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Coordinated and Distinct Functions of Velvet Proteins in *Fusarium verticillioides*

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Velvet-domain-containing proteins are broadly distributed within the fungal kingdom. In the corn pathogen *Fusarium verticillioides*, previous studies showed that the velvet protein *F. verticillioides* VE1 (FvVE1) is critical for morphological development, colony hydrophobicity, toxin production, and pathogenicity. In this study, tandem affinity purification of FvVE1 revealed that FvVE1 can form a complex with the velvet proteins *F. verticillioides* VelB (FvVelB) and FvVelC. Phenotypic characterization of gene knockout mutants showed that, in the case of FvVE1, FvVelB regulated conidial size, hyphal hydrophobicity, fumonisin production, and oxidant resistance, while FvVelC was dispensable for these biological processes. Comparative transcriptional analysis of eight genes involved in the ROS (reactive oxygen species) removal system revealed that both FvVE1 and FvVelB positively regulated the transcription of a catalase-encoding gene, *F. verticillioides* CAT2 (FvCAT2). Deletion of FvCAT2 resulted in reduced oxidant resistance, providing further explanation of the regulation of oxidant resistance by velvet proteins in the fungal kingdom.

The filamentous fungus *Fusarium verticillioides* (synonym *Fusarium moniliforme*, teleomorph *Gibberella moniliformis*, synonym *Gibberella fujikuroi* mating population A) is one of the most common corn pathogens. Infection by this fungus can cause stalk and ear rot on corn (1). This fungus can produce many toxic metabolites, such as fumonisins, fusarins, and bikaverin. Fumonisins, including FB₁ (fumonisin B₁, the most abundant fumonisin in corn), FB₂, FB₃, and FB₄, are among the most important mycotoxins in grains and are able to inhibit ceramide synthase (2, 3). Fumonisins can cause neural tube and craniofacial defects in mouse embryo culture (4). A gene cluster comprised of 17 genes is involved in fumonisin biosynthesis in *F. verticillioides* (5–7). Among these genes, disruption of *FUM1*, *FUM6*, and *FUM8* abolishes fumonisin production (5, 7).

A velvet-domain-containing protein, *F. verticillioides* VE1 (FvVE1), regulates the biosynthesis of fumonisins in *F. verticillioides* (8). FvVE1 is the orthologue of *Aspergillus nidulans* VeA, the first characterized velvet-domain-containing protein (9). VeA contains one velvet domain, one nuclear localization signal sequence (NLS) at the N terminus, one nuclear export signal (NES) at the end of the velvet domain, and one PEST domain at the C terminus (10, 11). Velvet-domain-containing proteins are widely conserved in fungal species, particularly in ascomycetes (10, 12, 13). In addition to VeA, filamentous fungi also possess other velvet-domain-containing proteins, including VelB, VelC, and VosA. VelB in *A. nidulans* has one noncontinuous velvet domain, in which an insertion of 99 amino acids was found, but has no nuclear localization signal sequence. It shuttles between the cytoplasm and nucleus, dependent on VeA (14). VelC in *A. nidulans* contains a single velvet domain at its C terminus. VosA in *A. nidulans* contains a velvet domain and TAD (transcription activation domain) with a NLS between them (15–17). Recently, a DNA binding motif was found in the velvet domain of VosA (17). All of these velvet-domain-containing proteins form the velvet family (10, 18, 19). Previous studies demonstrated that VelB can interact with VeA and VosA (14, 16), and VelC can interact with VosA in *A. nidulans* (20). Unlike the case with *A. nidulans*, VosA is not present in *Fusarium oxysporum*, in which all three velvet proteins, VeA, VelB, and VelC, can interact with each other (21). These studies indicate that the composition of velvet protein complex varies among fungi. Further investigation of velvet protein complexes in other fungi will be helpful in fully understanding the composition, function, and evolution of velvet protein complexes in the fungal kingdom.

Velvet proteins are involved in many important biological processes. First, some velvet proteins in pathogenic fungi have been found to be critical for virulence. Deletion of VeA orthologue-encoding genes reduced the virulence of *F. verticillioides* in corn and the virulence of *Fusarium graminearum* in wheat (8, 22). Additionally, the absence of VeA1 in *Histoplasma capsulatum* and deletion of vea and velB orthologues in *F. oxysporum* also decreased virulence in immunosuppressed mice (21, 23). Second, velvet proteins regulate sexual development and secondary metabolism. Deletion of vea orthologue completely impaired the formation of sexual fruiting bodies or resistant structures and the production of mycotoxins in *A. nidulans*, *Aspergillus flavus*, *Aspergillus parasiticus*, *F. verticillioides*, *Fusarium fujikuroi*, *Penicillium chrysogenum*, and *Cochliobolus heterostrophus* (24–31). Similar functions of VelB were also found in *F. oxysporum* (21). Third, VeA orthologues in *Fusarium* spp. are also critical for controlling conidial size and hyphal hydrophobicity (21, 22, 28), and the similar functions were also found in VelB in *F. oxysporum* (21).
Fourth, velvet proteins are required for oxidant resistance in \textit{A. nidulans}. Deletion of velvet-protein-coding genes, including \textit{veA}, \textit{velB}, and \textit{vosA}, made conidia hypersensitive to H$_2$O$_2$ in \textit{A. nidulans} (16). The \textit{velB} deletion mutants and \textit{vosA} deletion mutants were more sensitive to H$_2$O$_2$ than \textit{veA} mutants in \textit{A. nidulans} (16). The regulatory mechanism of velvet proteins in oxidant resistance has been investigated for \textit{Cochliobolus heterostrophus}, which demonstrated that the \textit{VeA} orthologue ChVe1 positively regulates expression of the catalase-encoding gene \textit{C. heterostrophus CAT3} (\textit{ChCAT3}) but not other catalase-encoding genes, including \textit{ChCAT1} and \textit{ChCAT2} (30).

In this study, we found, using a tandem-affinity-purification-based approach, that FvVe1, the VelB orthologue \textit{F. verticillioides} \textit{VelB} (\textit{FvVelB}), and the VelC orthologue \textit{F. verticillioides} \textit{VelC} (\textit{FvVelC}) form a complex in \textit{F. verticillioides}. By phenotypic analysis of deletion mutants and transcriptional analysis, we determined the roles of the \textit{FvVe1} and \textit{FvVelC} proteins in the biosynthesis of fumonisins, conidial size control, and hyphal hydropobicity. Furthermore, we revealed an association between velvet proteins and the oxidative stress response in \textit{F. verticillioides} and showed a possible regulatory mechanism of velvet proteins for oxidative stress resistance.

