UvWHI2 is Required for Stress Response and Pathogenicity in Ustilaginonidea Virens

Shuai Meng
China National Rice Research Institute
Jiehua Qiu
China National Rice Research Institute
Meng Xiong
China National Rice Research Institute
Zhiquan Liu
China National Rice Research Institute
Jane Sadhna Jagernath
China National Rice Research Institute
Huanbi Shi
China National Rice Research Institute
Yanjun Kou (Kouyanjun241@hotmail.com)
China National Rice Research institute https://orcid.org/0000-0003-4189-0962

Original article

Keywords: Whi2, Rice false smut, Ustilaginoidea virens, Pathogenesis, Secondary spore

Posted Date: November 5th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-101297/v1

UvWHI2 is required for stress response and pathogenicity in *Ustilaginonidea virens*

Shuai Meng*, Jiehua Qiu*, Meng Xiong*, Zhiquan Liu, Jane Sadhna Jagernath, Huanbin Shi#, Yanjun Kou#

State Key Laboratory of Rice Biology, China National Rice Research Institute, Hangzhou. China, 311400

*Shuai Meng, Jiehua Qiu, Meng Xiong contributed equally to this work.

#For Correspondence: Huanbin Shi; Email: shihuanbin@caas.cn; Yanjun Kou; Email: kouyanjun241@hotmail.com

Abstract:

Background: *Ustilaginoidea virens* causes rice false smut disease, which emerges as a worldwide disease of rice. At present, some stress response related genes have been identified in *U. virens*, but it is not clear whether and how defects of stress responses affect the pathogenesis processes of *U. virens*. To answer this question, the function of a general stress response factor Whi2 was analyzed in *U. virens*.

Results: In this study, we identified UvWhi2 as a homolog of *Saccharomyces cerevisiae* Whi2 in *U. virens*. The relative expression level of *UvWHI2* was significantly up-regulated during infection, suggesting that UvWhi2 may be involved in pathogenesis. Furthermore, knockout of *UvWHI2* showed decreased the mycelial growth, increased in conidiation in the PS (potato sucrose) medium and a defect in pathogenicity. In addition, the RNA-Seq and phenotypic analysis showed that UvWhi2 is involved in response to oxidative, hyperosmotic, cell wall stresses, and nutrient limitation. Further studies revealed that the defects of stress responses of the Δ*Uvwhi2* mutant affected the formation of secondary spores on the nutrient limited surface and the rice surface, resulting in a significant reduction of pathogenicity of *U. virens*.

Conclusions: Our results suggest that UvWhi2 is necessary for fungal growth, stress responses, and the formation of secondary spores in *U. virens*. In addition, the defects

of stress responses could affect the formation of secondary spores on the rice surface, and then compromise the pathogenicity of *U. virens*.

Keywords: Whi2, Rice false smut, *Ustilaginoidea virens*, Pathogenesis, Secondary spore

Introduction

Rice false smut disease is caused by the ascomycete filamentous fungus *Ustilaginoidea virens*, which has emerged to be one of the most devastating rice fungal diseases in the rice-cultivated areas (Fan et al., 2016, Liang et al., 2018, Guo et al., 2019, Qiu et al., 2019, Meng et al., 2020, Sun et al., 2020). The occurrence of rice false smut seriously affects high and stable yield and quality of rice (Lu et al., 2015, Zheng et al., 2017, Lin et al., 2018). During the booting period, the rice spikelets were infected by the *U. virens*, which hindered the nutrition transportation and the normal development of grains, resulting in the increase of empty chaff rate and the decrease of 1000-grain weight (Yu et al., 2015, Tang et al., 2019). False smut balls contain various toxins, such as *Ustilaginoidins* and *Ustiloxins*, which could inhibit the assembly of microtubules in eukaryotic cells, disrupt cell mitosis and cause pathological changes in animal organs and plants (Lu et al., 2015, Zheng et al., 2016, Sun et al., 2017, Wang et al., 2017).

