Corresponding author:** Dr. Lorena Almagro; e-mail: lorena.almagro@um.es; Department of Plant Biology, Faculty of Biology, University of Murcia, Campus de Espinardo, E-30100 Murcia, Spain.

Phone: +34868884904

ABSTRACT

Elicitors induce defense responses that resemble those triggered by pathogen attack, including the synthesis of phytoalexins and pathogen-related proteins, which are accumulated in the extracellular space. In this work we analyze the changes in the secretome of *Vitis vinifera* cv. Monastrell cell cultures. This refers to the secreted proteome obtained from cell suspension cultures, in response to treatment with cyclodextrins and methyl jasmonate, separately or in combination using label-free quantitative approaches. Of the proteins found, thirty-three did not show significant differences in response to the different treatments carried out, indicating that these proteins were expressed in a constitutive way in both control and elicited grapevine cell cultures. These proteins included pathogenesis-related proteins 4 and 5, class III peroxidases, NtPRp-27, chitinases and class IV endochitinases, among others. Moreover, eleven proteins were differentially expressed in the presence of cyclodextrins and/or methyl jasmonate: three different peroxidases, two pathogenesis related protein 1, LysM domain-containing GPI-anchored protein 1, glycerophosphoryl diester phosphodiesterase, reticulon oxidase, heparanase, β -1,3-glucanase and xyloglucan endotransglycosylase.

Treatments with cyclodextrins reinforced the defensive arsenal and induced the accumulation of peroxidase V and xyloglucan endotransglycosylase. However, elicitation with methyl jasmonate decreased the levels of several proteins such as pathogenesis related protein 1, LysM domain-containing GPI-anchored protein 1, cationic peroxidase, and glycerophosphoryl diester phosphodiesterase, but increased the levels of new gene products such as heparanase, β -1,3 glucanase, reticulon oxidase, and peroxidase IV, all of which could be used as potential biomarkers in the grapevine defense responses.

KEYWORDS: cyclodextrins, methyl jasmonate, pathogenesis-related protein, secretome, *Vitis vinifera* cv. Monastrell.

1. INTRODUCTION

Plant strategies to combat external attack include constitutive and inducible mechanisms. Structural barriers or reservoirs of antimicrobial compounds represent constitutive defenses against tissue colonization. However, induced defense responses require the activation of defense-related genes to produce phytoalexins and pathogenesis-related protein (PR-proteins) (Belchí-Navarro et al., 2012). Most PR-proteins are induced by the action of pathogen-derived compounds, plant-derived molecules or elicitors. These proteins show antimicrobial activities through their hydrolytic activities on cell walls and are involved in plant defense reactions (Kawano and Furuichi, 2007). In some cases, these defense responses are mediated by signaling molecules such as jasmonates, among them jasmonic acid and its more active derivative methyl jasmonate (MJ) (Zhao et al., 2005). These signaling molecules take part in plant defense reactions by increasing secondary metabolite production in grapevine (Tassoni et al., 2005). The application of MJ to the surface of grapevine leaves induces the formation of lesions that mimic the typical hypersensitive response, and the initiation of defense responses such as an increase in phenolic compound production and the expression of defense-related genes (Faurie et al., 2009). Indeed, the presence of MJ resulted in stilbene accumulation in *V. vinifera* cell cultures (Tassoni et al., 2005). This signal molecule was also able to enhance the gene expression involved in the *trans*-resveratrol biosynthetic pathway in *V. vinifera* cell cultures cv. Monastrell (Lijavetzky et al., 2008, Almagro et al., 2014), and induced the accumulation of PR-proteins in grapevine (Wang et al., 2011; Martínez-Esteso et al., 2009). In fact, treatments with MJ enhanced the levels of PR-1 in leaves of *Vitis quinquangularis* and the accumulation of peroxidases and class III

and IV chitinases in *V. vinifera* cell cultures cv. Gamay (Wang et al., 2011; Martínez-Esteso et al., 2009).

For their part, cyclodextrins (CD), which are cyclic oligosaccharides consisting of seven to eight α -d-glucopyranose residues linked by $\alpha(1\rightarrow4)$ glucosidic bonds, act as elicitors, inducing defense responses in *V. vinifera* cell cultures, where they induced the production of *trans*-resveratrol and the expression of *phenylalanine ammonia lyase*, *cinnamate 4-hydroxylase*, *4-coumarate CoA ligase* and *stilbene synthase* genes (Almagro et al., 2014). Moreover, CD also increased the abundance of several proteins involved in grapevine defense reactions such as subtilisin-like protease, β -1,3-glucanase and peroxidase (Martínez-Esteso et al., 2009). The way in which CD act, seems to be related with their chemical similarity to the alkyl-derived oligosaccharides that are released from cell walls during fungal attack (Bru et al., 2006). Moreover, the effect of combined MJ and CD treatment on both the production of *trans*-resveratrol and the expression profile of the genes involved in its biosynthetic pathway in *V. vinifera* cv. Monastrell cell cultures has been well characterized (Lijavetzky et al., 2008; Belchí-Navarro et al., 2012; Almagro et al., 2014). However, the defense responses triggered by these two elicitors in the secretome of *V. vinifera* cv. Monastrell cell cultures is poorly known.

In *V. vinifera* cv. Monastrell cell cultures the extracellular medium is vital for cell life because plays a key role in interacting with environmental factors, including those required for defense responses and nutrition (Sabater-Jara et al., 2014). In this sense, the study of differentially expressed proteins in the secretome of *V. vinifera* cv. Monastrell cell cultures could improve our understanding of the role played by this dynamic cell wall compartment in grapevine defense responses. Among extracellular proteins, it is important to highlight the presence of PR-proteins, which are found constitutively in both plant tissues and cell cultures, and the fact that their levels

increase in the presence of biotic and abiotic elicitors (Sabater-Jara et al., 2014). In fact, some PR-proteins such as peroxidases, endochitinases and chitinases are often found in the secretome of cell cultures of *Taxus baccata*, *Zinnia elegans*, *Capsicum annuum*, among other (Novo-Uzal et al., 2009; Sabater-Jara et al., 2010).

Label-free quantitative approaches have gained significance in the proteomic field for their speed, high resolution, very high reproducibility, ease for use, sensitivity, suitability for large scale data processing and the ability to provide low-cost measurements of protein abundance in complex biological samples (Wang et al., 2011). For these reasons, the aim of this work was to investigate the effect of MJ and CD separately or in combination on the secretome of *V. vinifera* cv Monastrell cell cultures using label-free quantitative approaches.