**MATERIALS AND METHODS**

**Strains and media.** All \textit{F. verticillioides} strains used in this study are listed in Table 1. YPG medium (0.3% yeast extract, 1% peptone, and 2% glucose), PDA medium (20% potato, 2% glucose, and 1.5% agar), and YPGA medium (0.3% yeast extract, 1% peptone, 2% glucose, and 1.5% agar) were used for characterization of vegetative growth and asexual development. The regeneration medium (yeast extract, 0.1%; casein hydrolysate, 0.1%; sucrose, 0.8 M; agar, 1.6%) was used for protoplast transformation. All cultures were grown at 28°C.

**Target gene knockout.** Target gene replacement was performed as detailed in Fig. S1 to S4 in the supplemental material, using the split-marker approach and homologous gene replacement method (32, 33). The gene replacement cassette containing \textit{hph} (hygromycin phosphotransferase gene) was introduced into the wild-type strain by protoplast transformation. PCR analyses for \textit{hph} and the target gene were used to confirm the knockout strains (primers are listed in Table S1).

**Construction of complemented strains.** The plasmid pKN, containing the neomycin phosphotransferase gene, which confers G418 resistance, was used to construct the vectors for complementation. A DNA fragment containing the corresponding gene and its native promoter and terminator regions was inserted into the multiple cloning sites of the plasmid. The constructed vectors were then introduced into the deletion mutant by protoplast transformation, and fungal transformants were selected on regeneration medium containing G418 (80 μg/ml).

**Construction of the strain expressing FvVE1-Myc-His.** In order to identify proteins associated with FvVe1 in \textit{F. verticillioides} in vivo, a plasmid in which a 5×c-Myc–6×His coding sequence was added as a tag to the 3’ end of the \textit{F. verticillioides} VE1 (\textit{FvVe1}) gene (see Fig. S5A in the supplemental material) was constructed and then transformed into the \textit{Fvvel} deletion mutant. The transformants were verified by Western blotting with anti-c-Myc antibody (see Fig. S5B). A complemented transformant that showed wild-type phenotypes for conidial size and hyphal hydropobicity (see Fig. S5C) was chosen for purification of FvVe1-Myc-His.

**Purification of FvVe1-Myc-His proteins.** The \textit{Fvvel} \textit{[FvVE1-MYC-HIS]} complemented strain and the wild-type strain (negative control) were cultured for approximately 17 h in constant darkness in YPG liquid medium. The purification procedure followed the method previously reported (34). Fractions containing purified FvVe1-Myc-His proteins were immunoprecipitated by adding 40 μl of anti-c-Myc antibody-attached beads (c-Myc [9E10] agarose conjugate sc-40 AC; Santa Cruz Biotechnology). The precipitates of FvVe1-Myc-His samples were analyzed by SDS-PAGE (4% to 15%), which was subsequently silver stained following the manufacturer’s instructions (ProteoSilver Plus; Sigma). Specific bands were excised and subjected to tryptic digestion and liquid chromatography-tandem mass spectrometry (LC-MS/MS).

**HPLC-MS analysis of fumonisins.** Extracts from cultures of the wild type, \textit{Fvvel}, \textit{FvvelB}, and \textit{FvvelC} deletion mutants, and \textit{FvvelB}[\textit{FvvelB}] complemented strains grown on cracked corn medium in constant darkness for 11 days were analyzed by high-performance liquid chromatography-mass spectrometry (HPLC-MS). Fumonisins were first extracted according to the acetonitrile method described by Rotthaus et al. (35). HPLC-MS analysis of fumonisins was performed as described previously by Silva et al. (36) with some modifications.

Liquid chromatography separation was performed on an Agilent 1200 Series system (Agilent, USA) using an Agilent Zorbax Extend-C18 1.8-μm, 2.1- by 50-mm column. With a total flow rate of 0.5 ml/min, mobile phase A consisted of water with 0.3% formic acid, and mobile phase B consisted of methanol with 0.5% formic acid. The gradient began with 65% mobile phase B for 4 min, changed to 95% B over 4 min, and maintained a constant level at 95% B for 7 min. The injection volume was set to 10 μl.

Mass spectra were acquired using an Agilent 6520 accurate-mass quadrupole time-of-flight (Q-TOF) MS system equipped with an electrospray ionization (ESI) source. All MS experiments were detected in the positive ionization mode. For Q-TOF/MS conditions, fragmentor and capillary voltages were kept at 280 and 3,500 V, respectively. Nitrogen was supplied as the nebulizing and drying gas. The temperature of the drying gas was set at 300°C. The flow rate of the drying gas and the pressure of the nebulizer were 13.0 liters/min and 30 lb/in², respectively. Full-scan spectra were acquired over a scan range of m/z 300 to 800. Fumonisins were identified by comparison to an FB1 standard, as well as the previously described molecular weight and fragmentation spectrum (35, 36).

**H$_2$O$_2$ and menadione sensitivity test.** Conidia developed on YPG medium were harvested into distilled water, and the final concentration of conidial suspensions was then adjusted to 10$^5$ conidia/ml. Three microliters of conidial suspension was inoculated on YPGA medium with or without 25 μg/ml menadione or 0.27 mM H$_2$O$_2$. Colony diameters were recorded after 72 h of incubation at 28°C in the dark. The relative inhibition rate of each strain was calculated as follows: 100 × (mean colony diameter on control plates – mean colony diameter on oxidant-added plates)/mean colony diameter on control plates.

**RNA extraction and qRT-PCR analysis.** Mycelia were harvested and immediately frozen and ground into fine powder in liquid nitrogen. RNA extraction, cDNA synthesis, and quantitative reverse transcription-PCR (qRT-PCR) analysis exactly followed methods previously described (37, 38). Each cDNA sample was analyzed in triplicate, and the average threshold.

| TABLE 1 Strains used in this study |
|-----------------------------------|
| Species   | Strain name or description | Genotype | Source |
|-----------|---------------------------|----------|--------|
| \textit{F. verticillioides} | WT | Wild type | This study |
| Fvvel mutant | $\Delta$fvvel | This study |
| Fvvel[\textit{FvVE1}] mutant | $\Delta$fvvel:fvvel-\textit{Smyc-his} | This study |
| FvvelB mutant | $\Delta$fvvelB | This study |
| FvvelB[\textit{FvVELB}] mutant | $\Delta$fvvelB:fvvelB | This study |
| FvvelC mutant | $\Delta$fvvelC | This study |
| Fvac2 mutant | $\Delta$fvac2 | This study |
| Fvac2[\textit{FvCAT2}] mutant | $\Delta$fvac2-2:fvac2-2 | This study |
| \textit{N. crassa} | FGSC 4200 | Wild type | FGSC |
| FGSC 11532 | $\Delta$cat-2 | FGSC |
old cycle was calculated. Relative expression levels were calculated using the $2^{-\Delta \Delta CT}$ method (39). The results were normalized to the expression level of $\beta$-tubulin. The primer pairs used for the qRT-PCR assay are shown in Table S2 in the supplemental material.

