**VvMJE1 of the Grapevine (*Vitis vinifera*) VvMES Methylsterase Family Encodes
for Methyl Jasmonate Esterase and Has a Role in Stress Response**

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Abstract

The known members of plant methyl esterase (MES) family catalyze the hydrolysis of a C-O ester linkage of methyl esters of several phytohormones including indole-3-acetic acid, salicylic acid and jasmonic acid. The genome of grapevine (*Vitis vinifera*) was found to contain 15 *MES* genes, designated *VvMES1-15*. In this report, *VvMES5* was selected for molecular, biochemical and structural studies. *VvMES5* is most similar to tomato methyl jasmonate esterase. *E.coli*-expressed recombinant *VvMES5* displayed methyl jasmonate (MeJA) esterase activity, it was renamed *VvMJE1*. Under steady-state conditions, *VvMJE1* exhibited an apparent K_m value of 92.9 μM with MeJA. *VvMJE1* was also shown to have lower activity with methyl salicylate (MeSA), another known substrate of the MES family, and only at high concentrations of the substrate. To understand the structural basis of *VvMJE1* in discriminating MeJA and MeSA, a homolog model of *VvMJE1* was made using the X-ray structure of tobacco SABP2, which encodes for methyl salicylate esterase, as a template. Interestingly, two bulky residues at the binding site and near the surface of tobacco SABP2 are replaced by relatively small residues in *VvMJE1*. Such a change enables the accommodation of a larger substrate MeJA in *VvMJE1*. The expression of *VvMJE1* was compared in control grape plants and grape plants treated with one of the three stresses: heat, cold and UV-B. While the expression of *VvMJE1* was not affected by heat treatment, its expression was significantly up-regulated by cold treatment and UV-B treatment. This result suggests that *VvMJE1* has a role in response of grape plants to these two abiotic stresses.

Key words: methylesterase, methyl jasmonate, grapevine, homology modelling, abiotic stress

Introduction

Many metabolites undergo methylation and demethylation under specific physiological conditions. These metabolites include the phytohormones indole-3 acetic acid (IAA), salicylic acid (SA) and jasmonic acid (JA). The methylation of IAA, SA and JA is catalyzed by carboxyl methyltransferases (D'Auria et al., 2003). In several flowering plants with a sequenced genome, these carboxyl methyltransferase genes, collectively called the *SABATH* family, were found to form a mid-sized family with 20-30 members (D'Auria et al., 2003; Zhao et al., 2007; Zhao et al., 2008; Zhao et al., 2010; Zhao et al., 2013). The moss *Physcomitrella patens* contains four *SABATH* genes (Zhao et al., 2012). The methylation of IAA, SA and JA is catalyzed by different members of the *SABATH* family, with individual members exhibiting distinct substrate specificity. This suggests that gene duplication followed by functional divergence is responsible for the evolution of the *SABATH* family (Zhao et al., 2008). As the reverse reaction of methylation, the demethylation of the methyl esters of IAA, SA and JA is catalyzed by methylesterase (MES), which belongs to the α/β hydrolase superfamily (Nardini and Dijkstra, 1999). The plant MES family has been better studied in *Arabidopsis* (Yang et al., 2008). It is interesting to note that the *Arabidopsis* genome contains similar number of *MES* and *SABATH* genes (20 and 24 respectively). In comparison to the *SABATH* genes that methylate IAA, SA and JA, which have been studied in a variety of plant species and tissues (Qin et al., 2005; Ross et al., 1999; Seo et al., 2001), our knowledge of the *MES* family that demethylate MeIAA, MeSA and MeJA is still limited.

