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Full Length Research Paper

Potential novel *bZIP-like* gene for resistance to *Erysiphe necator* identified in Chinese wild *Vitis pseudoreticulata*

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In this study, a novel *bZIP*-like gene was isolated from Chinese wild *Vitis pseudoreticulata* W. T. acc. Baihe-35-1. The full-length complementary deoxyribonucleic acid (cDNA) sequence of the gene was 1583 bp including 159 bp 5' untranslated region (UTR), 365 bp 3' UTR and a 1083 bp ORF which encodes a polypeptide of 360 amino acids with a molecular weight of 38.662 kDa. The deduced amino acid sequence shares an overall 46 to 69.8% sequence similarity with bZIP from other plants. Therefore, we designated this gene as *V. pseudoreticulata bZIP* (*VpbZIP-like*). The expression of *VpbZIP-like* was induced 12 h post inoculation (hpi) by *Erysiphe necator*, but transiently decreased, then increased in these two genotypes and its expression was lower in highly resistant genotype Baihe-35-1 than in susceptible genotype Hunan-1 at 24, 48 and 72 hpi. We further tested whether the expression was also a response to plant signaling molecules. Results indicate that the susceptible genotype Hunan-1 showed higher expression of *VpbZIP-like* than the highly resistant genotype Baihe-35-1 after exogenous application of methyl jasmonate (MeJA), salicylic acid (SA) and ethephon (Eth). Moreover, tissue specific expression pattern of *VpbZIP-like* was analyzed by reverse transcription-polymerase chain reaction (RT-PCR). Results reveal that it was in lower lever in flower than in leaf, stem, tendril and fruit. The CDS of *VpbZIP-like* was inserted into the prokaryotic expression construct pGEX-4T-1, and then transformed into *Escherichia coli* BL21-code induced by isopropyl-b-D-thiogalactopyranoside (IPTG) which resulted in the production of a Mr. 64 kDa of GST- VpbZIP-like fusion protein displayed in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Key words: Chinese wild *Vitis,* bZIP, gene expression, signaling molecules, fusion protein expression.

INTRODUCTION

European grapevine (*Vitis vinifera* L.), is an economically important crop grown worldwide for winemaking, table grapes, raisins, liquors and juice, and is susceptible to fungal pathogens. Powdery mildew (PM) of grapevine, caused by *Erysiphe necator* Burr, is the most economically important fungal disease of *V. vinifera*, causing reduced yield and loss of wine quality. As part of the diverse East Asia *Vitis* gene pool, China has more than 35 *Vitis* species occurring naturally. Furthermore, Chinese wild *Vitis* have extremely high resistance to *E. necator* (Wang et al., 1995).

Therefore, it is crucial to improve elite cultivars of *V.*

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vinifera through the identification of resistant genes from Chinese wild *Vitis* species. To gain an insight into the novel genes and investigate the resistance mechanism in Chinese Wild *Vitis pseudoreticulata* clone Baihe-35-1, messenger ribonucleic acid (mRNA) differential display was employed to explore the differential expression of resistant gene to the disease. In a previous study, a partial *bZIP-like* fragment was identified from the leaves upon infection with *E. necator*. In this study, the full-length complementary deoxyribonucleic acid (cDNA) sequence of this gene was cloned from Baihe-35-1 using a 5′ and 3′ rapid amplification of complementary deoxyribonucleic acid (cDNA) ends (RACE) strategy and designated as *V. pseudoreticulata bZIP* (*VpbZIP-like*). We further analyzed its expression profile in response to *E. necator* and signaling molecules. In addition, the successful expression of *VpbZIP-like* in fusion with GST of *Schistosoma japonicur* in *E. coli* was also demonstrated.

MATERIALS AND METHODS

Two grapevine genotypes tested in this study were maintained in the grape germplasm resources orchard of Northwest A and F University, Yangling, China which included two *E. necator*-resistant and E. *necator*-susceptible grapevine genotypes. The *E. necator* resistant grapevine genotype was *Vitis pseudoreticulata* acc. Baihe-35-1. The susceptible genotype was *V. pseudoreticulata* acc. Hunan-1.

When shoots of the grapevine vines were 30 to 40 cm in length, the third to sixth fully expanded young leaves below the apex were selected for *E. necator* and signaling molecule treatments (Li et al., 2010). The inoculation of *E. necator* on leaves were conducted according to the protocol previously described by Wang et al. (1995), and was repeated three times on three independent plants for each genotypes.

Leaf selection and treatment with defense signaling molecules SA, MeJA and ethephon (Eth) for three genotypes were performed as described (Li et al., 2010).