**RESULTS**

**Identification of FvVE1-associated proteins.** In order to identify proteins associated with FvVE1 in *F. verticillioides* in vivo, proteins from the strain expressing 5×c-Myc–6×His-tagged FvVE1 were extracted, enriched by using a nickel column, precipitated by an anti-c-Myc antibody, and isolated by electrophoresis. Three protein bands seen in the FvVE1-Myc-His strain but not in the wild-type strain were cut for protein identification by mass spectrometry analysis (Fig. 1A). Two independent experiments were carried out, and proteins that were commonly identified in both experiments were chosen for further analysis in this study. These identified proteins included all three velvet proteins encoded by the *F. verticillioides* genome. Based on previously described phylogenetic analysis (21), these velvet proteins are the VeA orthologue FvVE1 (FVEG_09521), the VelB orthologue FvVelB (FVEG_01498), and the VelC orthologue FvVelC (FVEG_05214) (Fig. 1B). In addition, three other proteins that had not previously been implicated in interactions with velvet family members were also identified. Based on sequence homology, these proteins are likely homologues of CPSF5 (cleavage and polyadenylation specificity factor 5), UreG (urease accessory protein), and yeast Hrp1p. We gave FVEG_02467 the name *F. verticillioides* Hrp1 (FvHrp1) (Fig. 1B).

**Velvet proteins regulate morphogenesis.** In order to analyze the function of each individual velvet protein, single gene knockout mutants for FvVELB and FvVELC were generated by homologous gene replacement with hygromycin as the selective marker. The phenotypes of each mutant were compared with those of the wild-type strain (Fig. 2). FvvelB and FvvelC mutants showed phenotypes similar to those of the wild-type strain, which can form abundant fluffy aerial hyphae. Second, colonies of both the FvvelB mutant and the FvvelC mutant were light brown, while colonies of the wild type were white (Fig. 2A). Third, the ratios of macroconidia (40 to 60 μm in length) to microconidia (4 to 6 μm) in both the FvvelB mutant and the FvvelC mutant were dramatically higher than in the wild type. The wild-type strains produced significantly more macroconidia than macroconidia, while the FvvelB mutant and the FvvelC mutant produced more macroconidia than microconidia. The ratios between macroconidia and microconidia were similar between the FvvelB mutant and the FvvelC mutant. The FvvelC mutant, however, showed phenotypes similar to those of the wild-type strain in colony morphology and conidiation characteristics (Fig. 2B and C). Thus, FvVE1 and FvVE1B play almost equal roles in balanced production of two different types of conidia, while FvVE1C is dispensable in this process.

Due to the absence of aerial hyphae, the hydrophobicity of the cell surface was investigated. Water droplets (30 μl each) with or without acid fuchsin were placed on the surfaces of 7-day-old colonies of wild-type, Fve1, FvvelB, FvvelC, and complemented strains grown on PDA medium. On colonies of wild-type, FvvelB, and complemented strains, the water droplets remained on the surface of the mycelium without extending or being absorbed for quite a long time (at least 1 h). In contrast, the strains carrying mutations in Fvve1 and FvvelB displayed a phenotype quite different from those of wild-type and FvvelC strains. The water droplets on the Fvvel1 and FvvelB strains were immediately spread and absorbed (Fig. 2A). Fvvel1 regulates expression of hydrophobin genes (28). Transcriptional analysis by qRT-PCR showed that compared with findings for the wild-type strain and the FvvelB[FvvelB] strain, the transcriptional levels of HYD3 and HYD4 were dramatically low in the FvvelB strain, and HYD1 and HYD2 were completely blocked by FvvelB (Fig. 2D). These data,
together with findings of our previous study (28), demonstrated that, as in the case of FvVE1, FvVelB is also critical for normal morphological development and cell surface hydrophobicity in *F. verticillioides*. Thus, similar to FvVE1, FvVelB is critical in maintaining normal hydrophobicity of cells in *F. verticillioides*.

Velvet proteins regulate fumonisin biosynthesis. FvVE1 plays a key role in regulating biosynthesis of fumonisins (8). To determine whether other members of the velvet complex also regulate fumonisin production, extracts from cultures of the wild-type, Fvve1, FvvelB, FvvelC, and FvvelB[FvVELB] strains, grown on cracked corn medium in constant darkness for 11 days, were analyzed by high-performance liquid chromatography-mass spectrometry (HPLC-MS). As shown in Fig. 3A, all fumonisins, including FB1, FB2, FB3, and FB4, were not detected in the Fvve1 mutant. In the FvvelB mutant, production of FB1, FB2, FB3, and FB4 was reduced by 70.8%, 84.2%, 87.3%, and 93.6%, respectively, compared with that of the wild type. The FvvelB[FvVELB] strain reverted to wild-type levels of fumonisin production. In the FvvelC mutant, fumonisins were at levels similar to those for the wild type.

We then examined transcriptional levels of FUM1 and FUM8 in the FvvelB, FvvelB[FvVELB], Fvve1, and wild-type strains. As shown in Fig. 3B, gene expression of FUM1 and FUM8 was dramatically reduced in the FvvelB and Fvve1 strains, but the reduction was more severe in the Fvve1 strain. Therefore, different components of the velvet complex differentially contribute to fumonisin biosynthesis. As previously shown (8), our data further verified that FvVE1 plays an essential role in the regulation of fumonisin biosynthesis, while FvVelB, although not essential for this process, also positively regulates fumonisin production. FvVelC, however, is dispensable for fumonisin biosynthesis.

Velvet proteins regulate oxidative stress tolerance. In *A. nidulans*, VelB and VosA play more important roles than VeA in tolerance against various stresses, including UV and H$_2$O$_2$, although all three velvet proteins contribute to tolerance to these stresses (16). Since ROS (reactive oxygen species) play a key role in plant-pathogen interactions, we examined the sensitivities of each velvet protein null mutant to H$_2$O$_2$ and menadione (a ROS-inducing chemical, 2-methyl-1,4-naphthoquinone, also known as vitamin K3). As shown in Fig. 4, the Fvve1 mutant was hypersensitive to H$_2$O$_2$ and menadione. On solid medium with 25 μg/ml menadione, the Fvve1 mutant could not form colonies, while the wild type and the FvvelB strain could. No significant difference in growth inhibition between the FvvelB mutant and the wild type were observed. On solid medium with 3.27 mM H$_2$O$_2$, growth inhibition rates of the Fvve1 mutant and the FvvelB mutant were significantly higher than that of the wild type (Fig. 4A). However, the FvvelB mutant was less sensitive to H$_2$O$_2$ than the Fvve1 mutant: the relative growth inhibition rates of the Fvve1 mutant and the FvvelB mutant were 46.3% and 18.7%, respectively (Fig. 4B). All these observations indicate that FvVE1 plays a more important role in oxidative stress resistance than FvVelB and the relative roles of FvVE1 and FvVelB of *F. verticillioides* are different from those of *A. nidulans* in the regulation of oxidative stress tolerance.
The FvvelC mutant displayed wild-type sensitivities to H2O2 and menadione (Fig. 4).