Thus far, only few pathogenesis-related proteins have been identified and characterized in *U. virens*, including the hypothetical protein UvPro1, the low-affinity iron transporter Uvt3277, two protein kinases UvPmk1 and UvCDC2, the cyclic adenosine monophosphate signaling proteins adenylate cyclase UvAc1 and phosphodiesterase UvPdeH, the Bax inhibitor UvBI-1, the effector Scre2 (Uv_1261), two transcriptional factors UvHox2 and UvCom1, the phosphatase UvPsr1, and the autophagy-related protein UvAtg8 (Lv et al., 2016, Zheng et al., 2017, Fang et al., 2019, Guo et al., 2019, Tang et al., 2019, Xie et al., 2019, Yu et al., 2019, Chen et al., 2020,

Meng et al., 2020, Xiong et al., 2020). Among these, UvPmk1, UvCdc2, UvPro1, UvBI-1, UvAc1, UvPdeh, UvPsr1, UvCom1, UvHox2, and UvAtg8 are involved in both various stresses responses and pathogenesis of *U. virens*. However, it is not clear whether and how defects of stress responses affect the pathogenesis processes of *U. virens.*

Whi2 (Whiskey2) is a general stress response factor. In yeast, ScWhi2 have been characterized to be involved in general stress response and coordinate nutrient status with cell cycle (Muller & Reichert, 2011, Sadeh et al., 2011, Teng et al., 2018). Deletion of *ScWHI2* causes decreased expression level of stress-associated genes, increased sensitivity to sodium ions, and disruption of the normal coordination of cell proliferation with nutrient availability (Kaida et al., 2002, Chen et al., 2018, Marsikova et al., 2020). In *Podospora anserina*, the Whi2 homolog has been shown to control nutrient sensing and the entrance of cells into the stationary phase and mycelial development (Timpano et al., 2016). In *Colletotrichum orbiculare*, *CoWHI2* was reported to play key roles in regulation of transition from biotrophic infection to necrotrophic infection via regulation of TOR (target of rapamycin) signaling, which is involved in the coordination of cell growth and proliferation with the availability of growth factors and nutrients (Harata et al., 2016). Until now, the function of Whi2 remains largly unknown in various organisms.

In this study, *UvWHI2*, the homologous of *S. cerevisiae WHI2* in *U. virens* was disrupted to characterize its function. RNA-Seq, qRT-PCR (quantitative Real-time PCR) and phenotypic analyses showed that UvWhi2 is involved in the growth, conidiation, pathogenicity, and various stresses response in *U. virens*. Furthermore, Δ*Uvwhi2* showed defects in the formation of secondary spores during germination on the nutrient limited rice surface, which highlights vital roles of UvWhi2 in stress response and pathogenicity in *U. virens*

Results

Identification of UvWhi2 in *U. virens*

To identify the homolog of Whi2 in *U. virens*, we used ScWhi2 (accession number NP_014686) sequence as a query to do a blastP search in GeneBank to obtain the most closely match protein, which is named as UvWhi2 (accession number KDB15335.1) (Fig. 1a). Sequence analysis with motif scan revealed that UvWhi2 contained a signal peptide and GSDH (Glucose/Sorbosone dehydrogenase; Members of this family are glucose/sorbosone dehydrogenases that possess a beta-propeller fold.) domain. The phylogenetic analysis of the amino acid sequences of Whi2 from *U. virens*, *Metarhizium robertsii*, *Aspergillus vadensis*, *Beauveria bassiana*, *Magnaporthe oryzae* and *S. cerevisiae*, showed that Whi2 is conserved in various fungi, and UvWhi2 is highly similar to MrWhi2 (Fig. 1b). In addition, the qRT-PCR analysis showed that the transcription level of *UvWHI2* at 3, 5 and 9 dpi (days post inoculation) were significantly higher than mycelia (Fig. 1c), suggesting that UvWhi2 may have important roles in the infection process of *U. virens*.