RNA isolation and purification

Total ribonucleic acid (RNA) was isolated using improved SDS/phenol method as described (Zhang et al., 2003). Genomic DNA was removed by DNase I (Promega, Madison, WI, USA). The concentration of total RNA was measured with an ultraviolet spectrophotometer at 260 nm. RNA purity was checked by determining the A_{260}/A_{280} ratio, and RNA integrity was examined by 1% agarose gel electrophoresis.

Cloning of *VpbZIP-like* and sequence analyses

In order to obtain the full-length cDNA of *VpbZIP-like*, 5'RACE and 3'RACE polymerase chain reaction (PCR) amplification were carried out according to the manufacturer's instructions (BD SMART™ RACE cDNA Amplification Kit) (Clontech, USA). The gene specific primers GSP1: 5′- TCT CCC ATA ACC ATT GAT TTC GCC GCA C-3′ for 5′RACE and GSP2:5′-GCC TAA GAA CGA TGC TTT GCT GGC TCA G-3′ for 3′RACE were designed based on the obtained partial sequence (Wang, 2004).

The resulting PCR products were cloned into the pGEM-T Easy Vector (Promega) and sequenced by TaKaRa Biotechnology (Dalian, China). The similarity analysis of nucleotide and amino acid sequences was carried out by using BLAST in NCBI (http://www.ncbi.nlm.nih.gov/blast).

Quantitative real-time PCR and semi-quantitative RT-PCR reaction

First-strand cDNA was synthesized from 1 µg of DNase treated total RNA using PrimeScript™ RTase Kit (TaKaRa Biotechnology). qRT-PCR was used to determine expression post PM inoculation and plant signal molecules treatments. Data analysis was conducted as described by Li et al. (2010). Tissues of stem, tendril, flowers (5 days after flowering), leaves and fruit (50 days after flowering) were collected from Baihe-35-1 and used for tissue-specific expression analysis using semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) technique. Grapevine *Actin1* (GenBank Acc no. AY680701) was amplified as internal control. Specific primers *VpbZIP-like* F: 5′- AAG CGG CTG AGA CAA CAA ACA C-3′ and R: 5′-GCT AAG TCT CAC TCC GAA CAG GC-3′ for *VpbZIP-like* and *Actin1* F: 5′- GAT TCT GGT GAT GGT GTG AGT- 3′ and R: 5′- GAC AAT TTC CCG TTC AGC AGT – 3′ for *Vpactin* was used for expression analysis. The RT-PCR products from each tissue were subject to electrophoresis on 1.8% agarose gel. The experiment was repeated three times with the similar results.

Construction of *VpbZIP-like* gene in the expression vector pGEX-4T-1

PGEX-4T-1/VpbZIP-like was transformed into the *E. coli* BL21 (DE3) strain. The positive clone was cultured in 5 ml Luria–Bertani (LB) liquid medium containing both ampicillin (100 mg/ml) and chloramphenicol (60 mg/ml), grown overnight at 37°C on a flat rotating incubator, then transferred to fresh medium (with both of the antibiotics) and incubation was continued. At the logarithmic phase (OD_{600} of 0.4 to 0.6), the bacterial culture was combined with isopropyl-b-D-thiogalactopyranoside (IPTG) at a final concentration of 0.1 mM, and then grown for additional 4 h at 37°C with shaking at 200 rpm. The recombinant protein was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Zhou et al., 2007).

RESULTS

Isolation and sequence analyses of *VpbZIP-like*

5′ RACE and 3′ RACE were employed to obtain the full-length cDNA sequence of *bZIP* gene. A band of 350 bp for 5′ RACE and 1500 bp for 3′ RACE were obtained by RACE strategy with primer GSP1 and GSP2, respectively. The full-length cDNA of 1583 bp was produced because of 272 bp overlapping sequences of 5′ and 3′ end fragments, which is designated as *VpbZIP-like*. *VpbZIP-like* contains a 1083 bp ORF, a 159 bp 5′ untranslated region (UTR) and a 365bp 3′ UTR that includes the putative polyadenylation signal AATAAA at position 1030 nt and a polyA tail (Figure 1). The translated sequence is 360 residues long with a molecular weight of 38.662 kDa, including a DUF1664 conserved domain. Multiple alignments of predicted amino acid sequences of *VpbZIP*-like and other bZIP