FvVE1 and FvVELB positively regulate FvCAT2 expression. To test how FvVE1 and FvVELB regulate antioxidant activity, transcriptional levels of eight genes involved in ROS detoxification during H2O2 treatment were comparatively analyzed in the Fvvel deletion mutant, the FvvelB deletion mutant, and the wild-type strain by qRT-PCR. These genes included GST (FVEG_07456; a glutathione S-transferase), GLT2 (FVEG_08420; encoding a glutathione transferase), GLRX3 (FVEG_07558; encoding a glutaredoxin), CCP (FVEG_01375; encoding a copper chaperone), putative-GST (pu-GST, FVEG_00418), catalase-peroxidase (FVEG_10866), CAT1 (FVEG_05591), and catalase-peroxidase 2 (FVEG_12888).

When mycelium was treated with 1.96 mM H2O2, all 8 genes showed transcriptional increases after 2 h of H2O2 treatment for the wild type, while after 4 h of treatment, their transcriptional levels were reduced relative to those after 2 h of treatment (Fig. 5A). Among these genes, FVEG_12888, which is predicted to encode catalase peroxidase 2, was the only gene showing a significant reduction in transcription upon Fvvel deletion mutant and the FvvelB deletion mutant, and the wild-type strain by qRT-PCR. These genes included GST (FVEG_07456; a glutathione S-transferase), GLT2 (FVEG_08420; encoding a glutathione transferase), GLRX3 (FVEG_07558; encoding a glutaredoxin), CCP (FVEG_01375; encoding a copper chaperone), putative-GST (pu-GST, FVEG_00418), catalase-peroxidase (FVEG_10866), CAT1 (FVEG_05591), and catalase-peroxidase 2 (FVEG_12888).

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were grown that of the wild type (Fig. 6A and B). However, when the strains were inoculated onto plates with or without 3.27 mM H₂O₂ or 25 μg/ml menadione. Images were captured after 72 h of incubation at 28°C in darkness. (B) Relative inhibition of colony growth by oxidants. Values of three replicates were used for statistical analysis. Means of inhibition rates were shown, and standard deviations were marked with bars. The significances (P < 0.01) between strains were estimated by the Waller-Duncan test with the software program SPSS and marked with the letters a, b, and c. Strains sharing any of these letters in common were not significantly different. The means without significant differences among strains were not marked.

FIG 4 Oxidant sensitivities of *F. verticillioides* strains. (A) The *Fve1*, *Fvel* [*FvVE1*-Myc-His], *FvelB*, *FvelB* [*FvVELB*], *FvelC*, and wild-type (WT) strains were inoculated onto plates with or without 3.27 mM H₂O₂ or 25 μg/ml menadione. Images were captured after 72 h of incubation at 28°C in darkness. (B) Relative inhibition of colony growth by oxidants. Values of three replicates were used for statistical analysis. Means of inhibition rates were shown, and standard deviations were marked with bars. The significances (P < 0.01) between strains were estimated by the Waller-Duncan test with the software program SPSS and marked with the letters a, b, and c. Strains sharing any of these letters in common were not significantly different. The means without significant differences among strains were not marked.

The above-described results with mycelium and conidia indicate that deletion of *Fve1* and *FvelB* impaired the normal transcription of several ROS-removal-related genes, and transcriptional profiles of affected genes shared some similarity in the *Fve1* deletion mutant and the *FvelB* deletion mutant, suggesting that the two velvet proteins coordinately activate the same set of genes for ROS detoxification. Overall, *Fve1* deletion had greater influence on the expression of these ROS-removal-related genes, providing an explanation of why the *Fve1* deletion mutant was more sensitive to oxidants than the *FvelB* deletion mutant.

**FvCat2 contributes to ROS antioxidant activity and morphological development.** All the above-described data indicate that FVEG_12888 is the most important gene regulated by velvet proteins among tested genes involved in antioxidant activity. Phylogenetic analysis (see Fig. S6 in the supplemental material) showed that FVEG_12888 is the orthologue of *Neurospora crassa* CAT-2 (40) and *A. fumigatus* CAT2 (41, 42), and thus it was named FvCat2 in this study.

To gain further insight into the function of FvCat2, a null mutant of FvCat2 was generated (see Fig. S4 in the supplemental material). H₂O₂ and menadione sensitivities between the wild type and the Fvcat2 deletion mutant were compared. On normal medium, the growth rate of the deletion mutant was similar to that of the wild type (Fig. 6A and B). However, when the strains were grown on the medium supplemented with H₂O₂ or menadione, growth of the Fvcat2 mutant displayed a higher level of inhibition than that of the wild type (Fig. 6A and B). The *N. crassa* gene knockout mutant for the FvCat2 orthologue CAT-2 also displayed increased sensitivity to H₂O₂ or menadione compared with that of the wild-type *N. crassa* strain (Fig. 6A and B), suggesting the general contribution of CAT-2 to oxidant resistance in filamentous ascomycetes.

The Fvcat2 mutant also exhibited several morphological defects, including reduced conidial production and increased conidial size relative to those of the wild type (Fig. 6D and E). The hydrophobicity of the hyphal surface was also slightly reduced compared with that of the wild type (Fig. 6C). Fumonisins were present at levels similar to those of the wild type (Fig. 6F).

All mutant phenotypes were restored in the complemented strains, confirming that the above phenotypes in the Fvcat2 mutant were caused by loss of the FvCAT2 gene. These data suggest that FvVE1-dependent expression of FvCat2 plays a role in maintaining normal oxidant resistance, conidial development, and cell hydrophobicity but has no influence on fumonisins production.

**DISCUSSION**

The velvet proteins are widely distributed in filamentous fungi. In *A. nidulans*, VeA can interact with VelB (14), VelB can interact with VosA (16), and VosA can interact with VelC (20). Yeast two-hybrid experiments demonstrated that all velvet proteins, including VeA, VelB, and VelC, can interact with each other in *Fusarium oxysporum* (21). In consistence with the results in *F. oxysporum* (21), here we demonstrated that FvVE1 (the VeA orthologue) can form a complex with other velvet proteins, FvVelB and FvVelC, in *F. verticillioides*. These phenomena, together with the fact that the *Fusarium* genus lacks VosA (21), indicate that the composition of velvet proteins and the interaction among velvet proteins in the *Fusarium* genus are different from those in the *Aspergillus* genus. Furthermore, this study identified several new proteins that possibly interact with velvet proteins, including CPSF5 (cleavage and polyadenylation specificity factor 5), UreG (urease accessory protein), and FvHrp1. The yeast FvHrp1 homologue Hrp1p is a sequence-specific RNA-binding protein required for mRNA 3’-end formation. Hrp1 shuttles between the nucleus and the cytoplasm...
However, this is the first time c-Myc has been used as a tag to isolate velvet proteins; whether the c-Myc tag has interaction with CPSF5, UreG, and FvHrp1 is unknown. Their interactions with velvet proteins still remain to be confirmed by other methods. Both CPSF5 and FvHrp1 are involved in RNA processing (43,45–49); if they interact with velvet proteins, the velvet protein complex might be also involved in posttranscriptional regulation.