2. MATERIAL AND METHODS

2.1 Plant material

Vitis vinifera cv. Monastrell calli were established in 1990, as described by Calderón et al., (1993), and grapevine cell cultures derived from them have been routinely maintained by periodical subcultures, as described by Belchí-Navarro et al. (2012).

2.2 Elicitation of *Vitis vinifera* cv. Monastrell cell cultures

V. vinifera cv. Monastrell cell cultures were elicited with 50 mM CD alone or in combination with 100 μ M MJ. Elicitation experiments were performed in triplicate using 14 day old grapevine cell cultures. For this, 4 g of washed cells (fresh weight) were transferred to 100 mL flasks and suspended in 20 mL of sterile fresh culture medium containing 50 mM CD alone or in combination with 100 μ M MJ followed by 96 h of incubation at 25 °C in darkness on a rotary shaker (110 rpm). Control treatments without elicitors were run in parallel. After elicitation, cells were separated from the culture medium under gentle vacuum and the spent medium was used to measure the *trans*-resveratrol content and to isolate proteins.

2.3 Protein extraction

Proteins from *V. vinifera* cv. Monastrell cell cultures were extracted as described by Martínez-Esteso et al. (2009). First, 4 ml of cell-free medium from the control or elicited cultures were frozen, thawed and then centrifuged. The supernatants were extracted with 25% ethylacetate. After removing the organic phase, the aqueous phase was supplemented with 2% (w/v) polyvinylpolypyrrolidone, and after 60 min incubation with shaking, the slurry was centrifuged at 8000×g for 15 min. The proteins in the supernatant were precipitated by adding trichloroacetic acid at a final concentration of 8% (w/v) and pelleted at 5000×g for 10 min. The pellets were washed three times in ice-cold methanol and three times in ice-cold acetone. The final dried precipitates were solubilized in buffer containing 7 M urea, 2 M thiourea, 50 mM DTT, 2% (w/v) CHAPS and 0.5% (v/v) IPG buffer. The protein was quantified by the Bradford method. Then, 30 µg of protein extract was digested with trypsin overnight. The peptides were desalted using a PepClean C-18 Spin Column (Agilent Technologies, USA) following the recommendations of the manufacturer. The extracted peptides were dried in vacuum and resuspended in 0.1% formic acid at a final concentration of 1 µg/µL.

2.4 MS and MS/MS spectra acquisition

LC-MS/MS analyses were performed using an Agilent 1100 HPLC-ChipCube/MS Interface coupled to an Agilent XCTplus Ion Trap mass spectrometer equipped with a nano-ESI source. Peptide separation was carried out using the ProtID-Chip-150 (II) connected to a 4 mm, 40 nL enrichment column, and an analytical separation column 150 mm x 75 µm (5 µm, ZORBAX 300SB-C18). Chromatography runs consisted of a 85 min linear gradient of 5-60% acetonitrile containing 0.1% (v/v) formic acid at a constant flow rate of 0.3 µL min⁻¹.

MS and MS/MS spectra were acquired in the standard enhanced mode (26000 $m/z/s$) and the ultrascan mode (8100 $m/z/s$), respectively. Mass spectrometer settings were as follows: ionization potential of 1.8 kV and an ICC smart target of 400000 or 150 ms of accumulation. MS/MS scans were performed using automated switching with a preference for doubly charged ions and a threshold of 105 counts and a 1.3 V fragmentation amplitude.

2.5 Label-free analysis of LC-MS

The LC-MS raw machine output files were converted to mzXML files and loaded in Progenesis LC-MS (Nonlinear Dynamics) v2.5 label-free analysis software. Quantification was done based on peak intensity. The Mascot search result files were used for protein identification. The identified and quantified proteins were filtered using a Mascot Score of 60 and ANOVA p -value < 0.009. The data file that yielded most features (peaks) was used as reference, against which the retention times of all other peaks and the intensities (areas under the peaks) were normalized. Experimental variations were corrected by calculating the robust distribution of all ratios. Once converted into an intensity list data, the features were filtered to include only features with charge state two or three, and which appeared in a retention time window of 20 to 60 min. The intensity lists were clustered according to the experimental groups (Control, MJ, CD and CD+MJ), and the average intensity ratios of the matched features were automatically calculated. To identify the proteins from which the features arise, all the MS/MS spectra collected were launched in a unique search by Mascot against the NCBI nr database using the following settings: 1 missed cleavage allowed; Viridiplantae as taxonomy; Cys carbamidomethylation as fixed modification; deamidation of asparagine or glutamine, oxidation of methionine and pyroglutamate formation of N-terminal glutamine or glutamate, as variable modifications; peptide tolerance 1.2 Da; fragment ion tolerance 0.6 Da; peptide

charge 2⁺ and 3⁺. The XML files resulting from the database search were imported into Progenesis LC-MS, which assigned peptide and protein identities to the corresponding features. The features were filtered by search score and by species, applying a MASCOT score of above 25 and "Vitis", respectively. Once peptides were assembled into proteins, assignment conflicts were resolved according to MASCOT score, the highest being the winner, or were left unresolved if the scores were the same, that is, the same sequence. Protein quantitative data were calculated as the average of their constitutive features and quantitative normalized data. Quantitation quality was determined by ANOVA test, whose p value was calculated based on the normalized data of the features.

2.6 Bioinformatic functional analysis

Gene ontology analysis of the identified proteins was carried out using Blast2GO v2.4.0³⁷. A file of FASTA format sequences of the identified and/or quantified protein set was batch retrieved from the NCBI website. Blast2GO was fed with the FASTA file and run: first, to incorporate sequence description by performing a BLASTp search against NCBI nr (e-value cutoff 1×10^{-50} , 100 for retrieved number of BLAST hits, 33 for HSP (highest scoring pair) length cutoff); second, to map GO, EC and Interpro terms, and finally, to annotate the sequences (E-Value Hit-Filter of 1×10^{-6} , a Hsp-Hit Coverage Cutoff of 0, an Annotation Cutoff of 55, and a GO Weight of 5). Automatic annotation performed by Blast2GO was manually revised to ensure accurate assignment.