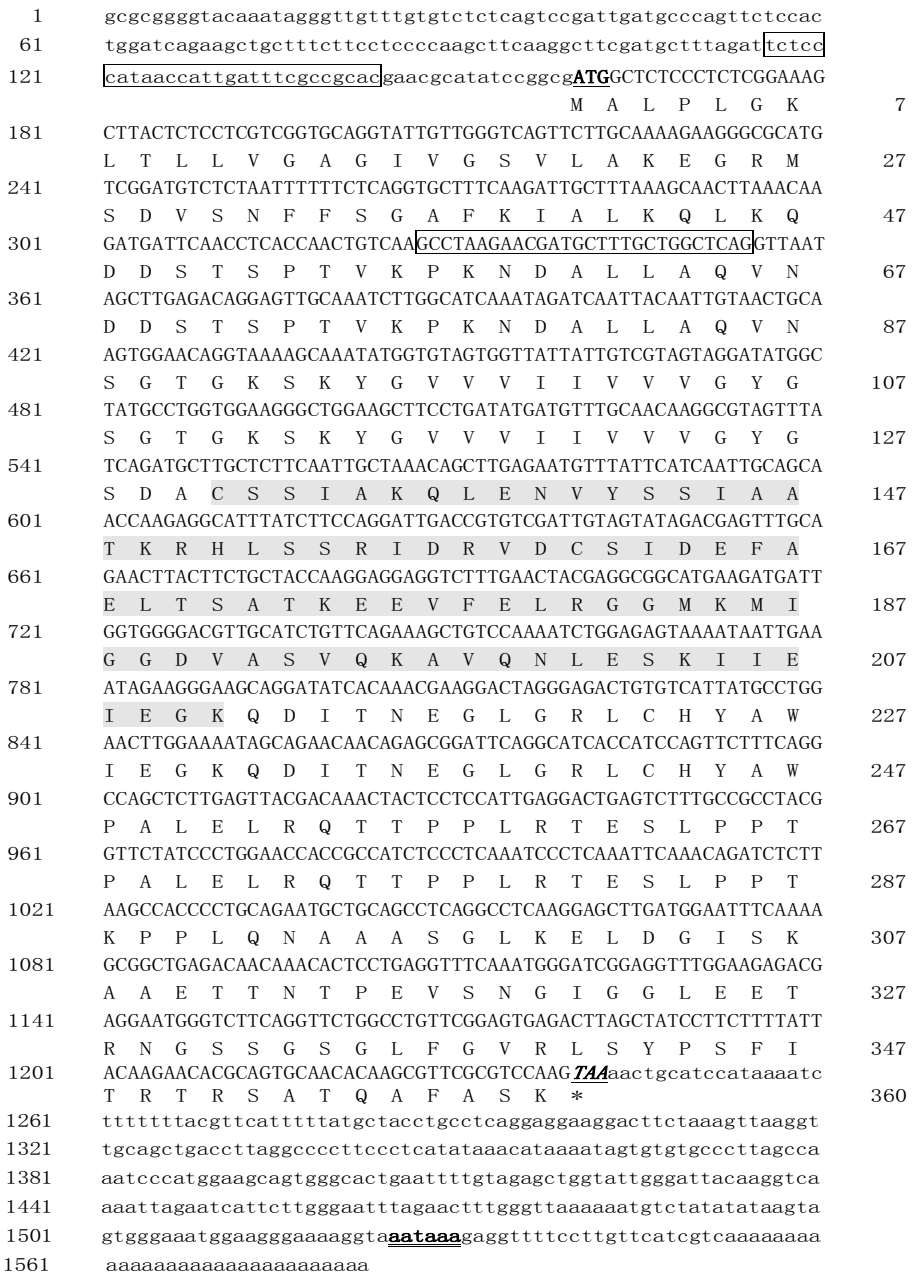

Figure 1. The full length cDNA sequence and deduced amino acid sequence of *VpbZIP-like*. Nucleotide positions are given on the left side of the sequence in the 5′ to 3′ orientation. The start codon ATG is underlined and the stop codon TAA is in italics and underlined. The deduced amino acid sequence is shown beneath the nucleotide sequence and the amino acids are numbered on the right side of the sequence, the DUF1664 conserved domain is grey-shaded. The sequences in the box are GSP primers. The polyadenylation signal AATAAA is the double-underlined sequences. This sequence has been deposited in GenBank (Accession No. HQ284196).

proteins indicated that *VpbZIP*-like shares an overall 46 to 69.8% sequence similar with *Glycine max bZIP55* (*GmbZIP55*:ABI34628.1), *Glycine max bZIP56* (*GmbZIP56*:ABI34646.1) and *Ricinus communis* DNA binding protein (*RcbZIP*:XP_002530014.1) (Figure 2).