The *MES* genes that encode enzymes for catalyzing the demethylation of MeSA have been isolated and characterized from tobacco (Kumar and Klessig, 2003), potato

(Manosalva et al., 2010), and poplar (Zhao et al., 2009). In poplar, MeSA esterase gene has two duplicated copies. The tobacco gene, also called *SABP2*, was shown to have a function in systemic acquired resistance (Park et al., 2007). The potato and poplar genes have been implicated in plant defense against biotic stresses (Manosalva et al., 2010; Zhao et al., 2009). For MeIAA esterase, the gene from Arabidopsis has been characterized in detail. It functions to activate the inactive MeIAA to IAA, the physiologically active form of auxin (Yang et al., 2008). MeJA esterase has been studied only in tomato (Stuhlfelder et al., 2004). Exogenous application of MeJA results in the down-regulation of tomato *MJE* (*LeMJE*) expression, suggesting that *LeMJE* has a role in regulating the JA signaling pathway (Stuhlfelder et al., 2004). For the MES members that have been biochemically studied in depth, individual members appear to show substrate specificity. The MeSA esterase from both tobacco and poplar showed highest activity towards MeSA. Only at high concentrations that are physiologically irrelevant, they showed activity towards MeIAA and MeJA (Forouhar et al., 2005; Zhao et al., 2009). In contrast, the Arabidopsis MeIAA esterase exhibited activity with only MeIAA not MeSA or MeJA (Yang et al., 2008). The crystal structure of tobacco MeSA esterase or SABP2 has been solved (Forouhar et al., 2005). However, our understanding of the structural basis that governs substrate specificity of the MES family is very limited. To understand the evolution of substrate specificity of the MES family and the biological function of individual members, it is important to isolate and characterize *MES* genes from a wide range of plant species.

In this study, we chose grapevine (*Vitis vinifera*) as a model species to study the MES family with a focus on MeJA esterase. Grapevine is one of the most important fruit crops

95 throughout the world (Martin et al., 2010). With a sequenced genome (The French–
96 Italian Public Consortium for Grapevine Genome Characterization, 2007), grapevine
97 serves as a model species for the study of fruit tree biology and physiology. The
98 jasmonate (including JA, MeJA and other forms of JA derivatives) signaling pathway
99 plays critical roles in plant developmental programs as well as plant interactions with the
100 environment. Jasmonates are important for root growth, flower development, and seed
101 germination (Creelman and Mullet, 1997). They have also known for their role as a
102 wound signal. When a plant is attacked by insects, jasmonate production is promoted,
103 which leads to plant defense responses against insects (Howe, 2005; Leon et al., 2001). In
104 addition to insect defense, jasmonates are also important for regulating plant defenses
105 against pathogens (Thomma et al., 1998; Vijayan et al., 1998). Many studies demonstrate
106 that jasmonates also have a role in the plant defense against several types of abiotic
107 stresses (Creelman and Mullet, 1995). In Arabidopsis, for example, exogenous
108 application of JA increases the freezing tolerance, while transgenic Arabidopsis blocking
109 the JA biosynthesis decreased plant freezing tolerance (Hu et al., 2013). In rice, increased
110 level of JA was observed in response to drought and cold stresses. Furthermore, many
111 genes involved in JA biosynthesis pathway were induced by drought and cold treatments
112 (Du et al., 2013). In wheat, one study showed that JA pretreatment obviously mitigated
113 the detrimental effect of UV-B (Liu et al., 2012). As a methyl ester of JA, MeJA has been
114 postulated to be the inactive or storage form of JA (Westfall et al., 2013). Demethylation
115 therefore would be the first step to activate MeJA. The methyltransferase that converts JA
116 to MeJA has been studied in several plants, including Arabidopsis (Seo et al., 2001),
117 poplar (Zhao et al., 2013), tomato (Tieman et al., 2010) and strawberry (Preuß et al.,

2014). In contrast, our knowledge about the enzyme that catalyzes the demethylation of MeJA to form JA is very limited. Therefore, the goal of this study is to identify and characterize the MeJA esterase gene from grapevine in order to understand its relatedness to tomato MJE, its biological function, and the structural basis governing substrate specificity of MES proteins.