3. RESULTS AND DISCUSSION

Proteomic analysis of secretome of *Vitis vinifera* cv Monastrell cell cultures elicited with cyclodextrins and/or methyl jasmonate

V. vinifera cell cultures exhibited a high capacity to produce *trans*-resveratrol extracellular accumulation when these cultures were elicited with CD and MJ (Belchí-

Navarro et al., 2012; Almagro et al., 2014). Consequently, the production of *trans*-resveratrol in the presence of these elicitors was analyzed in *V. vinifera* cv. Monastrell. *trans*-Resveratrol accumulation was greatest in grapevine cell cultures elicited with CD and MJ (data not shown), due to the increased expression of genes encoding the enzymes involved in the *trans*-resveratrol biosynthetic pathway (Lijavetzky et al., 2008). Since both the production of *trans*-resveratrol and the expression of the genes involved in its biosynthetic pathway have been studied in *V. vinifera* cv. Monastrell cell cultures treated with CD and MJ (Lijavetzky et al., 2008, Belchí-Navarro et al., 2012; Almagro et al., 2014), we studied the secretome of these *V. vinifera* cv. Monastrell cell cultures under elicitation conditions.

In this work, a label-free approach was used to identify extracellular proteins of grapevine cell cultures elicited with CD and/or MJ. For data analysis, Progenesis LC-MS software (NonLinear Dynamics), which quantifies proteins based on the intensity signals of their precursors and allows their identification by using their corresponding scans MS/MS, was used.

The selection of 2266 possible peptide precursors (features) was based on their charge (+2 or +3) and retention time (20-60 min). The peptide precursors with values of $p < 0.05$ and $p < 0.01$ in an ANOVA test were specifically labeled and analyzed. The searches were performed against the NCBI nr database. Each search was limited to proteins from species of *Vitis* and performed using the built-in decoy search option of Mascot. In addition to the proteins that are expressed differentially in the different treatments (p -value $< 0,009$), other proteins with a score higher than 60 and a p -value > 0.05 were identified. These proteins were expressed constitutively, and their levels did not change significantly in the presence of the different treatments. Amongst these proteins PR-4, class III peroxidases, PR-5, NtPRp-27, chitinases and class IV endochitinases should be noted (Table 1).

PR-4 proteins are classified as endochitinases (van Loon et al., 2006) because they have weak chitinase activity (Hawkins et al., 2015). These proteins are induced by attacks of pathogens such as *Botryosphaeria dothidea* in *Malus domestica* (Bai et al., 2013) as well as by holaphyllamine, a steroid, in *Arabidopsis* plants (Rao et al., 2002). PR-4 has also been found in the extrafloral nectar of acacia where it was seen to have a protective effect against fungal infections (González-Teuber et al., 2009). The presence of this protein in the grapevine culture medium agrees with the results obtained by Nogueira et al. (2007), who detected a PR-4 in embryogenic cell suspensions of cowpea and who suggested that this protein could play a role in the histodifferentiation of pro-embryogenic masses into somatic embryos.

Moreover, the presence of osmotin (PR-5) was found to be constitutive since it was expressed both in the control and elicited culture media in our grapevine cell cultures. Therefore, osmotin-like proteins are not only correlated with grapevine defense responses against elicitors but may also be expressed in non-elicited cell cultures due to the mechanical stress suffered by cell cultures when they are grown in continuously shaken flasks. In this way, osmotin proteins play an important role in the defense against pathogenic fungi (Misra et al., 2016; Gond et al., 2015) since they act by permeabilizing fungal cell membranes (Ullah et al., 2017). Osmotin-like proteins can also be induced by several hormonal signals, including abscisic acid or auxin, and environmental signals such as dehydration, salinity, or fungal infection (Ahmed et al., 2013). In addition, PR-5 proteins are also involved in growth and development processes so that they are considered constitutive (Zhu et al., 1995; Kim & Hwang, 2000), as occurred in our grapevine cell cultures, where thaumatin was found in both the control and elicited grapevine cell cultures (Table 1). This protein has also been found in *C. annuum* and *C. chinense* cell cultures (Sabater-Jara et al., 2010 and 2011) as well as in wheat and tobacco plants (Alam et al., 2014;

Okushima et al., 2000). In agreement with our results, NtPRp-27 was seen to be constitutively expressed in tobacco roots although this protein can also be induced by tobacco mosaic virus, wounding, drought, and by the application of ethylene, MJ, salicylic acid, and abscisic acid (Okushima et al., 2000; Elvira et al., 2008; Sabater-Jara et al., 2010).

Likewise, an endochitinase was detected in control and elicited grapevine cell cultures after 96 h of treatment (Table 1) probably due to the *in vitro* conditions in which grapevine cell cultures are grown. Plant chitinases are monomeric proteins with a molecular weight of 25-40 KDa. Class I, II and IV chitinases have homologous catalytic domains. This domain is homologous to wheat germ agglutinin and is responsible for binding the enzyme to chitin (Pusztahelyi et al., 2018). The different types of chitinases are differentiated by their structure, enzymatic properties, spatial and temporal localization, regulation and function (Kasprzewska, 2003). These enzymes are involved in plant defense responses as well as plant growth and development (Grover et al., 2012). Thus, chitinases have been identified in the secretome of non-elicited cell cultures from *Z. elegans*, *C. revoluta*, *T. baccata* cell cultures (Novo-Uzal et al., 2009), *Capsicum* sp cell cultures (Sabater-Jara et al., 2010, 2011) and *N. tabacum* cell cultures (Lippmann et al., 2009), which agrees to our finding in grapevine cell cultures. Moreover, endochitinases are able to hydrolyze N-acetyl glucosamine (chitin) polymers from the cell wall of fungi (Hamid et al., 2013). Harfouche et al. (2008) observed that an endochitinase was expressed in control *in vitro* chestnut cultures, and its abundance increased when salicylic acid was added to the cell cultures. The expression of endochitinase genes in untreated cells may be explained by the *in vitro* conditions, which to some extent may influence gene expression or the production of ethylene, which is accumulated at low concentrations in closed *in vitro* containers.

Figure 1 represents the distribution of all the proteins classified into their different categories, such as cellular compartment proteins, those with molecular functions and those involved in biological processes. As can be observed in the case of proteins classified according to their cellular compartment (Fig. 1A), most of the annotations correspond to proteins which are localized in endomembrane systems such as cytoplasmic vesicles delimited by membranes (37%) and proteins present in the vacuole (14%), or proteins anchored in the plasma membrane (5%). Another group of proteins are localized in the cell wall (8%), mitochondria (6%), and in the apoplast (20%) (Fig. 1A). The presence of annotations corresponding to proteins localized in the vacuoles, cytoplasmic vesicles and mitochondria can be explained by the multiple localization of these proteins during their biogenesis or by the existence of isoforms located in different cell compartments, whereas proteins detected in the extracellular medium of *Vitis* cell cultures mainly come from the apoplast. In fact, the spent medium of *Vitis* cell cultures provides a convenient, continuous and unique source of extracellular proteins that are easily obtained without cell disruption and so, without any cytosolic contamination (Delaunois et al. 2014). Indeed, the extracellular proteome refers to the secretome, which is the spent medium since it contains secreted proteins from cell cultures (Delaunois et al. 2014).