Induction of the *VpbZIP-like* gene in leaves by biotic and abiotic treatments

The expression of *VpbZIP-like* was induced 12 post inoculation (hpi) by *Uncinula necator* but transiently

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VpbZIP      MALPLG......KLTLLVGAGIVGSVLAKEGRMSDVSNFFSGAFKIALKQLK.QDDSTSPTVKPKNDALL      63
GmbZIP55    MALSLG......KLFILVGAGIAGSVIAKEGRLPDVSGLASGAFKVVLRQLKSDDPAPTVKKQPHNDALL      64
GmbZIP56    MALSLG......KLVILVSAGIAGSVIAKEGRLPDVSGLASGAFKVVLRQLKSDDPAPTVKKQPHNDALL      64
RcbZIP      MALPLG......KLTVLVGAGILGSVLAKEGRLPSVSDFVSGAFKIAFKQIKPQDSNSSSKSKPIDASLI      64

VpbZIP      AQVNSLRQELQILASNRSITIVTASGTGKSKYGVVVIIVVVG...YGYAWWKGWKLPDMMFATRRSLSDA     130
GmbZIP55    AQVNSLRQELQLLARDRSITIVNASGTGGRKYATVIVIVVVG...YGYVWWKGWKLPDLMFATRRGLSDA     131
GmbZIP56    AQVNSLRQELQLLARDRSITIVNASGTGGRKYITVIVIVVVG...YGYVWWKGWKLPDLMFATRRGLSDA     131
RcbZIP      AQVNSLQQELQLLASNRPITIVTASGTGAGKYGTIVLIVVVG...YGYVWWKGWKLPDMMFATRRSLSDA     131

VpbZIP      CSSIAKQLENVYSSIAATKRHLSSRIDRVDCSIDEFAELTSATKEE                             176
GmbZIP55    CTSIGNQMGKLYESIGDTKKKLSARINGLDKNLD............                             165
GmbZIP56    CTSIGNQMGKLYESIGDTKKKLSARINGLDKNLEECAAITESTREDD                             177
RcbZIP      CTSIAQQLENVYGSIRSTRRELSSNIEHMDATLDQVAALTANTREK                              177
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Figure 2. Multiple alignments of predicted amino acid sequences of *VpbZIP* and reported *bZIP* proteins from *GmbZIP55* (GenBank acc no. ABI34628.1), *GmbZIP56* (GenBank acc no. ABI34646.1) and *RcbZIP* (XP_002530014.1) respectively, using DNAMAN the multiple alignment programme. Gaps to optimize alignments are designated by dots (⋯). The consensus amino acid identity among all organisms is black color. The amino acids are numbered on the right side of the sequence.

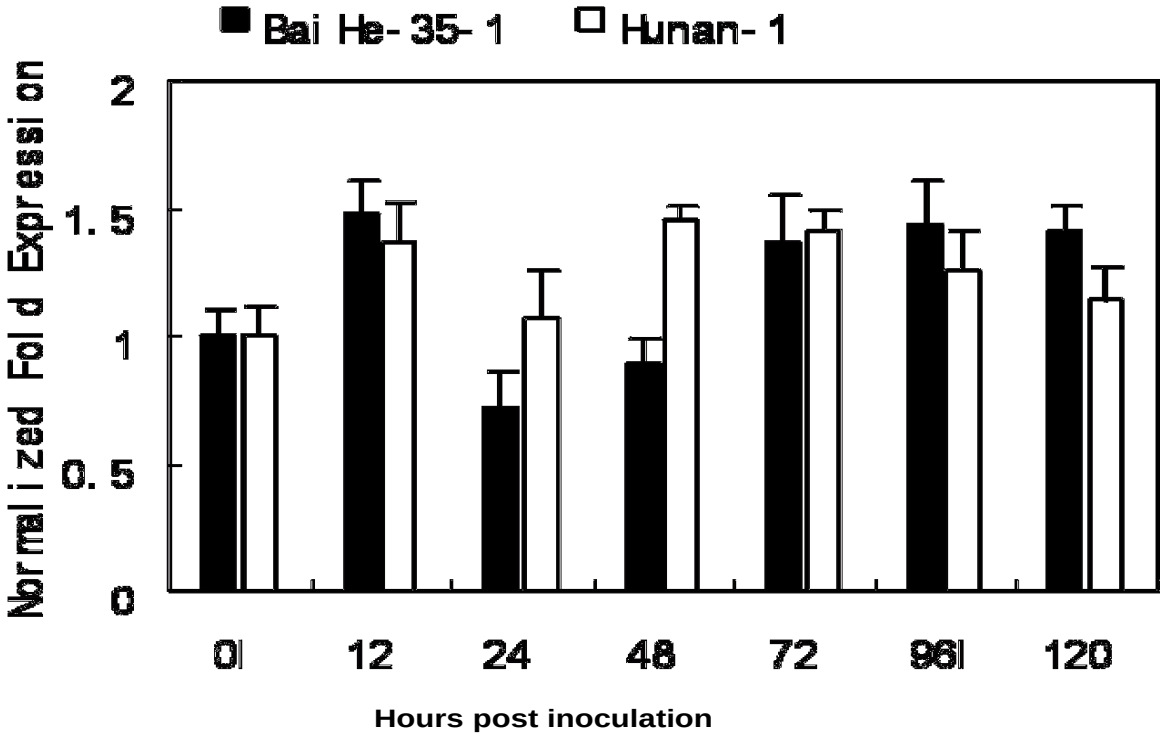

Figure 3. Expression profiles of *VpbZIP-like* following inoculation with *E. necator* in two grapevine genotypes.