Knockout and complementation of *UvWHI2* in *U. virens*

To analyze the function of *UvWHI2*, the *UvWHI2* deletion mutants was generated by replacing the targeted gene with *hygromycin resistant cassette* in the wild type (WT) strain HWD-2 (Fig. 2a). Then, qRT-PCR and Southern blot assays were performed to confirm the targeted gene deletion events and exclude ectopic integrations (Fig. 2b). Six Δ*Uvwhi2* transformants were obtained with similar phenotypes, and two mutants (Δ*Uvwhi2*-39 and 43) were chosen for the subsequent further experiments (Fig. 2b). To determine whether the altered phenotypes in the Δ*Uvwhi2* mutant were caused by deletion of the *UvWHI2*, the full-length gene copy of *UvWHI2* with its native promoter was inserted into the vector *pFGL823* and transformed into the Δ*Uvwhi2*-39 strain. The resultant Δ*Uvwhi2*-C strains were confirmed by PCR and qRT-PCR analyses, which

showed that the abundance of *UvWHI2* transcript was comparable to that of the WT strain (Fig. 2c, d). Furthermore, the colony morphology of Δ*Uvwhi2*-C strain was the same as that of the WT strain, suggesting that *UvWHI2* functionally complemented the phenotype of the Δ*Uvwhi2* strain.

Knockout of *UvWHI2* compromised fungal growth but enhanced conidiation under nutrient-rich condition

To explore the function of UvWhi2 in vegetative growth and colony morphology of *U. virens*, the growth of WT, Δ*Uvwhi2*, and Δ*Uvwhi2*-C strains were determined on the PSA (potato sucrose agar) medium cultured for 14 d. The Δ*Uvwhi2* mutant showed smaller colonies than the WT and complementation strains (Fig. 3a). Considering the reduction of colony size in the mutants, the dry weight of mycelium was measured after 7 d of the PS liquid culture to determine whether there was a difference in biomass between the WT and Δ*Uvwhi2* strains. The results showed that mycelial dry weight of the Δ*Uvwhi2* strains were lower than that of the WT strain (Fig. 3c). In contrast, the colony morphology and mycelia dry weight were rescued in the Δ*Uvwhi2*-C strain (Fig. 3b, c), suggesting that UvWhi2 plays important roles in the vegetative growth in *U. virens*.

The conidia play an important role during the infection processes of *U. virens* (Fan et al., 2016, Xie et al., 2019, Meng et al., 2020, Xiong et al., 2020). To analysis the function of *UvWHI2* in conidiation, the WT, Δ*Uvwhi2* and Δ*Uvwhi2*-C strains were cultured in the liquid PS medium for 7 d. Although the vegetative growth of Δ*Uvwhi2* strain reduced, the conidial production of the Δ*Uvwhi2* mutant was more than those of the WT and Δ*Uvwhi2*-C strains (Fig. 3d, e). These results showed that UvWhi2 acts as a negative regulator in the sporulation production process in nutrient rich medium.

UvWhi2 is required for pathogenesis in *U. virens*

To evaluate whether deletion of *UvWHI2* can affect the virulence of rice false smut, the WT, Δ*Uvwhi2*-39 and 43, and Δ*Uvwhi2*-C strains were inoculated into panicles of the susceptible rice cultivar Wanxian 98 (*Oryza sativa* L. *indica*). Three weeks after the inoculations, it was found that the number of smut balls formed in panicles inoculated with the Δ*Uvwhi2*-39 and 43 strains was significantly less than those of the WT and Δ*Uvwhi2*-C strains (Fig. 4a, b). Therefore, knockout of *UvWHI2* remarkably attenuated *U. virens* virulence, suggesting that UvWhi2 is a key regulator factor of the pathogenicity in *U. virens*.

The RNA-Seq and phenotypic analysis showed that UvWhi2 is involved in various stresses responses

To further explore the function of *UvWHI2* in *U. virens*, the RNA-Seq analysis was performed with the Δ*Uvwhi2* and WT strains. RNA-Seq analysis showed that the number of SDEGs (significantly differentially expressed gene, FDR adjusted $p \leq 0.05$ & absolute $\log_2$ fold ≥ 2) was 802, of which 515 were up-regulated and 287 were down-regulated in the Δ*Uvwhi2* mutant when compared with the WT strain (Table S1). And the SDEGs could be divided into three major functional groups: biological process, cellular component, and molecular function based on GO analysis (Fig. S1a). Notably, some peroxidase activity genes, chitin deposition genes, and peroxidase activity genes were shown in the differential expression genes (Fig. S1b).