This study also analyzed the relative contributions of FvVelB and FvVelC to morphological development, fumonisin biosynthesis, and oxidant resistance and compared their roles with those of the previous characterized FvVE1 (8, 26). Our results demonstrated that FvVE1 and FvVelB play similar roles in controlling balanced production of microconidia and macroconidia and in maintaining cell hydrophobicity. For fumonisin production, FvVE1 is essential. FvVelB is also important, but its absence did not completely abolish fumonisin production. Thus, its importance is less than that of FvVE1. In oxidant resistance, FvVE1 plays a more important role among velvet proteins than in other biological processes. All these facts indicate that the relative roles of VeA and VelB vary among different biological processes. It is possible that the velvet proteins might be able to form different protein complexes to fulfill different functions. In regulation of secondary metabolism, conidial development, and hyphal hydrophobicity, a complex composed of at least FvVE1 and FvVelB might be required. Regarding the oxidative stress response, FvVE1 alone or FvVE1 with proteins other than FvVelB or FvVelC might be sufficient. In addition, this study reveals the difference between Aspergillus and Fusarium in the relative contributions of VeA and VelB to oxidant resistance, since the contribution of VeA to oxidative stress resistance is lower than that of VelB in A. nidulans (16). VosA is required for trehalose accumulation in conidia and positively contributes to resistance to H2O2 and ultraviolet irradiation in A. nidulans (16). Since the transportation of VelB into nuclei depends on the formation of heterodimers with other velvet proteins with the nuclear localization signal (14, 16), the absence of VosA might make VeA more important in Fusarium than in Aspergillus. Although VelC negatively regulates conidial production in A. nidulans (20), similar to our observation for F. verticillioides, deletion of VelC caused less phenotypic alteration than that of VeA and VelB in A. nidulans and Aspergillus flavus (16, 18), indicating that VelC is less important than other velvet proteins.

Our study together with previous observations for C. heterosporus (30) provides a possible mechanism to explain how velvet proteins regulate oxidative stress resistance. The mechanisms of adaptation to oxidative stress are complicated in fungi. Several proteins with regulatory roles in oxidant resistance have been identified, but linkages among these regulatory proteins are not understood. Yap1p, a mammalian AP-1-like bZIP-type transcription factor in Saccharomyces cerevisiae, is the best-known regulator of oxidative stress resistance. Deletion of YAP1 or its orthologues increases sensitivity to oxidants in S. cerevisiae and other fungal species (50–53). Three new transcription factors, SRE, MIT-2, and MIT-4, were recently found to be important for resistance to menadione in N. crassa (54), but the mechanism by which they confer resistance is still unknown. In addition to these transcription factors, a mitogen-activated protein kinase (MAPK) pathway,

FIG 5 Transcriptional analysis of genes related to ROS scavenging during H2O2 treatment in the Fvve1, FvvelB, and wild-type (WT) strains as determined by qRT-PCR. (A) Results from mycelium. (B) Results from conidia. Transcriptional levels were calculated relative to those for the wild-type strain at the "0 h" time point. Values shown are means for three replicates. Standard deviations are indicated with error bars. The significances (P < 0.01) between strains were estimated by the Waller-Duncan test with the software SPSS and are marked with the letters a, b, and c. Strains sharing any of these letters in common were not significantly different. The means without significant differences among strains are not marked.
which is composed of StyI (MAPK), Wis1 (MAPKK), and Wis4 (MAPKKK) in Schizosaccharomyces pombe, is also implicated in the regulation of oxidative stress responses (55, 56). The StyI homologue SakA in Aspergillus fumigatus and the StyI homologue OS-2 and W1 homologue RRG-2 in roles (57, 58). In this study, we found that FvVE1 and FvVelB were also involved velvet proteins.

Possible explanation for the regulation of oxidant resistance by fumonisins production, and oxidant sensitivity, and suggested a regulatory proteins remains to be investigated.

Morphological development is influenced by environmental factors. Loss of the catalase FvCat2 and Cat1. However, the linkage between velvet proteins and the above-mentioned regulatory proteins remains to be investigated.

In summary, this study revealed the composition of the velvet protein complex in F. verticillioides, functionally characterized individual velvet proteins in conidial size, hyphal hydrophobicity, fumonisins production, and oxidant sensitivity, and suggested a possible explanation for the regulation of oxidant resistance by velvet proteins.

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REFERENCES

1. Leslie JF, Pearson CAS, Nelson PE, Tousson TA. 1990. Fusarium spp. from corn, sorghum, soybean fields in the central and eastern United States. Phytopathology 80:343–350. http://dx.doi.org/10.1094/Phyto-80-368

2. Nelson PE, Desjardins AE, Plattner RD. 1993. Fumonisins, mycotoxins produced by Fusarium species: biology, chemistry, and significance. Annu. Rev. Phytopathol. 31:233–252. http://dx.doi.org/10.1146/annurev.phyto.31.090193.001313.

3. Desai K, Sullards MG, Allegood J, Wang E, Schmelz EM, Hartl M, Humpf H-U, Liotta DC, Peng Q, Merrill AH, Jr. 2002. Fumonisins and fumonisin analogs as inhibitors of ceramide synthase and inducers of apoptosis. Biochim. Biophys. Acta 1585:386–392. http://dx.doi.org/10.1016/S0962-8988(01)00981-7

4. Marasas WFO, Riley RT, Hendricks KA, Stevens VL, Sadler TW, Gelineau-van Waes J, Missmer SA, Cabrera J, Torres O, Gelderblom WCA, Allegood J, Martinez C, Maddox J, Miller JD, Storr L, Sullards MC, Roman AV, Voss KA, Wang E, Merrill AH. 2004. Fumonisins disrupt sphingolipid metabolism, folate transport, and neural tube development in embryo culture and in vivo: a potential risk factor for human neural tube defects among populations consuming fumonisin-contaminated maize. J. Nutr. 134:711–716.

5. Bojja RS, Cerny RL, Proctor RH, Du L. 2004. Determining the biosynthetic sequence in the early steps of the fumonisin pathway by use of three gene-disruption mutants of Fusarium verticillioides. J. Agric. Food Chem. 52:2855–2860. http://dx.doi.org/10.1021/jf035429z.