decreased, and then increased in the two genotypes. But its expression was lower in highly resistant genotype Baihe-35-1 than in susceptible genotype Hunan-1 at 24, 48 at 72 hpi (Figure 3). We further tested whether the expression of this gene also response to plant signaling molecules. Results show that expression was induced by SA, MeJA and Eth almost in the two genotypes (Figure 4). The transcripts of *VpbZIP-like* in the highly resistant genotype Baihe-35-1 had the same trend as the susceptible genotype Hunan-1 but, the susceptible genotype Hunan-1 showed higher expression of *VpbZIP-like* than the highly resistant genotype Baihe-35-1 after exogenous application of MeJA, SA and Eth (Figure 4). In Hunan-1, expression of *VpbZIP-like* was induced by three signaling molecules SA, MeJA and Eth; the maximum induction presented 6 h post three signaling molecules

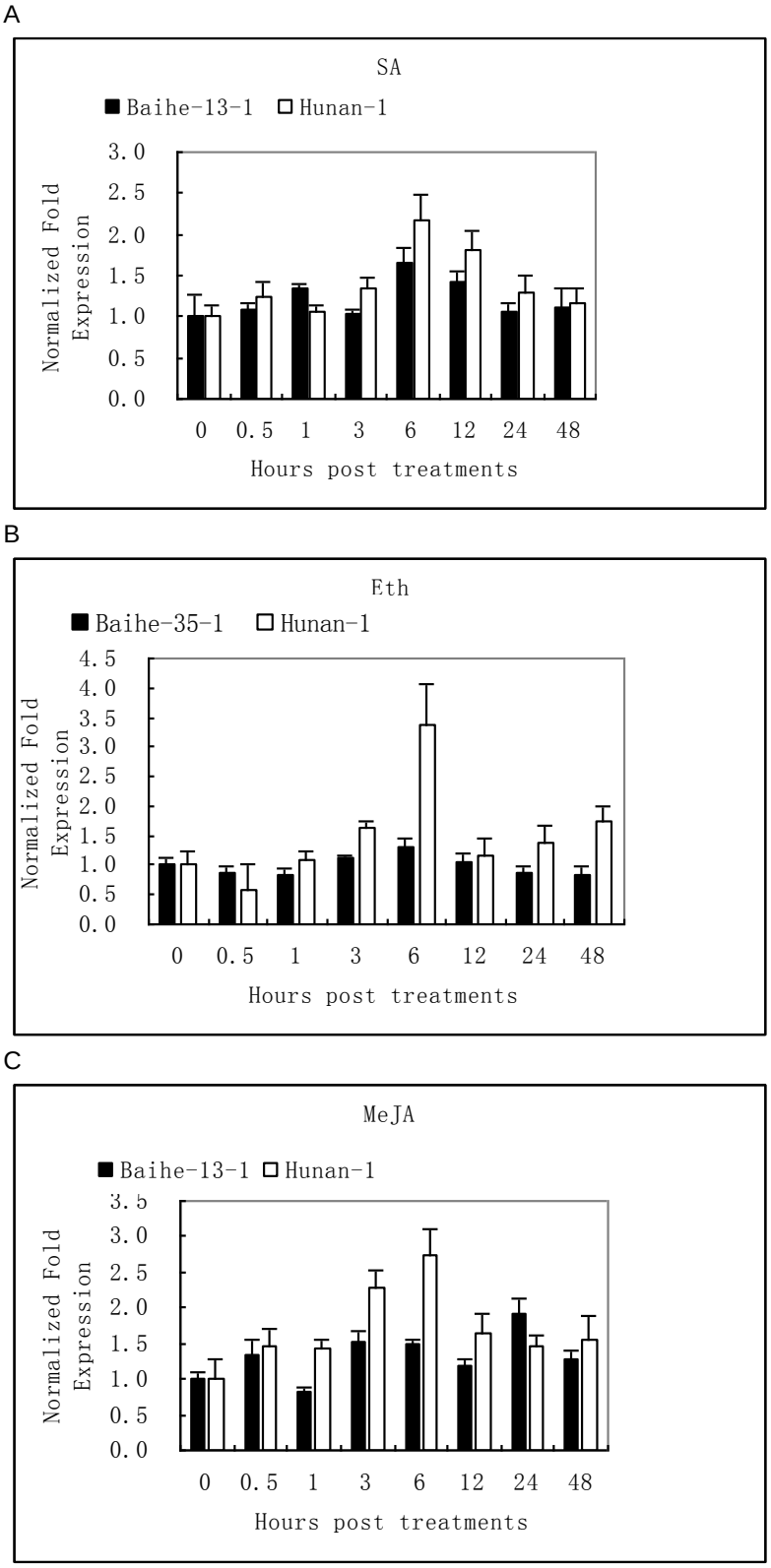

Figure 4. Expression profiles of *VpbZIP-like* in response to plant defense signaling molecules. Expression of *VpbZIP-like* was the response to SA (a), MeJA (b) and Eth (c) treatments in the leaves of Chinese wild *Vits pseudoreticulata* acc. Baihe-35-1, and Chinese wild *V. pseudoreticulata* acc. Hunan-1.

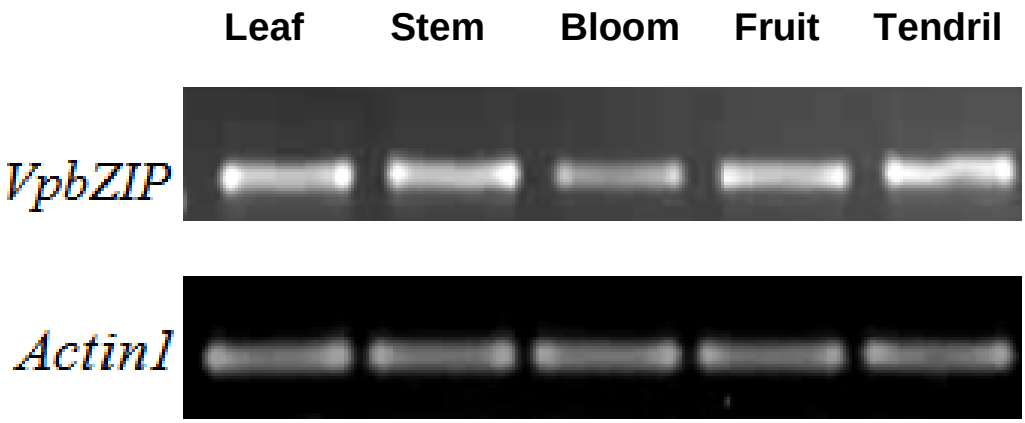

Figure 5. Expression pattern analysis of *VpbZIP-like* in different organs by semi-quantitative RT-PCR. Total RNAs were from leaf, stem, tendril, flower, and fruit of *Vitis pseudoreticulata* acc. Baihe-35-1. The expression level of a *Actin1* gene was used as an internal control.