Synthesis of the laccase, peroxidase, chitin synthase, and hyperosmotic genes play an important role in stress response (Martin et al., 2007, Song et al., 2010, Li et al., 2016, Zheng et al., 2016). Comparing with the WT strain, the expression level of chitin deposition genes (*Uv8b_7958*, *Uv8b_7948*, *Uv8b_4757*, and *Uv8b_3223*) of the Δ*Uvwhi2* strain were significantly lower (Fig. 5a), suggesting that UvWhi2 may be involved in the cell wall stresses response. Then, the WT and Δ*Uvwhi2* strains were cultured on the PSA with 0.03% Sodium dodecyl sulfate (SDS), 120 μg/mL Calcofluor

white (CFW) and 120 μg/mL Congo red (CR), which are cell wall disruptors, to determine the sensitivity of indicated strain to these agents. The results showed that the Δ*Uvwhi2* strains were more sensitive to the SDS, CFW and CR than the WT strain (Fig. 5d, e). In addition, the expression levels of laccase and peroxidase activity genes (*Uv8b_1784*, *Uv8b_990*, *Uv8b_6387*, *Uv8b_4892*, and *Uv8b_4252*) were significantly reduced in the Δ*Uvwhi2* strain (Fig. 5c). Moreover, the growth of both Δ*Uvwhi2* strains was more strongly inhibited by the presence of 0.03% H_2O_2 (Oxidative stress agent) than that of the WT and Δ*Uvwhi2-C* strains (Fig. 5d, e). In contrast, the expression levels of hyperosmotic activity genes (*Uv8b_1888* and *Uv8b_6309*) were notably increased compared with the WT strain (Fig. 5b). Consistently, in the presence of 0.4 M NaCl or 0.7 M sorbitol, the growth of the WT strain was reduced by 70%, but that of Δ*Uvwhi2* mutants reduced by approximate 35%, indicating Δ*Uvwhi2* was less sensitive to osmotic stresses than the WT strain (Fig. 5d, e). In conclusion, these results suggested that *UvWHI2* contributes to the responses to cell wall, oxidative and osmotic stresses in *U. virens*.

In addition, the RNA-Seq and qRT-PCR results also showed that the expression levels of sugar-related genes (*Uv8b_1668* and *Uv8b_1099*) in the Δ*Uvwhi2* mutant strain were lower than the WT strain (Fig. 6a). We assumed that UvWhi2 may be involved in the regulation of cell growth and proliferation in response to nutrient stress as Whi2 in other fungus (Kaida et al., 2002, Leadsham et al., 2009, Chen et al., 2018). To confirm this hypothesis, the WT, Δ*Uvwhi2*, and Δ*Uvwhi2*-C strains were cultured on the SD (synthetic dropout medium), SD-G (synthetic dropout medium without glucose), and SD-N (synthetic dropout medium without nitrogen) plates. After 15 d of incubation, comparing with the WT strain on the SD medium, the Δ*Uvwhi2* strains were significantly reduced in mycelia growth on both the SD-G and SD-N plates (Fig. 6b, c). In contrast, the complementation strain Δ*Uvwhi2*-C showed similar phenotypes as the WT strain under all observed stress conditions. All these results indicated that UvWhi2

is involved in the regulation of nutrient stresses responses in *U. virens*.

Defects in the stress response compromised the secondary spore formation on the rice surface in the Δ*Uvwhi2* mutant

In order to further determine whether the defects in stress response in the Δ*Uvwhi2* mutant affect pathogenesis, we carefully observed the germination processes. We found that the Δ*Uvwhi2* mutant had a significant increase in the formation of secondary spores on the PSA medium plate (Fig. 7a). In contrast, when the conidia were germinated on the agarose plate without other nutrient supplementation, the formation of secondary spores was sharply decreased (Fig. 7b). Moreover, secondary spore formation of the Δ*Uvwhi2* mutant was even rarer on the rice surface (Fig. 7c), which might have limited nutrient and oxidative stress (Fan et al., 2014, Wang et al., 2019). These results suggested that the secondary spore formation of the Δ*Uvwhi2* mutant is compromised under the nutrient limited condition and rice surface. And we inferred that the highly decreased secondary spore formation on the rice surface is one of the main reasons for the lost pathogenicity in the Δ*Uvwhi2* mutant.