Materials and methods

Plant material stress treatments and chemicals

Herbaceous cuttings of *V. vinifera*, cv. Chardonnay obtained from field grown plants were rooted in intermittent mist beds in a greenhouse with 27°C heat applied from underneath. Rooted plants were transplanted into one gallon pots filled with a mix of Yolo sandy loam, perlite, and peat (1:1:1). Plants were maintained at 24°C to 32°C with supplemental lighting to maintain an average day and night cycle of 18 h and 6 h, respectively. To ensure uniform shoot growth, after four weeks' growth, plants were cut back to two buds and re-grown for 4 weeks prior to treatments. Control plants were grown in a greenhouse under the same growth conditions indicated above. For heat treatment, six experimental plants each individually representing an experimental unit was transferred into a walk-in growth chamber maintaining a constant temperature of 37 °C supplemented with the same lights as in a greenhouse. Multiple leaves were collected from three grapevines after one hour and another three plants after five hours of heat treatments. For cold treatment, similarly, six grapevines were transferred from 27°C

temperature conditions in the greenhouse to the same growth chamber but maintained at 1.5°C. Leaves from grapevines exposed to cold treatment were collected from three plants after one hour and from another three plants after five hours. For UV-B (280-320 nm) radiation treatment, six plants were placed in a UV-exposure cabinet. The UV spectrum at the top of plants was adjusted to 0.6 W/m² according to the method previously described (Allen et al., 1997). Plants were exposed for 1 hour and 5 hours, respectively. Leaves were collected at the end of the treatments. Sampling of control plants was conducted at the same time. The duration of each treatment time was determined based on previous studies (Lin et al., 2006). For all treatments above, harvested leaves were immediately immersed into liquid N₂ for later RNA isolation. MeJA, MeSA and other chemicals used for enzyme assays were all purchased from Sigma-Aldrich (St. Louis, MR, USA).

Database search and sequence analysis

To identify putative grape SABP2-like genes, the protein sequence of tobacco SABP2 (accession: AY485932) was used as a query to search the genome sequence database of grape (<http://www.plantgdb.org/VvGDB/>) using BlastP algorithm (Altschul et al., 1990). Multiple protein sequence alignments were made using the ClustalX2.0 program (Larkin et al., 2007), and displayed using GeneDoc (Nicholas et al., 1997). A maximum likelihood tree was constructed with MEGA version 6 (Tamura et al., 2013), and 1,000 replicates bootstrap was used to assess the reliability of branches.

Cloning full-length cDNA of VvMJE1

Total RNA was extracted from leaf tissues of *V. vinifera*, cv. Chardonnay using the RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) and DNA contamination was removed with an on-column DNase (Qiagen, Valencia, CA, USA) treatment. Total RNA (1.5 µg) was reverse-transcribed into first-strand cDNA in a 15 µL reaction volume using the first strand cDNA synthesis Kit (Amersham Biosciences, Piscataway, NJ, USA) as previously described (Chen et al., 2003). VvMJE1 full-length cDNA was amplified with the first strand cDNA using the forward primer 5'-ATGGAGAAAAGAGAGAGGCATTTTGTG-3' and the reverse primer 5'-CTAAGAATAACTCTCAACTATTTTCATAC-3'. PCR was set as follows: 94 °C for 2 min followed by 30 cycles at 94 °C for 30 s, 60 °C for 45 s and 72 °C for 1 min 30 s, and a final extension at 72 °C for 10 min. The PCR product was separated on 1.0% agarose gel. The target band was sliced from the gel and purified using QIAquick Gel Extraction Kit (Qiagen, Valencia, CA, USA). The PCR product was cloned into pET100/D-TOPO vector using the protocol recommended by the vendor (Invitrogen, Carlsband, CA, USA). The cloned cDNAs in pET100/D-TOPO vector were fully sequenced.

Purification from E. coli-expressed recombinant proteins

To express VvMJE1, the corresponding protein expression construct were transformed into *E. coli* strain BL21 (DE3) CodonPlus (Stratagene, La Jolla, CA, USA). Protein expression was induced by IPTG for 18 h at room temperature and the cells were lysed by sonication. His-tagged VvMJE1 protein was purified from *E. coli* cell lysate using Ni-NTA agarose following manufacturer's instruction (Invitrogen, Carlsband, CA, USA).

The purity of the protein was verified by SDS-PAGE and the concentration of the protein determined by the Bradford assay (Bradford, 1976).