Delaunois et al. (2013) performed a proteomic analysis of the grapevine leaf apoplastic fluid, and described that most of the proteins found in the apoplastic fluid were related to plant defense mechanisms and the cell wall metabolism (50.7% PR-proteins, 16% proteases, 11.9% cell wall modifying enzymes and 2.4% peroxidases). The same authors described that the main PR-proteins detected in grapevine leaf apoplastic fluid were osmotin, chitinases, glucanases, thaumatin-like proteins and peroxidases, as was the case in the secretome obtained from elicited spent medium in our experiments (Table 1).

As regards the proteins classified according to their molecular functions (Fig. 1B), most of the annotations correspond to peroxidase type proteins (15%), calcium- and hemo-binding proteins (29%), or electron transport proteins (15%). Other annotations correspond to PR-proteins induced as defense responses against pathogens or abiotic stresses such as cold, drought, osmotic stress or UV light (Agarwal and Agarwal, 2010; Tian et al., 2015). Among these proteins, it is worth noting those which have chitinase (10%), endopeptidase (6%), transferase (6%) and hydrolase (6%) activities, as well as chitin-binding proteins (10%).

Moreover, the annotations correspond to the biological processes (Fig. 1C) showed the presence of proteins related to plant defense responses against abiotic stimuli (6.41%), innate immune responses (8.33%) and oxidative stress (7.69%). Another group of annotations is related to cell and carbohydrate catabolism (8.33 and 6.41%, respectively), as well as other metabolic processes which occur in the cell wall (7.05%), and polymers (7.05%) and polysaccharides metabolism (9.61%). The last group of annotations corresponds to plant processes related to development (8.33%) (Fig. 1C).

In order to study the differential levels of proteins, we selected those peptides whose ANOVA had a *p* value of <0.05 (Figs. 2-6). The most abundant protein was peroxidase V from *V. vinifera* (Fig. 2A; gi|225455195) in *V. vinifera* cv Monastrell cell cultures treated with CD alone or in combination with MJ, their abundance being 35 and 14 times higher, respectively, than in control treatments. Moreover, we also found a protein like cationic peroxidase of *A. thaliana* (Figure 2B; gi|225439625), which was strongly expressed in the control treatments, and to a lesser extent, in CD-treated cells (2.7 times less than in control treatments). However, the abundance of this peroxidase was significantly reduced in the treatment with MJ. In addition, peroxidase IV (gi|225434381) was identified in the treatment with MJ alone and in its

combination with CD, where its abundance was 8 times higher in the combined treatment than in MJ-treated grapevine cells (Fig. 2C). These types of peroxidases are class III peroxidases induced in response to stress, injury or pathogens, and belong to PR-9 protein subfamily according to the classification of van Loon et al. (2006), their functions being to limit the spread of infection through the development of structural barriers or the production of ROS and/or reactive nitrogen species (Passardi et al., 2004). In addition, these peroxidases can produce the oxidation of *trans*-resveratrol in the presence of H₂O₂ exogenous. In fact, an extracellular medium obtained from elicited *Vitis* cell culture was able to convert all the *trans*-resveratrol present into *trans*-resveratrol dimers when exogenous H₂O₂ was added (Martínez-Esteso et al., 2009).

In addition, the treatment with MJ alone induced a decrease in the levels of some proteins, while elicitation with CD alone or in combination with MJ led to protein accumulation. This phenomenon was also observed in culture media obtained from elicited *V. vinifera* cv Gamay analyzed with 2D-electrophoresis gels (Martínez-Esteso et al., 2009). Although MJ decreased the abundance of PR-1 proteins (Fig. 3, gi|225429117 and gi|163914225), their content was constitutive since these proteins were also detected in control treatments, and to a lesser extent, in CD-treated cells. These PR-1 proteins were seen to be expressed constitutively in tomato (Tornero et al., 1997) and they have also been described in *Arabidopsis* plants in the presence of salicylic acid and *Trichoderma* sp. (Wu et al., 2012; Brotman et al., 2013).

The inhibitory effect of MJ on the levels of some apoplastic proteins such as LysM domain-containing GPI-anchored protein 1 from *V. vinifera* was also observed (Fig. 4A; gi|225459538). In fact, MJ decreased its abundance 67 fold with respect to the control treatments (Fig. 4A). In the plant kingdom, proteins containing LysM domains act as receptors that bind chitin which is the major component of the cell wall of fungi,

so these proteins with LysM domains have been associated with plant defense responses against fungi (Zhang et al., 2009). In our grapevine cell cultures, LysM domain-containing GPI-anchored protein 1 can be considered as constitutive because it is present in both control and CD-treated cells. Likewise, a glycerophosphoryl diester phosphodiesterase was also down-regulated in the presence of MJ (Fig. 4B; gi|147811111) but it was abundant in control and CD-treated cells. Glycerophosphoryl diester phosphodiesterase is found in a wide variety of organisms and contains highly conserved domains, which are essential for its enzymatic activity (van der Rest et al., 2004). These proteins which seem to be involved in the metabolism of phospholipids, are located on the cell surface where they participate in the cell wall organization and the cell signaling pathway (Hayashi et al., 2008; Lino et al., 2016). Therefore, our results indicate that glycerophosphoryl diester phosphodiesterase is expressed constitutively and would be involved in the cell wall organization as well as in the cell signaling pathway led by CD and MJ in grapevine cell cultures.

On the other hand, there are two proteins strongly induced in the presence of MJ, a reticulon oxidase (gi|147846526) and a protein similar to heparanase (gi|147862366) (Fig. 5A and Fig. 5B, respectively). Reticulon oxidase catalyzes the formation of (S)-esculerin from (S)-reticulon, which is involved in the production of benzophenanthridine alkaloids. These compounds have cytotoxic activity and their production increases in response to pathogenic attack (Liu et al., 2015). In this sense, Custers et al. (2004) observed that the addition of salicylic acid induced the accumulation of reticulon oxidase in leaves of lettuce and sunflower. We also found sequences which had homologies with the protein 3, similar to a heparanase of *A. thaliana* (Fig. 5B). This protein is synthesized in the Golgi apparatus in the form of proenzyme, and is then transferred to the endosomes for its transport to the cell

surface, where it exerts its glucuronidase activity, participating in the metabolism and remodelling of the cell wall. These results indicated that MJ led to the activation of plant defense responses in grapevine increasing the production of PR-proteins, particularly, reticulon oxidase and heparanase.