Discussion

Rice false smut has posed a serious threat to the yield and quality of rice in recent years, so it is important to study the pathogenesis of *U. virens*. Here, we identified a general stress response factor Whi2 in *U. virens*. According to our results, UvWhi2 play important roles in the hyphal growth, conidiation, various stresses responses, and pathogenicity.

In *U. virens*, a series of genes related to pathogenicity have been identified, but the pathogenic mechanism of this fungus is still largely unknown (Zheng et al., 2016, Zheng et al., 2017, Yu et al., 2019, Xiong et al., 2020, Lv et al., 2016, Tang et al., 2019, Xie et al., 2019). In this study, deletion of *UvWHI2* highly reduced the pathogenicity of

U. virens to rice. Through a series of analysis, we found that, in addition to the compromised fungal growth, the reduced pathogenicity in Δ*Uvwhi2* mutant was likely caused by highly reduced formation of secondary spores on the rice surface. During pathogenic process of *U. virens*, the formation of secondary spores tends to greatly increase the amount of inoculums available to infect rice plants (Fan et al., 2014). In addition, the deletion mutant of *UvATG8*, which encodes an ubiquitin-like protein required for autophagy-independent function, has defects in formation of secondary spores and showed the loss of pathogenicity, suggesting that the formation of secondary spores play an important role in the infection of *U. virens* (Meng et al., 2020). In the Δ*Uvwhi2* mutant, secondary spore formation of the Δ*Uvwhi2* mutant was rarer on the rice surface. Thus, we inferred that the main reason for the highly reduced of pathogenicity of the Δ*Uvwhi2* mutant may be the decrease of secondary spore formation.

In *U. virens*, UvPmk1, UvCdc2, UvPro1, UvBI-1, UvAc1, UvPdeh, UvPsr1, UvCom1, UvHox2, and UvAtg8 are involved in both various stresses responses and pathogenesis (Lv et al., 2016, Guo et al., 2019, Tang et al., 2019, Xie et al., 2019, Yu et al., 2019, Meng et al., 2020, Xiong et al., 2020). However, it is not clear whether and how defects of stress responses affect the pathogenesis processes of *U. virens.* This study analyzed the role of a general stress response factor Whi2 to reveal the relationship between the stress response and pathogenicity in *U. virens.* In yeast, the homolog of Whi2, ScWhi2, has been identified to be involved in various stress responses (Mendl et al., 2011, Harata et al., 2016, Teng et al., 2018). ScWhi2 interacts with the protein ScPsr1 to activate STRE-mediated genes, possibly via the dephosphorylation of the Msn2 transcription factor (Kaida et al., 2002, Boeckstaens et al., 2014), and then regulates the downstream stress response. In *P. anserine*, PaWhi2 is also likely affected in nutrient sensing and regulate the vegetative growth (Timpano et al., 2016). In this study, the results of comparative RNA-Seq analysis showed that some stress response genes were differential expressed in the WT and Δ*Uvwhi2* mutant

strains. Consistent with the RNA-Seq results, knockout of *UvWHI2* reduced the sensitive to salinity stress, increased the sensitive to oxidative stress and cell wall stresses, and decreased the hyphal growth under nutrient limited conditions, indicating that UvWhi2 is also involved in various stresses responses in *U. virens*. Furthermore, we noted that deletion of *UvWHI2* enhanced secondary spore formation on the PSA plate, on the contrary, reduced secondary spore formation on the nutrient limited surface, including the agarose plate without other nutrient supplementation and the rice surface. These results suggest that the defects of stress responses in the Δ*Uvwhi2* mutant affected the formation of secondary spores on the nutrient limited rice surface, leading to a severe reduction in pathogenicity of *U. virens*.