MeJA esterase activity assay

A two-step radiochemical esterase assay was performed to determine the substrate specificity of VvMJE1 following a protocol previously reported (Forouhar et al., 2005; Zhao et al., 2009). The first step reaction was performed with 40 µl volume containing 50 mM Tris-HCl, pH 7.5, 1 mM substrate (MeJA or MeSA) and 0.5 µl purified enzyme for 30 min at 25°C, after that the enzyme was inactivated with boiling. The second step reaction started with the addition of 3 µM ¹⁴C-SAM with a specific activity of 51.4 mCi/mmol (Perkin Elmer, Boston, MA, USA), 120µM SAM and purified MT (JMT or SAMT). The assay was kept at room temperature for 30 min. The reaction was stopped by the addition of 150 µl ethyl acetate, vortexed, and phase-separated by a 1 min centrifugation at 14,000g. The upper organic phase was counted using a liquid scintillation counter (Beckman Coulter, Fullerton, CA, USA). Three independent assays were performed for each of the two potential substrates MeJA and MeSA.

Determination of kinetic parameters of VvMJE1

The increase in reaction rate with increasing concentrations of MeJA was evaluated with the radiochemical assay described above and was found to obey Michaelis-Menten kinetics. Appropriate enzyme concentrations and incubation times were determined in time course assays so that the reaction velocity was linear during the assay period. To determine *K_m* for MeJA, the concentrations of MeJA were independently varied in the

range from 5 to 100 μ M. Lineweaver-Burk plots were made to obtain apparent K_m values and maximum velocity values, as previously described (Chen et al., 2003). Final values are an average of three independent measurements.

Homology Model of VvMJE1

A homology model was built based on the X-ray structure of SABP2 (PDB ID: 1IXKL) using the homology modeling program in the molecular operation environment (Molecular Operating Environment (MOE), 2013.08; Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2015). Salicylic acid (SA) in the model was built based on the superposition of the model with the X-ray structure of the SABP2-SA complex (1Y7I).

Expression analysis of VvMJE1 using qRT-PCR

Total RNA was extracted from the leaves of experimental plants using ABI RNAqueous® Kit (Applied Biosystems, USA). About 200 ng of total RNA per sample was reverse-transcribed into cDNA using the SuperScript® First-Strand Synthesis System (Invitrogen, Life Technologies, USA). cDNA concentrations were then adjusted to 50 ng/ μ l measured by the PicoGreen method. Two μ l of cDNA was used for gene expression analysis with *VvMJE1* forward 5'-ATGGAGAAAAGAGAGAGGCA-3' and reverse primers, 5'-CTCTCCATAGCAACCGATACG-3'. Real-time PCR was performed using SYBR-Green PCR Master Mix (Applied Biosystems, CA) on a 7500 Real-Time PCR System (Applied Biosystems, CA) with the following cycle conditions: 95°C for 10 min followed by 40 cycles of 95°C for 15s, 56°C for 45s and 72°C for 45s.

V. vinifera actin gene with forward primer 5'-CTGAGTTCTAACTTGCTCCTTTGAC-3' and reverse primer 5'- ATAGTTCTTCTCAACTGAGGAGCTG-3' was also included as the endogenous control/normalization. The expression level of *VvMJE1* in untreated control was arbitrarily set at 1.0.

Statistical analysis

Statistical analysis of *VvMJE1* expression in control and treated plants was conducted using SAS (Version 9.2) (SAS Institute, NC) based on three biological replicates and three technical replicates. Levels of significance were calculated using Student's t-test at $p < 0.05$.

Results

Identification and sequence analysis of grape MJE

To identify the complete *MES* gene family from the fully sequenced grapevine genome (The French–Italian Public Consortium for Grapevine Genome Characterization, 2007), the protein sequence of tobacco SABP2 was initially used to search the genome database of grapevine using the BLASTP algorithm (Altschul et al., 1990). Fifteen genes encoding proteins bearing significant similarity to known MES proteins were identified in the grape genome (Supplemental Table S1). They were designated *VvMES1-15*.

To understand the evolutionary relationship between *VvMESs* and other known esterase proteins, a phylogenetic tree was constructed consisting of all 15 putative

VvMESs, AtMESs, NtSABP2, potato methyl salicylate esterase (StMES1) and LeMJE (Fig. 1). Three VvMES members are closely related with LeMJE.