Among the proteins that were activated in response to stress in *Vitis*, a β -1,3-glucanase (Fig. 6A; gi|37992763) was strongly induced by MJ or CD, although its content was reduced by half when both elicitors were added to grapevine cell cultures. Martínez-Esteso et al. (2009) also found a β -1,3-glucanase in *V. vinifera* cv Gamay cell suspensions in both control and CD treatments. These PR-proteins were also detected in *C. annuum* cell cultures elicited with MJ (Sabater-Jara et al., 2010) as well as in cell cultures of *Zinnia elegans*, *Cycas revoluta* and *Taxus baccata* (Novo-Uzal et al., 2009). Linthorst et al. (1991) also showed that genes encoding β -1,3-glucanases, which were constitutively found in healthy tobacco plants, were strongly induced after tobacco mosaic virus infection or salicylate treatment. These results agree with ours since the levels of β -1,3-glucanase (gi|37992763) increased in the presence of MJ and CD, indicating that this protein is clearly involved in grapevine defense responses.

Finally, a xyloglucan endotransglycosylase of *Populus tremula* x *Populus tremuloides* (gi| 225462505) was found when *V. vinifera* cv Monastrell cell cultures were elicited with MJ or CD alone (Fig. 6B). The activity of xyloglucan endotransglycosylases is often closely correlated with cell elongation (Braidwood et al. 2014; Lee et al. 2018). In fact, the overexpression of xyloglucan endotransglycosylase from *Brassica campestris* in *Arabidopsis* revealed that the transgenic plant had an elongated stem length (Shin et al., 2006). In addition, these proteins have also been associated with defense responses since Albert et al. (2004) demonstrated that the accumulation and activity of xyloglucan endotransglycosylases were correlated with the tomato-parasite

interaction. In line with these findings, an increase in xyloglucan endotransglycosylase protein was registered after 96 h of treatment with CD or MJ, suggesting that this protein could be involved in the plant defense mechanism of grapevine plants against different types of elicitors.

4. Conclusion

In conclusion, the secretome of *V. vinifera* cv. Monastrell cell cultures contain constitutive defense related-proteins; however, elicitation with CD reinforces the defensive arsenal, and markedly induces peroxidase accumulation. Moreover, elicitation with MJ decreased the levels of PR-1, LysM domain-containing GPI-anchored protein 1, cationic peroxidase and glycerophosphoryl diester phosphodiesterase but increased the abundance of new gene products such as heparanase, β -1,3 glucanase and reticulon oxidase, which could be used as potential biomarkers.

5. Author Contribution Statement

SBN, RBM and MAP conceived and designed research. SBN conducted experiments. SBN and LA analyzed data. SBN and LA wrote the manuscript. All authors read and approved the manuscript.

Acknowledgements

This work has been supported by the Ministerio de Economía y Competitividad (No. BIO2017-82374-R) and Fundación Seneca-Agencia de Ciencia y Tecnología de la Región de Murcia (No. 19876/GERM/15). University of Alicante's lab is a member of Proteored, PRB3 and is supported by grant PT17/0019, of the PE I+D+i 2013-2016, funded by ISCIII and ERDF.

References

- Agarwal, P., Agarwal, P.K., Joshi, A.J., Sopory, S.K., Reddy, M.K., 2010. Overexpression of PgDREB2A transcription factor enhances abiotic stress tolerance and activates downstream stress-responsive genes. *Mol. Biol. Rep.* 37, 1125-1135.
- Ahmed, N.U., Park, J.I., Jung, H.J., Kang, K.K., Lim, Y.P., Hur, Y., Nou, I.S., 2013. Molecular characterization of thaumatin family genes related to stresses in *Brassica rapa*. *Sci. Hortic.* 152, 26-34.
- Alam, M.A., Hongpo, W., Hong, Z., Ji, W.Q., 2014. Differential expression of resistance to powdery mildew at the early stage of development in wheat line N0308. *Genet. Mol. Res.* 13, 4289-4301.
- Albert, M., Werner, M., Proksch, P., Fry, S.C., Kaldenhoff, R., 2004. The cell wall modifying xyloglucan endotransglycosylase/hydrolase LeXTH1 is expressed during the defence reaction of tomato against the plant parasite *Cuscuta reflexa*. *Plant Biol.* 6, 402-407.
- Almagro, L., Carbonell-Bejerano, P., Belchí-Navarro, S., Bru, R., Martínez-Zapater, J.M., Lijavetzky, D., Pedreño, M.A., 2014. Dissecting the transcriptional response to elicitors in *Vitis vinifera* cells. *PLoS one* 9: e109777.
- Bai, S., Dong, C., Li, B., Dai, H., 2013. A PR-4 gene identified from *Malus domestica* is involved in the defense responses against *Botryosphaeria dothidea*. *Plant Physiol. Biochem.* 62, 23-32.
- Belchí-Navarro, S., Almagro, L., Lijavetzky, D., Bru, R., Pedreño, M.A., 2012. Enhanced extracellular production of *trans*-resveratrol in *Vitis vinifera* suspension cultured cells by using cyclodextrins and methyl jasmonate. *Plant Cell Rep.* 31, 81-89.
- Braidwood, L., Breuer, C., Sugimoto, K., 2014. My body is a cage: mechanisms and modulation of plant cell growth. *New Phytol.* 201, 388-402.

- Brotman, Y., Landau, U., Cuadros-Inostroza, A., Takayuki, T., Fernie, A.R., Chet, I., Viterbo, A., Willmitzer, L., 2013. *Trichoderma*-plant root colonization: escaping early plant defense responses and activation of the antioxidant machinery for saline stress tolerance. *PLoS pathogens* 9:e1003221.
- Bru, R., Sellés, S., Casado-Vela, J., Belchí-Navarro, S., Pedreño, M.A., 2006. Modified cyclodextrins are chemically defined glucan inducers of defense responses in grapevine cell cultures. *J. Agric. Food Chem.* 54, 65-71.
- Calderón, A.A., Zapata, J.M., Muñoz, R., Pedreño, M.A., Ros, Barceló. A., 1993. Resveratrol production as a part of the hypersensitive-like response of grapevine cells to an elicitor from *Trichoderma viride*. *New Phytol.* 124, 455-463.
- Custers, J.H.H.V., Harrison, S.J., Sela-Buurlage, M.B., van Deventer, E., Lageweg, W., Howe, P.W., van der Meijs, J., Ponstein, A.S., Simons, B.H., Melchers, L.S., Stuiver, M.H., 2004. Isolation and characterization of a class of carbohydrate oxidases from higher plants, with a role in active defence. *Plant J.* 39, 147-160.
- Delaunois, B., Colby, T., Belloy, N., Conreux, A., Harzen, A., Baillieul, F., Clément, C., Schmidt, J., Jeandet, P., Cordelier, S., 2013. Large-scale proteomic analysis of the grapevine leaf apoplastic fluid reveals mainly stress-related proteins and cell wall modifying enzymes. *BMC Plant Biol.* 13, 24-39.
- Delaunois, B., Jeandet, P., Clément, C., Baillieul, F., Dorey, S., Cordelier, S., 2014. Uncovering plant-pathogen crosstalk through apoplastic proteomic studies. *Front Plant Sci.* 5, 249-267.
- Elvira, M.I., Galdeano, M.M., Gilardi, P., Garcia-Luque, I., Serra, M.T., 2008. Proteomic analysis of pathogenesis related proteins (PRs) induced by compatible and incompatible interactions of pepper mild mottle virus (PMMoV) in *Capsicum chinense* L3 plants. *J. Exp. Bot.* 59, 1253-1265.