6. Brown DW, Butchko RA, Busman M, Proctor RH. 2007. The Fusarium verticillioides FUM gene cluster encodes a Zn(II)2Cys6 protein that affects...
FUS gene expression and fumonisin production. Eukaryot. Cell 6:1210–1218. http://dx.doi.org/10.1128/EC.00400-06.

7. Seo J-A, Proctor RH, Plattner RD. 2001. Characterization of four clustered and coregulated genes associated with fumonisin biosynthesis in Fusarium verticillioides. Fungal Genet. Biol. 34:155–165. http://dx.doi.org/10.1016/S1382-4896(01)12999-0.

8. Myung K, Li S, Butchko RA, Busman M, Proctor RH, Abbas HK, Calvo AM. 2009. FvVE1 regulates biosynthesis of the mycoxin fumonisins and fusarins in Fusarium verticillioides. J. Agric. Food Chem. 57:5089–5094. http://dx.doi.org/10.1021/jf900783u.

9. Käfer E. 1965. Origins of translocations in Aspergillus nidulans. Genetics 52:217.

10. Bayram O, Braus GH. 2012. Coordination of secondary metabolism and development in fungi: the velvet family of regulatory proteins. FEMS Microbiol. Rev. 36:1–24. http://dx.doi.org/10.1111/j.1574-6976.2011.00285.x.

11. Stinnett SM, Espeso EA, Coboño I, Araújo-Bazán L, Calvo AM. 2007. Aspergillus nidulans VeA subcellular localization is dependent on the importin α carrier and on light. Mol. Microbiol. 63:242–255. http://dx.doi.org/10.1111/j.1365-2958.2006.05506.x.

12. Calvo AM. 2008. The VeA regulatory system and its role in morphological and chemical development in fungi. Fungal Genet. Biol. 45:1053–1061. http://dx.doi.org/10.1016/j.fgb.2008.03.014.

13. Myung K, Zitomer NC, Duval M, Glenn AE, Riley RT, Calvo AM. 2012. The conserved global regulator VeA is necessary for symptom production and mycotoxin synthesis in maize seedlings by Fusarium verticillioides. Plant Pathol. 61:152–160. http://dx.doi.org/10.1111/j.1365-3639.2011.02540.x.

14. Bayram O, Krappmann S, Ni M, Bok JW, Kummer A, Bok JW, Helma G, Kummer A. 2012. Coordination of secondary metabolism and development of the maize pathogen Cochliobolus heterostrophus. PLoS Pathog. 8:e1002542. http://dx.doi.org/10.1371/journal.ppat.1002542.

15. Kopke K, Hoff B, Bloemendal S, Katsochorowksi A, Kamereward J, Kück U. 2013. Members of the Penicillium cryagensum velvet complex play functionally opposing roles in the regulation of penicillin biosynthesis and conidiation. Eukaryot. Cell 12:299–310. http://dx.doi.org/10.1128/EC.01212-12.

16. You B-J, Lee M-H, Chung K-R. 2009. Gene-specific disruption in the filamentous fungus Cercospora nicotiana using a split-marker approach. Arch. Microbiol. 191:615–622. http://dx.doi.org/10.1007/s00203-009-0489-4.

17. Kück U, Hoff B. 2010. New tools for the genetic manipulation of filamentous fungi. Appl. Microbiol. Biotechnol. 86:51–62. http://dx.doi.org/10.1007/s00253-009-1416-7.

18. He Q, Cheng P, He Q, Liu Y. 2005. The COP9 signalosome regulates the Neurospora circadian clock by controlling the stability of the SCFFWD-1 complex. Genes Dev. 19:1518–1531. http://dx.doi.org/10.1101/gad.1322205.

19. Rottinghaus GE, Coatey CE, Minor HC. 1992. A rapid, sensitive thin layer chromatography procedure for the detection of fumonisins B1 and B2. J. Vet. Diagn. Invest. 4:326–329. http://dx.doi.org/10.1177/10406378920040316.

20. Silva L, Fernández-Franzón M, Font G, Pena A, Silveira I, Lino C, Márquez J. 2009. Analysis of fumonisins in corn-based food by liquid chromatography with fluorescence and mass spectrometry detectors. Food Chem. 112:1031–1037. http://dx.doi.org/10.1016/j.foodchem.2008.06.080.

21. Zhang Y, Zhang Z, Zhang X, Zhang H, Sun X, Hu C, Li S. 2012. CDR4 is the major contributor to azole resistance among four Pdr5p-like ABC transporters in Neurospora crassa. Fungal Biol. 116:48–854. http://dx.doi.org/10.1016/j.funbio.2012.05.002.

22. Sun X, Zhang H, Zhang Z, Wang Y, Li S. 2011. Involvement of a helix-loop-helix transcription factor CHC-1 in CO2-mediated conidiation suppression in Neurospora crassa. Fungal Genet. Biol. 48:1077–1086. http://dx.doi.org/10.1016/j.fgb.2011.09.003.

23. Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25:402–408. http://dx.doi.org/10.1016/S1046-2023(01)00265-9.

24. Chary P, Navin DO. 1989. Evidence for three differentially regulated catalase genes in Neurospora crassa: effects of oxidative stress, heat shock, and development. J. Bacteriol. 171:2646–2652.

25. Paris S, Wysong D, Debeaupuis J-P, Shibuya K, Philippe B, Diamond RD, Latgé JP. 2003. Catalases of Aspergillus fumigatus. Infect. Immun. 71:3551–3562. http://dx.doi.org/10.1128/IAI.71.6.3551-3562.2003.

26. Calera JA, Paris S, Monod M, Hamilton AJ, Debeaupuis JP, Dacquin M, López-Medrano R, Leal F, Latgé JP. 1997. Cloning and disruption of the antifungal catalase gene of Aspergillus fumigatus. Infect. Immun. 65:4718–4724.