Among these three genes, VvMES5 was identified to be the most similar to LeMJE, sharing 77% similarity with LeMJE at the protein level. Although the protein encoded by VvMES5 has been annotated as grapevine salicylic acid-binding protein 2-like protein (XM_010648292.1), it displayed specific MeJA esterase activity (see the next section). Because the most majority of grapevine esterase proteins have not been analyzed biochemically, it was not possible to rule out the possibility that other grapevine esterase proteins might have MeJA esterase activity. Thus, VvMES5 was renamed VvMJE1.

A full-length cDNA of VvMJE1 was cloned via RT-PCR from grape leaf. The sequence of 783 bp of VvMJE1 encodes a protein of 261 amino acids with an expected molecular weight of 29.59 KDa. VvMJE1 shares 60% and 50% identities with LeMJE and NtSABP2 respectively at the amino acid level (Fig. 2). It also contains the catalytic triad (Ser82, His239, and Asp211) that forms part of the active site pocket (Fig. 2). These three residues are highly conserved among VvMJE1, LeMJE and NtSABP2 (Fig. 2). The putative PI of VvMJE1 is 7.7.

Biochemical properties of VvMJE1

Recombinant protein of VvMJE1 was expressed in *E.coli* from its corresponding full length cDNA. In the vector pET100/D-TOPO, the full-length cDNA of VvMJE1 was fused to an N-terminal sequence containing codons for six histidine residues. His-tagged VvMJE1 expressed in *E. coli* was then purified using Ni-NTA agarose to electrophoretic homogeneity (Fig. 3). Purified VvMJE1 was tested for esterase activity. When MeJA was

used as the substrate, VvMJE1 catalyzed the hydrolysis of the methyl group from MeJA to form JA. Under steady-state conditions, VvMJE1 exhibited apparent K_m value $92.9 \pm 0.6 \mu\text{M}$ with MeJA (Fig. 4).

Substrate specificity analysis of VvMJE1

In addition to MeJA, MeSA was also being tested as a potential substrate for VvMJE1 (Fig. 5). In the 1mM concentration of substrates, VvMJE1 had catalytic activity with MeSA and MeJA. The activity with MeSA was about 20% of that with MeJA. Because 1mM is beyond physiological concentration *in vivo*, we therefore examined the relative esterase activity of VvMJE1 in the presence of lower concentrations of MeSA and MeJA. In the 10 μM concentration of substrates, VvMJE1 had only the catalytic activity with MeJA. These results suggested VvMJE1 had the highly specific activity with MeJA in the more physiologically relevant conditions.

Structural feature of VvMJE1

The homology model of VvMJE1 is shown in Fig. 6. The model superposes quite well with the X-ray structure of tobacco SABP2 with a RMSD deviation of less than 1 Å (Fig. 6). The catalytic triad (Ser82, His239 and Asp211) align well with the corresponding residues (Ser81, His238 and Asp210) in SABP2; Ser82 is well positioned for the nucleophilic attack on substrates (Fig. 6B). It is of interest to note from the X-ray structure and structural model that some bulky residues at the binding site of SABP2 are replaced by relatively small residues in VvMJE1 (Fig. 6C). For instance, Trp131, Met149

and Phe155 on or near the protein surface of SABP2 are replaced by Ser131, Ile149 and Asp155, respectively in VvMJE1 (Fig 6D).

Expression analysis of VvMJE1 in grapevine leaves under stress conditions

To determine the expression patterns of *VvMJE1* under stress conditions, grapevine seedlings were treated with three abiotic stress factors: cold, heat, and UV-B. Leaf tissues were collected for gene expression analysis (Fig.7). Cold treatments at both 1 h and 5 h caused significant induction of *VvMJE1* expression. At 1 h, the expression increased more than two fold, and at 5 h, the expression increased eight fold. Likewise, UV-B treatments at both 1 h and 5 h led to significant up-regulation of *VvMJE1* expression, with an increase of approximately 6 fold and 10 fold respectively. In contrast, heat treatments at both time points did not change the expression of *VvMJE1* (Fig.7).