- Faurie, B., Cluzet, S., Méillon, J.M., 2009. Implication of signaling pathways involving calcium, phosphorylation and active oxygen species in methyl jasmonate-induced defense responses in grapevine cell cultures. *J. Plant Physiol.* 166, 1863-1877.
- Gond, S.K., Bergen, M.S., Torres, M.S., White, Jr. J.F., 2015. Endophytic *Bacillus spp.* produce antifungal lipopeptides and induce host defence gene expression in maize. *Microbiol. Res.* 172, 79-87.
- Gonzalez-Teuber, M., Eilmus, S., Muck, A., Svatos, A., Heil, M., 2009. Pathogenesis-related proteins protect extrafloral nectar from microbial infestation. *Plant J.* 58, 464-473.
- Grover, A., 2012. Plant chitinases: genetic diversity and physiological roles. *CRC Crit. Rev. Plant Sci.* 31, 57-73.
- Hamid, R., Khan, M.A., Ahmad, M., Ahmad, M.M., Abdin, M.Z., Musarrat, J., Javed, S., 2013. Chitinases: an update. *J. Pharm. Bioallied. Sci.* 5:21-29.
- Harfouche, A.L., Rugini, E., Mencarelli, F., Botondi, R., Muleo, R., 2008. Salicylic acid induces H₂O₂ production and endochitinase gene expression but not ethylene biosynthesis in *Castanea sativa in vitro* model system. *J. Plant Physiol.* 165, 734-744.
- Hawkins, L.K., Mylroie, J.E., Oliveira, D.A., Smith, J.S., Ozkan, S., Windham, G.L., Williams, W.P., Warburton, M.L., 2015. Characterization of the maize chitinase genes and their effect on *Aspergillus flavus* and aflatoxin accumulation resistance. *Plos One* 10:e0126185.
- Hayashi, S., Ishii, T., Matsunaga, T., Tominaga, R., Kuromori, T., Wada, T., Shinozaki, K., Hirayama, T., 2008. The glycerophosphoryl diester phosphodiesterase-like proteins SHV3 and its homologs play important roles in cell wall organization. *Plant Cell Physiol.* 49, 1522-1535.

- Kasprzewska, A., 2003. Plant chitinases-regulation and function. *Cellular and Molecular Biol. Lett.* 8, 809-824.
- Kawano, T., Furuichi, T., 2007. Salicylic acid as a defense-related plant hormone. In: *Salicylic acid: A plant hormone*. Springer, Dordrecht, pp. 277-321.
- Kim, J.K., Hwang, B.K., 2000. Pepper gene encoding a basic pathogenesis-related 1 protein is pathogen and ethylene inducible. *Physiol. Plant.* 108, 51-60.
- Lee, Y.K., Rhee, J.Y., Lee, S.H., Chung, G.C., Park, S.J., Segami, S., Maeshima, M., Choi, G., 2018. Functionally redundant LNG3 and LNG4 genes regulate turgor-driven polar cell elongation through activation of XTH17 and XTH24. *Plant Mol. Biol.* 97, 23-36.
- Lijavetzky, D., Almagro, L., Belchi-Navarro, S., Martinez-Zapater, J.M., Bru, R., Pedreño, M.A., 2008. Synergistic effect of methyl jasmonate and cyclodextrin on stilbene biosynthesis pathway gene expression and resveratrol production in Monastrell grapevine cell cultures. *BMC Res. Not.* 1, 132–140.
- Lino, B., Chagolla, A., de la Vara, L.E.G., 2016 Membrane proteins involved in transport, vesicle traffic and Ca^{2+} signaling increase in beetroots grown in saline soils. *Planta* 244, 87-101.
- Linthorst, H.J.M., 1991. Pathogenesis-related proteins of plants. *Crit. Rev. Plant Sci.* 10, 123-150.
- Lippmann, R., Kaspar, S., Rutten, T., Melzer, M., Kumlehn, J., Matros, A., Mock, H.P., 2009. Protein and metabolite analysis reveals permanent induction of stress defense and cell regeneration processes in a tobacco cell suspension culture. *Int. J. Mol. Sci.* 10, 3012-3032.
- Liu, M.H., Yang, B.R., Cheung, W.F., Yang, K.Y., Zhou, H.F., Kwok, J.S.L., Liu, G.C., Li, X.F., Zhong, S., Lee, S.M., Tsui, S.K., 2015. Transcriptome analysis of leaves,

roots and flowers of *Panax notoginseng* identifies genes involved in ginsenoside and alkaloid biosynthesis. *BMC Gen.* 16, 265-277.

Martinez-Esteso, M.J., Sellés-Marchart, S., Vera-Urbina, J.C., Pedreño, M.A., Bru-Martinez, R., 2009. Changes of defense proteins in the extracellular proteome of grapevine (*Vitis vinifera* cv. Gamay) cell cultures in response to elicitors. *J. Proteomics* 73, 331-341.

Misra, R.C., Kamthan, M., Kumar, S., Ghosh, S., 2016. A thaumatin-like protein of *Ocimum basilicum* confers tolerance to fungal pathogen and abiotic stress in transgenic *Arabidopsis*. *Sci. Rep.* 6:25340.

Nogueira, F.C.S., Gonçalves, E.F., Jereissati, E.S., Santos, M., Costa, J.H., Oliveira-Neto, O.B., Soares, A.A., Domont, G.B., Campos, F.A.P., 2007. Proteome analysis of embryogenic cell suspensions of cowpea (*Vigna unguiculata*). *Plant Cell Rep.* 26, 1333-1343.