In conclusion, our results suggest that UvWhi2 is necessary for fungal growth, stress responses, conidiation, secondary spore formation, and pathogenicity in *U. virens*. In addition, the defects of stress responses could affect the formation of secondary spores on the rice surface, and then affect the pathogenicity of *U. virens.*

Materials and methods

Sequence analysis

The sequences of the genes and proteins used in this study were downloaded from the National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/). The motif scan was performed online (https://myhits.isb-sib.ch/cgi-bin/motif_scan). The protein sequence alignments were processed using EPSript 3.0. The following phylogenetic analyses were performed using MEGA 5.0 with a neighbor-joining algorithm method (Robert & Gouet, 2014).

Fungal growth

The *U. virens* wild-type strain HWD-2 was a kind gift from the Huazhong agriculture university (Hubei, Wuhan). The *U. virens* strain was cultured at a constant temperature

of 28°C on the PSA (potato 200 g/L, sucrose 20 g/L, and agar 20 g/L). For liquid culture, the mycelia were shaken in the PS (200 g/L potato and 20.0 g/L sucrose) medium at 170 rpm and 28℃ for 7 d. The mycelia growth, mycelia dry weight, conidia concentration was measured as described (Meng et al., 2020). The conidial germination experiments were performed on the PSA, agarose plates, and the rice sheath respectively. Three independent biological experiments were performed with three replicates each time.

Construction of the Δ*Uvwhi2* strains and complementation analyses

To construct the *UvWHI2* deletion mutant strain using the gene replacement strategy, the 1026 bp upstream and 989 bp downstream flanking sequences of *UvWHI2* were amplified from the genomic DNA of the HWD-2 strain using the primer pairs Uvwhi2-5F/R and Uvwhi2-3F/R (Table S2). Then, these flanking fragments of *UvWHI2* were cloned into vector *pFGL821* (Addgene: 58224, www.addgene.org) (Xiong et al., 2020). For the complementation, the *UvWHI2* fragment, including coding region, promoter and 3'-UTR regions of *UvWHI2* (Table S2), was inserted into the vector *pFGL823*, which was derived from the replacement of *hygromycin phosphotransferase* gene cassette in *pFGL821* by *neomycin resistance gene*. *Agrobacterium*-mediated transformation was applied for genetic transformation in *U. virens* following the protocol (Yu et al., 2015). The correct transformants of Δ*Uvwhi2* and complementation assay were ascertained using Southern blot and qRT-PCR analyses.

Southern blotting, RNA isolation and qRT-PCR analyses

For Southern blot analysis, the genomic DNA of the WT strain and mutants were extracted and digested with *Xho* I. Then the digested products of genomic DNA were size fractionated through 0.8% agarose gel and mounted onto positively charged nylon membrane (GE Healthcare, Buckinghamshire, UK). The purified probe UvWHI2-probe-F/R (Table S2) was DIG-labeled with Labeling Reagents (GE Healthcare,

309 Buckinghamshire, UK) to hybridize with the digested products of the WT and Δ*Uvwhi2*
310 strains. All the hybridization process was executed following the manufacturing
311 instruction of Amersham^{TM} AlkPhos Direct Labeling Reagents (GE Healthcare,
312 Buckinghamshire, UK). Then, ChemiDoc XRS+ system (Bio-Rad, Hercules, USA) was
313 used to detect the signals of Southern Blotting.
314
315 The total RNA of the fungal mycelia was extracted using the Fungal RNA Kit 200
316 (OMEGA). The first-stranded cDNA was synthesized with a reverse transcription kit
317 (TaKaRa, Japan), and then TB Green^{TM} Premix Ex Taq^{TM} (Tli RnaseH Plus) was used
318 for qRT-PCR analysis. The *β-Tubulin* gene was used as the endogenous reference gene
319 (Table S2). The relative expression levels of *UvWHI2* gene were calculated using the
320 $2^{-\Delta\Delta}$Ct method.
321
322 **Pathogenicity assay**
323 For the pathogenicity assay, liquid culture of mycelia and conidia (1×10^6 conidia/mL)
324 were mixed together, and broken down in a juice blender. Then, the conidia suspension
325 was injected into the panicles of selected rice cultivar Wanxian 98 (a susceptible rice
326 cultivar, *Oryza sativa* L. *indica*) at the booting stage. The inoculated plants were
327 cultivated under 12 h light/dark conditions at 25℃ and 95% humidity. Then, the
328 phenotype of smut balls was measured and scanned at 21 dpi. All the experiments in
329 this part were repeated three times in each test.
330
331 **RNA-Seq library preparation and Illumina sequencing**
332 Total RNA was extracted from seven-day-old mycelia and conidia of WT and Δ*Uvwhi2*-
333 39 strains using the Fungal RNA Kit 200 (OMEGA). A total of 3 μg RNA and the
334 NEBNext® UltraTM RNA Library Prep Kit was used to generate RNA-Seq
335 transcriptome libraries for sequencing on Illumina® (NEB, USA) following
336 manufacturer's recommendations.