treatment (Figure 4). In contrast, the transcripts of *VpbZIP-like* in the highly resistant genotype Baihe-35-1 were observed to change appreciably over the time course of the experiment. Tissue specific expression of *VpbZIP-like* gene was analyzed by RT-PCR. Results reveal that the expression of *VpbZIP-like* is in lower level in flower than in leaf, stem, tendril and fruit (Figure 5).

Expression of the GST-VpbZIP-like fusion protein in *E. coli*

The recombinant plasmid pGEX-4T-1-*VpbZIP-like* was first confirmed by digestion of *Eco*RI I and *Xho*I, and then Using cDNA from RT-PCR as a template, the *VpbZIP-like* CDS was amplified with primer F1 (5′-GCTGAATTCATGGCTCTCCCTCTCGG- 3′) and primer R1 (5′ - GCGCTCGAGTTACTTGGA CGCGAAC- 3′). The sequences underlined are the recognition sites of the restriction enzymes *Eco*RI and *Xho*I. The reaction was performed at 98°C for 30 s, followed by 35 cycles at 94°C for 30 s, at 58°C for 30 s and at 72°C for 2 min, followed by a final extension at 72°C for 10 min. The amplified PCR products were ligated into the pGEM-T easy vector. The positive plasmid was digested by *Eco*RI and *Xho*I, and ligated with the pGEX-4T-1 expression vector using DNA ligation Kit (TaKaRa Biotechnology). The recombinant vector pGEX-4T-1 was identified by restriction digestion and the *VpbZIP-like* insert was verified by sequencing. The correct recombinant prokaryotic expression vector was named as pGEX-4T-1/VpbZIP-like.