27. Kessler MM, Henry MF, Shen E, Zhao J, Gross S, Silver PA, Moore CL. 1997. Hrp1, a sequence-specific RNA-binding protein that shuttles between the nucleus and the cytoplasm, is required for mRNA 3′-end forma-
tion in yeast. Genes Dev. 11:2545–2556. http://dx.doi.org/10.1101/gad.11
19.2545.
44. Lange A, Mills RE, Devine SE, Corbett AH. 2008. A PY-NLS nuclear
744 targeting signal is required for nuclear localization and function of the
Saccharomyces cerevisiae mRNA-binding protein Hrp1. J. Biol. Chem. 283:
12926–12934. http://dx.doi.org/10.1074/jbc.M800898200.
45. Hollerer I, Grund K, Hentze MW, Kulosik AE. 2014. mRNA 3’end
747 processing: a tale of the tail reaches the clinic. EMBO Mol. Med. 6:16–26.
http://dx.doi.org/10.1002/emmm.201303300.
46. Leeper TC, Qu X, Lu C, Moore C, Varani G. 2010. Novel protein-
748protein contacts facilitate mRNA 3’-processing signal recognition by
Rna15 and Hrp1. J. Mol. Biol. 401:334–349. http://dx.doi.org/10.1016/j
jmbl.2010.06.032.
47. González CI, Ruiz-Echevarria MJ, Vasudevan S, Henry MF, Peltz SW.
750 2000. The yeast hnRNP-like protein Hrp1/Nab4 marks a transcript for
nonsense-mediated mRNA decay. Mol. Cell 5:489 – 499.http://dx.doi.org
10.1016/S1097-2765(00)80443-8.
48. Mandel CR, Kaneko S, Zhang H, Gebauer D, Vethantham V, Manley
754 JL, Tong L. 2006. Polyadenylation factor CPSF-73 is the pre-mRNA 3’-
end-processing endonuclease. Nature 444:953–956. http://dx.doi.org/10
10.1038/nature05363.
49. Kyburz A, Friedlein A, Langen H, Keller W. 2006. Direct interactions
between subunits of CPSF and the U2 snRNP contribute to the coupling of
pre-mRNA 3’ end processing and splicing. Mol. Cell 23:195–205. http:
//dx.doi.org/10.1016/j.molcel.2006.05.037.
50. Chen D, Wilkinson CRM, Watt S, Penkett CJ, Toone WM, Jones N,
Bährler J. 2008. Multiple pathways differentially regulate global oxidative
stress responses in fission yeast. Mol. Biol. Cell 19:308–317. http://dx.doi
.org/10.1091/mbc.E07-08-0735.
51. Lev S, Hadar R, Amedeo P, Baker SE, Yoder OC, Horwitz BA. 2005.
Activation of an AP1-like transcription factor of the maize pathogen
Cochliobolus heterostrophus in response to oxidative stress and plant
signals. Eukaryot. Cell 4:443–454. http://dx.doi.org/10.1128/EC.4.2.443
454.2005.
52. Takahashi M, Yamashita K, Shiozawa A, Ichiishi A, Fukumori F,
Fujimura M. 2010. An AP-1-like transcription factor, NAP-1, regulates
expression of the glutathione-S-transferase and NADH: flavin oxi-
doreductase genes in Neurospora crassa. Biosci. Biotechnol. Biochem. 74:
746–752. http://dx.doi.org/10.1271/bbb.90790.
53. Gulshan K, Rovinsky SA, Coleman ST, Moye-Rowley WS. 2005. Oxi-
dant-specific folding of Yap1p regulates both transcriptional activation
and nuclear localization. J. Biol. Chem. 280:40524–40533. http://dx.doi
.org/10.1074/jbc.M504716200.
54. Zhu J, Yu X, Xie B, Gu X, Zhang Z, Li S. 2013. Transcriptomic
profiling-based mutant screen reveals three new transcription factors me-
diating mendadine resistance in Neurospora crassa. Fungal Biol. 117:422–
430. http://dx.doi.org/10.1016/j.funbio.2013.04.006.
55. Lushchak VI. 2010. Oxidative stress in yeast. Biochemistry (Mosc.) 75:
281–296. http://dx.doi.org/10.1134/S0006297910030041.
56. Moye-Rowley WS. 2003. Regulation of the transcriptional response to
oxidative stress in fungi: similarities and differences. Eukaryot. Cell 2:381–
389. http://dx.doi.org/10.1128/EC.2.3.381-389.2003.
57. Banno S, Noguchi R, Yamashita K, Fukumori F, Kimura M, Yamaguchi
I, Fujimura M. 2007. Roles of putative His-to-Asp signaling modules
HPT-1 and RRG-2, on viability and sensitivity to osmotic and oxidative
stresses in Neurospora crassa. Curr. Genet. 51:197–208. http://dx.doi.org
/10.1007/s00294-006-0116-8.
58. Du C, Sarfati J, Latge JP, Calderone R. 2006. The role of the sakA (Hog1)
and tscB (shl1) genes in the oxidant adaptation of Aspergillus fumigatus.
Med. Mycol. 44:211–218. http://dx.doi.org/10.1080/13693780500338886.
FIG S1 Targeted replacement of *FvVE1* gene. (A) Schematic diagram of homologous replacement of *FvVE1* by hygromycin resistance gene (*HPH*). A plasmid containing *HPH* gene flanked by up and down stream regions of the target gene was constructed. (B) Detection of *FvVE1* deletion of twenty independent transformants by PCR amplification using primers v1-3F and v1-3R. (C) Verification of the insertion of the replacement of *FvVE1* by hygromycin resistance gene by PCR using primers vC-5F and vC-5R.
FIG S2 Targeted replacement of *FvVELB* gene by hygromycin resistance gene (*HPH*) using split-marker approach. (A) Construction of the *FvVELB* knockout cassette by fusion PCR. (B) PCR amplification of *FvVELB* from eleven transformants using primers vB-5F and vB-5R. (C) Verification of the insertion of the replacement of *FvVELB* by hygromycin resistance gene by PCR using primers vB-6F and vB-6R (the left panel) and primers vB-7F and vB-7R (the right panel).
FIG S3 Targeted replacement of *FvVELC* gene. (A) Schematic diagram of homologous replacement of *FvVELC* by hygromycin resistance gene (*HPH*). A plasmid containing *HPH* gene flanked by up and down stream regions of the target gene was constructed. (B) Detection of *FvVELC* deletion of twenty three independent transformants by PCR amplification using primers vC-3F and vC-3R. (C) Verification of the insertion of the replacement of *FvVELC* by hygromycin resistance gene by PCR using primers vC-4F and vC-4R. (D) Verification of the insertion of the replacement of *FvVELC* by hygromycin resistance gene by PCR using primers vC-5F and vC-5R.