Novo-Uzal, E., Gómez-Ros, L.V., Hernández, J.A., Pedreño, M.A., Cuello, J., Ros-Barceló, A., 2009. Analysis of the soluble cell wall proteome of gymnosperms. *J. Plant Physiol.* 166, 831-843.

Okushima, Y., Koizumi, N., Kusano, T., Sano, H., 2000. Secreted proteins of tobacco cultured BY2 cells: identification of a new member of pathogenesis-related proteins. *Plant Mol. Biol.* 42, 479-488.

Passardi, F., Penel, C., Dunand, C., 2004. Performing the paradoxical: how plants peroxidases modify the cell wall. *Trends Plant Sci.* 9, 534-540.

Pusztahelyi, T., 2018. Chitin and chitin-related compounds in plant–fungal interactions. *Mycology* 1-13.

Rao, M.V., Lee, H., Davis, K.R., 2002. Ozone-induced ethylene production is dependent on salicylic acid, and both salicylic acid and ethylene act in concert to regulate ozone-induced cell death. *Plant Cell* 32, 447-456.

Sabater-Jara, A.B., Almagro, L., Belchí-Navarro, S., Ferrer, M.A., Ros-Barceló, A., Pedreño, M.A., 2010. Induction of sesquiterpenes, phytoesters and extracellular pathogenesis-related proteins in elicited cell cultures of *Capsicum annuum*. J. Plant Physiol. 167, 1273-1281.

Sabater-Jara, A.B., Almagro, L., Belchí-Navarro, S., Ros-Barceló, A., Pedreño, M.A., 2011. Methyl jasmonate induces extracellular pathogenesis-related proteins in cell cultures of *Capsicum chinense*. Plant Signal. Behav. 6, 440-442.

Sabater-Jara, A.B., Almagro, L., Pedreño, M.A., 2014. Induction of extracellular defense-related proteins in suspension cultured-cells of *Daucus carota* elicited with cyclodextrins and methyl jasmonate. Plant Physiol. Biochem. 77, 133-139.

Shin, Y.K., Yum, H., Kim, E.S., Cho, H., Gothandam, K.M., Hyun, J., Chung, Y.Y., 2006. BcXTH1, a *Brassica campestris* homologue of *Arabidopsis* XTH9, is associated with cell expansion. Planta 224, 32-41.

Tassoni, A., Fornalè, S., Franceschetti, M., Musiani, F., Michael, A.J., Perry, B., Bagni, N., 2005. Jasmonates and Na-orthovanadate promote resveratrol production in *Vitis vinifera* cv. Barbera cell cultures. New Phytol. 166, 895-905.

Tian, C., Jiang, Q., Wang, F., Wang, G.L., Xu, Z.S., Xiong, A.S., 2015. Selection of suitable reference genes for qPCR normalization under abiotic stresses and hormone stimuli in carrot leaves. PLoS One 10:e0117569.

Tornero, P., Conejero, V., Vera, P., 1994. A gene encoding a novel isoform of the PR-1 protein family from tomato is induced upon viroid infection. Mol. Gen Genet. 243, 47-53.

Ullah, A., Hussain, A., Shaban, M., Khan, A.H., Alariqi, M., Gul, S., Jun, Z., Lin, S., Li, J., Jin, S., Munis, M.F.H., 2017. Osmotin: a plant defense tool against biotic and abiotic stresses. Plant Physiol. Biochem. 123, 149-159.

- van der Rest, B., Rolland, N., Boisson, A.M., Ferro, M., Bligny, R., Douce, R., 2004. Identification and characterization of plant glycerophosphodiester phosphodiesterase. *Biochem. J.* 379, 601-607.
- van Loon, L.C., Rep, M., Pieterse, C.M., 2006. Significance of inducible defense-related proteins in infected plants. *Annu. Rev. Phytopathol.* 44, 135-162.
- Wang, Q., Zhang, Y., Gao, M., Jiao, C., Wang, X., 2011. Identification and expression analysis of a pathogen responsive PR-1 gene from Chinese wild *Vitis quinquangularis*. *Afr. J. Biotechnol.* 10, 17062-17069.
- Wu, Y., Zhang, D., Chu, J.Y., Boyle, P., Wang, Y., Brindle, I.D., De Luca, V., Després, C., 2012. The *Arabidopsis* NPR1 protein is a receptor for the plant defense hormone salicylic acid. *Cell Rep.* 1, 639-647.
- Zhang, X.C., Cannon, S.B., Stacey, G., 2009. Evolutionary genomics of Lys M genes in land plants. *BMC Evol. Biol.* 9, 183-196.
- Zhao, J., Davis, L.C., Verpoorte, R., 2005. Elicitor signal transduction leading to production of plant secondary metabolites. *Biotechnol. Adv.* 23, 283-333.
- Zhu, B., Chen, T.H., Li, P.H., 1995. Activation of two osmotin like protein genes by abiotic stimuli and fungal pathogen in transgenic potato plants. *Plant Physiol.* 108, 929-937.

Figure captions

Fig 1. Classification of identified proteins based on A) cellular compartment, B) molecular functions and C) biological processes.

Fig 2. Effect of cyclodextrins (CD) and/or methyl jasmonate (MJ) on peroxidase differentially expressed in elicited grapevine cell culture (cv. Monastrell). A) Peroxidase V from *V. vinifera* (gi|225455195), B) cationic peroxidase 1 from *A. thaliana* (gi|225439625), and peroxidase 4 from *V. vinifera* (gi|225434381).

Fig 3. Effect of cyclodextrins (CD) and/or methyl jasmonate (MJ) on PR-1 differentially expressed in elicited grapevine cell culture (cv. Monastrell). A) PR-1 from *V. vinifera* (gi|163914225) B) PR-1 from *V. vinifera* (gi|225429117).

Fig 4. Effect of cyclodextrins (CD) and/or methyl jasmonate (MJ) on A) LysM domain-containing GPI-anchored protein 1 from *V. vinifera* (gi|225459538) and B) glycerophosphoryl diester phosphodiesterase (gi|147811111) differentially expressed in elicited grapevine cell culture (cv. Monastrell).

Fig 5. Effect of cyclodextrins (CD) and methyl jasmonate (MJ) on A) reticulon oxidase from *V. vinifera* (gi|147846526) and B) heparanase (gi|147862366) differentially expressed in elicited grapevine cell culture (cv. Monastrell).