Functional expression of pGEX-4T-1/VpbZIP-like recombinant protein in *E. coli*

The recombinant plasmid pGEX-4T-1-*VpbZIP-like* was first confirmed by digestion of *Eco*RI I and *Xho*I, and then confirmed by sequencing. The result shows that the *VpbZIP-like* gene was correctly linked to the pGEX-4T-1. The *E. coli* BL21 cells harboring recombinant constructs was induced by 0.1 mM IPTG for 4 h at 37°C. A specific protein about 64 kD was observed in SDS-PAGE gel (Figure 6). It is identical to the predicted GST-VpbZIP-like fusion protein (ca.64 KDa) which was the molecular weight of *VpbZIP-like* (ca. 38 kDa) calculated from deduced amino acid sequence plus that of GST (ca.26 kDa). So the VpbZIP-like was successfully expressed in *E. coli* BL21.

DISCUSSION

The bZIP transcription factors belong to a large protein family. In this study, multiple alignments indicate that the novel bZIP-like share an overall 46 to 69.8% sequence similar with other bZIP proteins, but no bZIP conserved region was found, therefore, we designated the bZIP-like gene from wild Chinese *V. pseudoreticulata* as *VpbZIP-like*. A big part of DUF1664 conserved region was identified (Figure 1) by the analysis of amino acid sequences using Blast which indicated that this protein also belongs to DUF1664 superfamily. The region featured in this family is approximately 100 amino acids long, however, the function of other hypothetical plant DUF1664 protein members deposited in NCBI are still unknown so far.

The response to pathogenic attack requires large-scale transcriptional reprogramming of genes, and most of these processes have been proven to be induced by pathogens (Pandey and Somssich, 2009). In a previous report, there was a significant increase in the abundance of transcripts encoding enhanced disease susceptibility1, mitogen-activated protein kinase, WRKY, pathogenesis-related1, pathogenesis-related10, and stilbene synthase

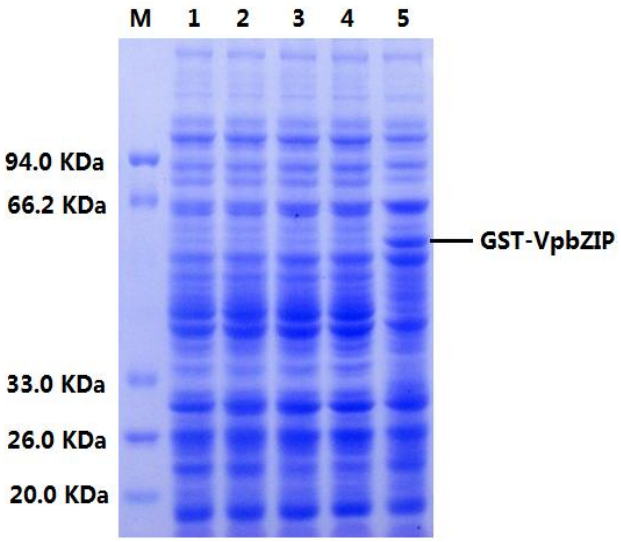

Figure 6. Expression of the GST-VpbZIP-like fusion protein in *E. coli*. M, protein marker; lanes 1, and 2, the *E. coli* BL21 cells harbored pGEX-4T-1 cultured by 0.1 mmol•L-1 IPTG for 4 h; lanes 3 and 4, the expression of the recombinant E. coli cells cultured without IPTG for 4 h; lane 5, the expression of the recombinant *E. coli* cells cultured with 0.1 mmol•L-1 IPTG for 4 h. The line indicates the fusion protein's bind.

in PM-infected *V. vinifera*, suggesting an induction of the basal defense response (Fung et al., 2008). Besides, several bZIP transcription factors are known to be involved in plant defense against microbial pathogens (Uno et al., 2000; Kang et al., 2002; Jakoby et al., 2002; Després et al., 2003; Thurow et al., 2005; Zhang et al., 2008; Guo et al., 2011). In agreement with the aforementioned result, the expression of *VpbZIP-like* was transiently induced in early stage by PM infection in leaves of the two grapevine genotypes tested (Figure 3).

Plant defense response to microbial attack is regulated through a complex network of signaling pathways thatinvolve SA, JA and Eth. These molecular signaling are typically considered to regulate different signal transduction pathways in plant disease resistance (Glazebrook, 2005).

The transcripts of *VpbZIP-like* in the highly resistant genotype Baihe-35-1 had the same trend as the susceptible genotype Hunan-1 but the susceptible genotype Hunan-1 showed higher expression of *VpbZIP-like* than the highly resistant genotype Baihe-35-1 after exogenous application of MeJA, SA, and Eth (Figure 4). These results indicate that *VpbZIP-like* may function as transcription factors that are essential components of the defense signaling pathways, since they regulate the expression of defense-related marker genes.