Discussion

Grapevine is the second plant species, after Arabidopsis, in which the entire methylesterase (*MES*) gene family has been identified. Despite the fact that the genome size of grapevine (~500 Mbp) is about four times of that of Arabidopsis (~135Mbp), the size of the grapevine *MES* family with 15 members is smaller than that of Arabidopsis, which contains 20 members (Yang et al., 2008). The larger size of the *MES* family of Arabidopsis is partly due to the presence of multiple tandem repeats of *MES* genes. Fifteen of the 20 members of the Arabidopsis *MES* family have biochemically tested for

esterase activity with four methyl esters of phytohormones: IAA, SA, JA and GA. While none of the 15 Arabidopsis MES proteins showed activity with GA, 11 showed activity with one or more of the three substrates IAA, SA and JA. This suggests that demethylation of methyl esters of phytohormones may have an important role in regulating the biological functions of the phytohormones (Westfall et al., 2013). Considering the critical roles of phytohormones to plant growth, development and interactions with the environment, it is not surprising to observe clustering of certain grapevine and Arabidopsis *MES* genes in phylogenetic analysis (Fig. 1).

Phylogenetic analysis showed that three VvMES proteins are clustered with LeMJE. Among these three VvMESs, VvMJE1 is most similar to LeMJE (Fig. 1). However, there is no an apparent ortholog of VvMJE1 and LeMJE in Arabidopsis, which implies independent evolution of MJE in grapevine/tomato and Arabidopsis lineages. VvMJE1 is the second MeJA esterase gene to be characterized in plants. In *in vitro* biochemical studies, VvMJE1 displayed highly specific esterase activity towards MeJA, supporting that MeJA is the *in vivo* substrate. The kinetic properties of VvMJE1 are comparable to those of other characterized MES proteins. The K_m of VvMJE1 for MeJA was 92.9 μM , which is about 1.5 times and 3 times higher than those of PtSABP2-1 and PtSABP2-2 (Zhao et al., 2009). In addition to MeJA, VvMJE1 also showed activity with methyl salicylate (MeSA), but only at high concentration of substrate. Like VvMJE1, some other characterized MES members also appear to show substrate preference. The MeSA esterase from both tobacco and poplar showed highest activity towards MeSA. Only at high concentrations that are physiologically irrelevant, they showed activity towards MeIAA and MeJA (Forouhar et al., 2005; Zhao et al., 2009). In contrast, the Arabidopsis

347 MeIAA esterase exhibited activity towards only to MeIAA not to MeSA and MeJA
348 (Yang et al., 2008). It will be interesting to examine whether VvMJE1 and other
349 members of the plant MES family have activity with other methyl esters produced by the
350 plant SABATH enzymes such as MeIAA, gibberellic acid methyl ester, methyl
351 farnesoate, methyl anthranilate and methyl nicotinate. If so, it will be interesting to
352 further ask whether such promiscuous activities are biologically relevant. Such
353 knowledge will be important for us to understand substrate specificity evolution of the
354 plant MES family.

355 The mechanism that governs substrate specificity of VvMJE1 is partly understood
356 through structure modeling (Fig. 6). For SABP2, the existence of the relatively large
357 hydrophobic residues such as Trp131, Met149 and Phe155 on or near the surface may
358 protect the substrate (MeSA) from solvent and prevent the relatively large substrates
359 (such as MeJA) from entering the active site or reaching the catalytic triad for the
360 catalysis, leading to substrate discrimination. Consistent with this suggestion, previous
361 studies on tobacco SABP2, PtSABP2-1 and PtSABP2-2 showed that these enzymes had
362 low or no activity on MeJA at physiologically relevant concentrations (Forouhar et al.,
363 2005; Zhao et al., 2007). The replacement of these bulky residues by the small residues in
364 VvMJE1 may allow the enzyme to bind MeJA and catalyze its reaction more efficiently.
365 For MeSA, the loss of the protection of Trp131, Met149 and Phe155 in VvMJE1 (Fig.
366 6D) exposes the substrate to the solvent. This could lead to a reduction of the catalytic
367 efficiency relative to MeJA, consistent with the experimental data reported here.