FIG S4 Targeted gene replacement of *FvCAT2* gene. (A) Schematic diagram of homologous replacement of *FvCAT2* by hygromycin resistance gene (*HPH*). A plasmid containing *HPH* gene flanked by up and down stream regions of the target gene was constructed. (B) Detection of *FvCAT2* deletion of four transformants by PCR amplification using primers cat2-3F and cat2-3R. (C) Verification of the insertion of the replacement of *FvCAT2* by hygromycin resistance gene by PCR using primers cat2-4F and cat2-4R (the left panel) and primers cat2-5F and cat2-5R (the right panel).
FIG S5 Construction of Fvve1 complemented strains expressing 5×cMyc-6×His tagged FvVE1. (A) Schematic diagram of the cassette expressing FvVE1-5×cMyc-6×His. (B) Verification of Fvve1[FvVE1-MYC-HIS] transformants by western blot with anti-Myc antibody. (C) The transformant Fvve1[FvVE1-MYC-HIS] which phenotypes restore to the wild type.
FIG S6 Phylogenic analysis of FVEG_12888 (catalase-peroxidase 2). CLUSTALW was used for protein alignment. Maximum Parsimony (MP) bootstrap consensus tree was constructed with MEGA4 software and the bootstrap values were 1000 replicates. Selected catalases and peroxidases were from *Aspergillus fumigatus* (Afu), *Neurospora crassa* (Nc), *Cochliobolus heterostrophus* (Ch), *Rhizoctonia solani* (Rhs) and *Fusarium verticillioides* (FVEG).
| Primer name | Sequence of primers (5’ → 3’) |
|-------------|-------------------------------|
| vB-1        | F: CCCCAACCTTTCTGTCTCCTT R: CCTCCACTAGCTCCAGCCAAGCCAGATAAGCCGATTTTGATCTGTGTC |
| vB-2        | F: GACAAAGATCAAATTCGCGTTACTGCCTGCTGGCTGGAGCTAGTGAGG R: GTATGGACCGATTTTGATCTGTC |
| vB-3        | F: GATGTAGAGGAGGCGTGGATATGCTCTT R: ATACAGACACACACCCCCGGAGAATAACCCCGGCGCATCTACTCTAATC |
| vB-4        | F: GTATGGACCGGTGTTTCTTGG R: GACAAAGATCAAATTCGCGTTACTGCCTGCTGGCTGGAGCTAGTGAGG |
| vB-5        | F: GATGTAGAGGAGGCGTGGATATGCTCTT R: ATACAGACACACACCCCCGGAGAATAACCCCGGCGCATCTACTCTAATC |
| vB-6        | F: GATGTAGAGGAGGCGTGGATATGCTCTT R: ATACAGACACACACCCCCGGAGAATAACCCCGGCGCATCTACTCTAATC |
| vB-7        | F: GATGTAGAGGAGGCGTGGATATGCTCTT R: ATACAGACACACACCCCCGGAGAATAACCCCGGCGCATCTACTCTAATC |
| vB-8        | F: GATGTAGAGGAGGCGTGGATATGCTCTT R: ATACAGACACACACCCCCGGAGAATAACCCCGGCGCATCTACTCTAATC |
| vB-9        | F: GATGTAGAGGAGGCGTGGATATGCTCTT R: ATACAGACACACACCCCCGGAGAATAACCCCGGCGCATCTACTCTAATC |
| vC-1        | F: GATGTAGAGGAGGCGTGGATATGCTCTT R: ATACAGACACACACCCCCGGAGAATAACCCCGGCGCATCTACTCTAATC |
| vC-2        | F: GATGTAGAGGAGGCGTGGATATGCTCTT R: ATACAGACACACACCCCCGGAGAATAACCCCGGCGCATCTACTCTAATC |
| vC-3        | F: GATGTAGAGGAGGCGTGGATATGCTCTT R: ATACAGACACACACCCCCGGAGAATAACCCCGGCGCATCTACTCTAATC |
| vC-4        | F: GATGTAGAGGAGGCGTGGATATGCTCTT R: ATACAGACACACACCCCCGGAGAATAACCCCGGCGCATCTACTCTAATC |
| vC-5        | F: GATGTAGAGGAGGCGTGGATATGCTCTT R: ATACAGACACACACCCCCGGAGAATAACCCCGGCGCATCTACTCTAATC |
| cat2-1      | F: GATGTAGAGGAGGCGTGGATATGCTCTT R: ATACAGACACACACCCCCGGAGAATAACCCCGGCGCATCTACTCTAATC |
| cat2-2      | F: GATGTAGAGGAGGCGTGGATATGCTCTT R: ATACAGACACACACCCCCGGAGAATAACCCCGGCGCATCTACTCTAATC |
| cat2-3      | F: GATGTAGAGGAGGCGTGGATATGCTCTT R: ATACAGACACACACCCCCGGAGAATAACCCCGGCGCATCTACTCTAATC |
| cat2-4      | F: GATGTAGAGGAGGCGTGGATATGCTCTT R: ATACAGACACACACCCCCGGAGAATAACCCCGGCGCATCTACTCTAATC |
| cat2-5      | F: GATGTAGAGGAGGCGTGGATATGCTCTT R: ATACAGACACACACCCCCGGAGAATAACCCCGGCGCATCTACTCTAATC |
| cat2-6      | F: GATGTAGAGGAGGCGTGGATATGCTCTT R: ATACAGACACACACCCCCGGAGAATAACCCCGGCGCATCTACTCTAATC |
| v1-1        | F: GATGTAGAGGAGGCGTGGATATGCTCTT R: ATACAGACACACACCCCCGGAGAATAACCCCGGCGCATCTACTCTAATC |
| Gene     | Sequences of primers (5’ → 3’) | Gene     | Sequences of primers (5’ → 3’) |
|----------|---------------------------------|----------|---------------------------------|
| HYD1     | F: CCAACAATGCTTTGCAGGAAAA       | FVEG_1288| F: AAGCAGCTCAAGCAGGGTTCTCTCT    |
|          | R: ACTGATCAAACAGGGCTTCAAG       |          | R: TAGCAGCAACACCACCGAGAAACAAA   |
|          | F: GCCAAAACACCTCTACACAA         |          | F: TCATTGCGTTGTCACACTCTCA       |
|          | R: CTGATAGCAACGCAGGAAGAG        |          | R: ACGAAGAAAGTGGTGGTCAGAGA      |
| HYD2     | F: CAGCAACACGGGCAAACAA          | CAT1     | F: GCATTCAACTCGGCTGAACTTTGA    |
|          | R: CATCAGACTCGGCACTGAA          |          | R: GAGGTGCGGAAACGCAGGAAAGG     |
|          | F: CCAAGGAGTTCAGAGAGGTAGTGG    | GST      | F: GACCGAGAAATGAGAGCACAGA      |
|          | R: TTAAGCAGTAACACCCGAGAAGG     |          | R: GCATAGCACCACTCTCA           |
| HYD4     | F: AGACCTTGACTGCAGGAAAC         | putative-GST | F: TAACGCCAAGGCAACATATCCACGA |
|          | R: AGGATGCCTTTGTAAGAAGATAG     |          | R: TGCATGAAGATGCAAGGCAAGCCACC |
| FYM1     | F: GCCATTGACTTGGCTGATGTTTC      | GLT-2    | F: TGGACTGCTGCTGTGATGT        |
|          | R: ACTTTGACGGGCTTGAAGG         |          | R: GCATAATCTCGCCGACCTTC       |
| FYM5     | F: GTTCTCAATTCTATGCTACTG       | GLR      | F: AGCTCGACCAAAACACTC         |
|          | R: GAAGCTTTGCAACGAGGTATAC      |          | R: CGTCGAAACTCTGCTCA          |
| tublin   | F: ATTCACCAACATCCAGACAGCC      | CCP      | F: GCTTCAACGATACGAGGATT       |
|          | R: GAACCTGCTGAAACACGCTTCGAA    |          | R: GCATTGGTCAGAAACGGTAG       |