Conclusion

A novel *VpbZIP-like* gene was isolated from wild Chinese *Vitis*, its expression was induced by PM infection and plant molecular signaling, indicated that *VpbZIP-like* is potential gene for resistance to PM in grapevine. Further study will focus on how *VpbZIP-like* function as a transcription factors in grapevine PM resistance from transcription level and protein level.

ACKNOWLEDGEMENTS

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Abbreviations: qRT-PCR, Quantitative reverse transcriptase-polymerase chain reaction; **semi-quantitative RT-PCR,** semi-quantitative reverse transcription-polymerase chain reaction; ***VpbZIP-like*****,** *V. pseudoreticulata bZIP*; **SA,** salicylic acid; **MeJA,** methyl jasmonate; **Eth,** ethephon; **hpi,** hours post inoculation; **dpi,** days post inoculation; **hpt,** hours post treatment; **daf,** days after flowering; **RACE,** rapid amplification of complementary deoxyribonucleic acid (cDNA) ends; **DDRT-PCR,** messenger ribonucleic acid (mRNA) differential display reverse transcription-PCR; **SDS–PAGE,** sodium dodecyl sulfate polyacrylamide gel electrophoresis; **IPTG,** isopropyl-b-D-thiogalactopyranoside.

REFERENCES

Després C, Chubak C, Rochon A, Clark R, Bethune T, Desveaux D, Fobert P R (2003). The *Arabidopsis* NPR1 disease resistance protein is a novel cofactor that confers redox regulation of DNA binding activity to the basic domain/leucine zipper transcription factor TGA1. Plant Cell Online, 15: 2181.

Fung RWM, Gonzalo M, Fekete C, Kovacs LG, He Y, Marsh E, Mcintyre LM, Schachtman DP, Qiu WP (2008). Powdery mildew induces defense-oriented reprogramming of the transcriptome in a susceptible but not in a resistant grapevine. Plant Physiol., 146: 236-249.

Glazebrook J (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Ann. Rev. Phytopathol., 43: 205-227.

Guo M, Chen Y, Du Y, Dong Y, Guo W, Zhai S, Zhang H, Dong S, Zhang Z, Wang Y (2011). The bZIP transcription factor MoAP1 mediates the oxidative stress response and is critical for pathogenicity of the rice blast fungus *Magnaporthe oryzae*. PLoS Pathogens, 7.

Jakoby M, Weisshaar B, Dröge-Laser W, Vicente-Carbajosa J, Tiedemann J, Kroj T, Parcy F (2002). bZIP transcription factors in *Arabidopsis*. Trends Plant Sci., 7: 106-111.

Kang J, Choi H, Im M, Kim S Y (2002). *Arabidopsis* basic leucine zipper proteins that mediate stress-responsive abscisic acid signaling. Plant Cell Online, 14: 343.

Li H, Xu Y, Xiao Y, Zhu Z G, Xie X Q, Zhao H Q, Wang Y J (2010). Expression and functional analysis of two genes encoding transcription factors, *VpWRKY1* and *VpWRKY2*, isolated from Chinese wild *Vitis pseudoreticulata*. Planta, 232:1325-1337.

Pandey SP, Somssich IE (2009). The role of WRKY transcription factors in plant immunity. Plant Physiol., 150:1648-1655.

Thurow C, Schiermeyer A, Krawczyk S, Butterbrodt T, Nickolov K, Gatz C (2005). Tobacco bZIP transcription factor TGA2.2 and related factor TGA2.1 have distinct roles in plant defense responses and plant development. Plant J., 44: 100-113.

Uno Y, Furihata T, Abe H, Yoshida R, Shinozaki K, Yamaguchi-Shinozaki K (2000). *Arabidopsis* basic leucine zipper transcription factors involved in an abscisic acid-dependent signal transduction pathway under drought and high-salinity conditions. Proc. Nat. Acad. Sci., 97: 11632.

Wang Y, Liu Y, He P, Chen J, Lamikanra O, Lu J (1995). Evaluation of foliar resistance to *Uncinula necator* in Chinese wild *Vitis* species. Vitis, 34: 159-164.

Zhang JJ, Wang YJ, Wang XP (2003). An improved method for rapidly extracting total RNA from *Vitis*. Journal of Fruit Sci. Chin. Eng. abst., 20:178-181

Zhang Y, Zhang G, Xia N, Wang X J, Huang L L, Kang Z S (2008). Cloning and characterization of a bZIP transcription factor gene in wheat and its expression in response to stripe rust pathogen infection and abiotic stresses. Physiol. Mol. Plant Pathol., 73: 88-94.

Zhou B J, Wang X P, Wang Y J (2007). cDNA cloning, expression, protein purification, and characterization of a novel glyoxal oxidase related gene from *Vitis pseudoreticulata*. Biologia Plantarum, 51: 458-466.