368 MeJA has been shown to be involved in a diverse array of plant growth and
369 development as well as plant interactions with the environment (Creelman and Mullet,

1997). However, the mode of action of MeJA is not fully understood. There is growing evidence suggesting that MeJA is a volatile compound that functions as the signal molecule for the JA transportation. It is likely converted back to JA to function (Stitz et al., 2011). The identification of *VvMJE1* appears to strengthen this hypothesis. Recent reports imply that JA signal pathway is involved in abiotic defense. For instance, exogenous application of JA in *Arabidopsis* significantly enhanced plant freezing tolerance (Hu et al., 2013). In rice, cold stress resulted in increase of JA and induction of JA biosynthesis genes but heat stress caused the reduction of JA levels (Du et al., 2013). In response to UV-B radiation, the increased JA content has been shown to enhance the expression of stress-related genes (Liu et al., 2012). The changes of the concentrations of JA are hypothesized to impact the level of JA-Ile, the bioactive form of jasmonates (Fonseca et al., 2009).

Based on this existing knowledge, we tested whether *VvMJE1* has a role in defense against abiotic stresses by examining the expression of *VvMJE1* in grapevines under three types of stress: heat, cold and UV-B radiation (Fig. 7). The expression of *VvMJE1* was not significantly affected by heat treatment. In contrast, the expression of *VvMJE1* in grapevines was significantly induced by both cold treatment and UV-B radiation (Fig. 7). It can be speculated that induced expression of *VvMJE1* increased the levels of JA by converting MeJA to JA, which is consistent with the observed changes of the levels of JAs/JA biosynthesis in other plants under abiotic stresses in previous studies (Du et al., 2013; Liu et al., 2012).

In a brief summary of this study, we identified the 15 MES genes in the grapevine genome and showed that one of them, *VvMJE1*, encodes methyl jasmonate esterase.

393 VvMJE1 can distinguish between methyl jasmonate and methyl salicylate. The structural
394 basis for such substrate specificity was partly understood through homology modeling.
395 Moreover, the induced expression of *VvMJE1* by cold treatment and UV-B treatment
396 indicate that this gene has a role in stress response.
397
398

Figure Legends:

Fig. 1 Phylogenetic analysis of plant methyl esterases. The tree was constructed with protein sequences of 15 grapevine MES members (VvMES1-VvMES15), 20 Arabidopsis MES members (AtMES1-AtMES20; Yang et al., 2008); PtSABP2-1, *Populus trichocarpa* SABP2-1 (estExt_fgenes4_pm.C_LG_VII0354); PtSABP2-2, *Populus trichocarpa* SABP2-2 (eugene3.00070971); LeMJE, *Lycopersicon esculentum* MJE (Accession: NM_001246884.1); StMES1, *Solanum tuberosum* MES1 (XP_006360243) and NtSABP2, *Nicotiana tabacum* SABP2 (accession # AY485932). OPE, a sterol esterase from fungus *Ophiostoma piceae* (accession # PDB: 4BE9_B) was used as outgroup. VvMES5 is methyl jasmonate esterase (VvMJE1). Numbers at nodes indicate bootstrap values calculated over 100 replicates. Branches are drawn to scale with the bar indicating 0.5 substitutions per site.

Fig. 2 Alignment of deduced amino acid sequences of VvMJE1 with tomato methyl jasmonate esterase (LeMJE) and tobacco SABP2 (NtSABP2). Conserved or semi-conserved amino acids in three sequences were shown in white letters on black or grey backgrounds, respectively. The arrows indicate the three conserved amino acids that form a catalytic triad.

Fig. 3 SDS-PAGE of purified recombinant VvMJE1. His-tagged VvMJE1 expressed in *E. coli* was purified as described in Materials and Methods. Lane M contained protein molecular weight markers. Lane A contained VvMJE1 crude extract, and lane B

contained 1 μ g of purified VvMJE1 protein. Arrow indicates the purified VvMJE1. The gel was stained with Coomassie Blue.

Fig. 4 Steady-state kinetic measurements of VvMJE1 using methyl jasmonate (MeJA) as the substrate. One example of the Lineweaver-Burk plot is shown. The K_m value shown is not absolute. However, it was apparent under our specified reaction conditions.