Fig 6. Effect of cyclodextrins (CD) and methyl jasmonate (MJ) on A) β -1,3-glucanase from *V. riparia* (gi|37992763) and B) xyloglucan endotransglycosylase of *Populus tremula* x *Populus tremuloides* (gi| 225462505) differentially expressed in elicited grapevine cell culture (cv. Monastrell).

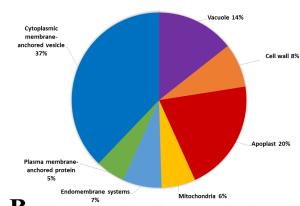
Table 1. Proteins expressed in the secretome of grapevine cell cultures after 96 h of treatment with a score higher than 60.

ACCESSION	PEPTIDE COUNT	SCORE	DESCRIPTION ¹
gi 225459180	18	1064.61	Cationic peroxidase [<i>Arabidopsis thaliana</i>]
gi 225439623	11	486.13	Cationic peroxidase [<i>Arabidopsis thaliana</i>]
gi 225453022	9	421.87	PR-4 [<i>Vitis vinifera</i>]
gi 147838866	8	382.11	Class III Peroxidase [<i>Vitis vinifera</i>]
gi 225430555	8	325.23	Aspartyl protease family protein At5g10770 [<i>Arabidopsis thaliana</i>]
gi 225434078	5	291.98	Class IV Chitinase [<i>Vitis vinifera</i>]
gi 3511147	4	280.44	PR-4 [<i>Vitis vinifera</i>]
gi 225441373	8	260.67	β -1,3-glucanase [<i>Vitis riparia</i>]
gi 147814943	3	206.84	α -amilase [<i>Vitis vinifera</i>]
gi 2306811	6	182.45	Class IV Chitinase [<i>Vitis vinifera</i>]
gi 6273385	3	178.63	Thaumatin 1SCULTL2 [<i>Vitis vinifera</i>]
gi 225434050	3	172.20	Class IV Chitinase [<i>Vitis vinifera</i>]
gi 225432965	5	167.36	Protease inhibitor [<i>Vitis vinifera</i>]
gi 1839046	3	156.66	Osmotin-type protein [<i>Vitis vinifera</i>]
gi 147860594	2	155.29	Class III peroxidase [<i>Vitis vinifera</i>]
gi 147823287	2	142.92	Trypsin inhibitor [<i>Vitis vinifera</i>]
gi 225432971	2	137.06	Protease inhibitor [<i>Vitis berlandieri</i> x <i>Vitis riparia</i>]
gi 2306813	6	133.60	Class IV Endochitinase [<i>Vitis vinifera</i>]
gi 147784683	2	132.07	Pathogenesis-related protein NtPRp27 [<i>Vitis vinifera</i>]
gi 225457600	3	131.48	β -1,3-glucanase [<i>Vitis vinifera</i>]
gi 225439627	3	127.74	Cationic peroxidase 1 [<i>Vitis vinifera</i>]
gi 225427492	2	123.57	Acyl-CoA-binding protein [<i>Vitis vinifera</i>]
gi 225428879	3	112.3	Cysteine proteinase RD21A [<i>Vitis vinifera</i>]
gi 225453525	2	111.77	Putative expansin-B2 [<i>Vitis vinifera</i>]
gi 225470066	3	106.52	2S Albumin storage proteins [<i>Vitis vinifera</i>]

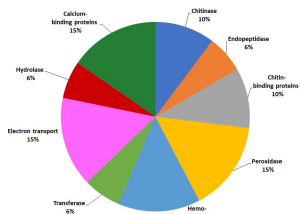
gi 147805839	2	98.57	Class I Chitinase [<i>Vitis vinifera</i>]
gi 225441645	3	85.32	β -1,3-glucanase [<i>Vitis vinifera</i>]
gi 225451467	1	72.91	Peroxidase 55 [<i>Arabidopsis thaliana</i>]
gi 225447360	2	72.69	Cysteine-rich repeat secretory protein 38 [<i>Vitis vinifera</i>]
gi 225456525	2	70.15	Similar to MtN19-like protein [<i>Vitis vinifera</i>]
gi 225445051	1	64.87	Acid phosphatase 1-like [<i>Arabidopsis lyrata</i> subsp. <i>lyrata</i>]
gi 1549400	1	64.59	β -1,3-glucanase [<i>Vitis vinifera</i>]
gi 225448120	2	62.27	Serine-type endopeptidase inhibitor [<i>Vitis vinifera</i>]

¹This description is that captured in B2GO³⁷; the accession numbers corresponds to *Vitis* species.

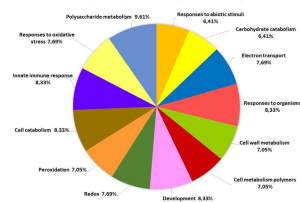
A Classification of identified proteins based on relevant biological processes by cellular compartment

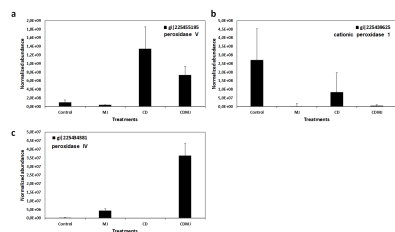


B Classification of identified proteins based on relevant biological processes by molecular functions

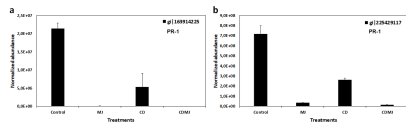


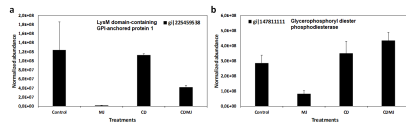
C Classification of identified proteins based on relevant biological processes by biological processes

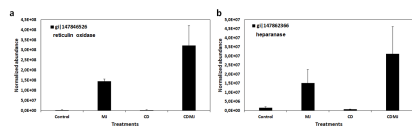




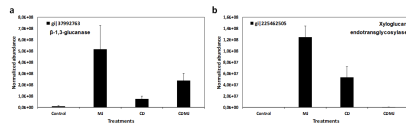
ACCEPTED MANUSCRIPT







ACCEPTED MANUSCRIPT



Proteins were differentially expressed with cyclodextrins and/or methyl jasmonate

Cyclodextrins reinforces defensive arsenal of grapevine cells

Cyclodextrins induced peroxidase and xyloglucan endotransglycosylase expression

Methyl jasmonate increased the expression of pathogenesis-related proteins.

ACCEPTED MANUSCRIPT

Contribution

SBN, RBM and MAP conceived and designed research. SBN conducted experiments. SBN and LA analyzed data. SBN and LA wrote the manuscript. All authors read and approved the manuscript.

ACCEPTED MANUSCRIPT