Fig. 5 Substrate specificity of VvMJE1. Relative methyl esterase activity of VvMJE1 was measured with MeJA and MeSA at three different concentrations (10 μ M, 100 μ M, and 1 mM). The activity with MeJA at each of the substrate concentrations was set at 100%. The activity ratios between MeJA and MeSA are indicated. All results were obtained from three independent measurements.

Fig. 6 Homology Model of VvMJE1. (A) Superposition of the homology model of VvMJE1 (Magenta ribbon) with the X-ray structure of SABP2 (Cyan. PDB: IXKL). RMSD=0.9 $\text{\AA}$ based on main chain atoms. SA is also shown in space filling based on its position in the SABP complex (11Y7). (B) Comparison of the catalytic triad (Ser82, His239 and Asp211) of VvMJE1 (Magenta) and SABP2 (Cyan). SA is shown in grey. (C) Comparison of the binding pockets for VvMJE1 (Magenta) and SABP2 (Cyan). (D) Space filling model of SABP2. W131, M149 and F155 are in pink color and SA in grey.

Fig. 7 Expression analysis of *VvMJE1* under stress conditions. Seedlings of grapevine were treated with cold, heat and UV-B for 1h and 5h. Leaves were collected and used for

quantitative RT-PCR analysis. Three biological replicates and three technical replicates were performed for gene expression analysis. The expression levels were presented as mean + SE. Bars marked with asterisk (*) indicate that VvMJE1 showed a significant difference in expression levels in treated vs. control plants ($p < 0.05$).

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584

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N. Zhao, H. Lin and F. Chen: conceived the project. N. Zhao, S.Q. Lan, Q.D. Jia, X. Chen, H. Guo: performed the experiments and analyzed the data. N. Zhao and F. Chen: wrote the draft manuscript. All authors contributed to manuscript revision.

Figure 1

VvMJE1 : MEKRER--HFVLVHGACHGAWCWYKVTTFILRSAGHKVTALDLAAAGANGKRLDELNSISDY : 59
 LeMJE : MEKGDKNHFVLVHGACHGAWCWYKVVTTILRSEGHKVSVLDMAASGINPKHVDDLNSMADY : 60
 NtsABP2 : MK--EGKHfVLVHGACHGGWSYKLLKPLLEAAGHKVTALDLAASGTDLRKIEELRTLYDY : 58

↓

VvMJE1 : HEPLMKEMTSLVAGEKVLVAHSLGGVSVSVAMERFPQKISVAVFVSAYMPGPDFNLSTV : 119
 LeMJE : NEPLMEFMNSLPQLERVVLVGHSMGGINISLAMEKFPQKIVVAVFVTAFMPPGPDNLVAL : 120
 NtsABP2 : TLPLMELMEISLSADEKVLVGHSLGGMNLGLAMEKYPQKIYAAVFLAAFMPDSVHNSSFV : 118

VvMJE1 : YQELHQRRQG-ASKDTQYTFDRGSNNPPTSIIIFSPEDLAAKLYQLSPPEDLTLATILMRP : 178
 LeMJE : GQQYNQQVE--SHMDTEFVYNNQDKAPTSLVLGPVVLATNFYQLSPPEDLTLATYLVLRP : 178
 NtsABP2 : LEQYNERTPAENWLDTQFLPYGSPPEELTSMFFGPKFLAHKLYQLCSPEDLALASSLVLRP : 178

↓

VvMJE1 : TKLFRGENLLKETTVTREKYGTVRRVYIVCDKDNILKED-FQRWMIKNNPSEDEVKVMGS : 237
 LeMJE : VPLFDESILLANTTISKEYGSVHRVYVCDKDNVLKEQQFQKWLINNNEPDEVQLIHNA : 238
 NtsABP2 : SSLMED-LSKAKYFTDERFGSVKRVYIVCTEDKGIPEE-FQRWQIDNIGVTEAIEIKGA : 236

↓

VvMJE1 : DHMPMFskPLDLCAYLQEIVEsYS : 261
 LeMJE : DHMVMFskPRDLSSCLVMISQKYY : 262
 NtsABP2 : DHMAMLCePQKLcASlLEIAHKYN : 260

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7