For the RNA-Seq data analysis, the reads containing adapter and ploy-N and low-quality reads were removed to obtain clean reads base on their error rate, Q20, Q30, and GC contents. Reference genome and gene model annotation files were downloaded from genome website directly (https://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/687/475/GCA_000687475.1_Assembly_for_version_1_of_the_Villosiclava_virens_genome/). Then, the eatureCounts v1.5.0-p3 was used to map the clean reads to reference genome. And each gene expression level was based on the FPKM (Fragments Per Kilobase per Million). Differential expression genes of two samples was obtained using the DESeq2 software with FDR adjusted $p \leqslant 0.05$ & absolute log2 fold $\geqslant 2$. Gene Ontology (GO) enrichment analysis was performed under the Bonferroni-corrected P-value $\leqslant 0.05$ compared with the whole-transcriptome background. GO functional enrichment analysis was finished on the website (https://www.omicshare.com/tools/).

Abiotic stresses response analysis

To test the sensitivity to various abiotic stresses, the WT, Δ*Uvwhi2*, and Δ*Uvwhi2*-C strains were cultured at 28℃ for 15 d on the PSA and PSA with 0.03% H_2O_2, 0.4 M NaCl (Solarbio, S8210), 0.7 M sorbitol (Coolaber, CS10381), 0.03% SDS (Sigma, L3771), 120 μg/mL CFW (Sigma, F3543), and 120 μg/mL CR (Solarbio, IC1000) to measure the colony diameters. The formula of inhibition rate was calculated as follow: Inhibition rate = (average of strain colony diameters on the PSA - the average of strain colony diameters on the PSA with different chemicals)/average of the strain colony diameters on the PSA × 100%. For the nutrient starvation assay, the SD (Yeast nitrogen base without amino acids 1.7 g/L, Ammonium sulfate, 5g/L glucose 20g/L, and agar 20 g/L) medium, SD-G (Yeast nitrogen base without amino acids 1.7 g/L, Ammonium sulfate, 5g/L glucose 20g/L, and agar 20 g/L), and SD-N (Yeast nitrogen base without amino acids 1.7 g/L, glucose 20 g/L, and agar 20 g/L) plates were used. All the experiments were performed three times with three replicates.

Abbreviations

Whi2, Whiskey2; CFW, calcofluor white; CR, congo red; d, days; dpi, days post inoculation; Kb, kilobases; *F. graminearum, Fusarium graminearum*; *M. oryzae, Magnaporthe oryzae*; mins, minutes; PS, potato sucrose medium; PSA, potato sucrose agar medium; SD, synthetic dropout medium; SD-G, synthetic dropout medium without glucose; SD-N, synthetic dropout medium without nitrogen; *S. cerevisiae*, *Saccharomyces cerevisiae*; SDS, Sodium dodecyl sulfate; *U. virens*, *Ustilaginoidea virens*; *P. anserina*, *Podospora anserina*; WT, wild type; qRT-PCR, quantitative real-time polymerase chain reaction.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional files.

Competing interests

There is no conflict of interest.

Funding: This research was funded by Zhejiang Provincial Natural Science Foundation of China, grant number "LQ19C140004" and key R&D project of Zhejiang Province, grant number "2019C02018".

Authors' contributions

YJK and HBS planned and designed the research. SM, JHQ, MX, JSJ, and HBS performed experiments, conducted field work, and analyzed data etc. SM, MX, and YJK wrote the manuscript. All authors read, revised and approved the final manuscript.

Acknowledgements

This project was supported by the Chinese Academy of Agricultural Sciences under the "Elite Youth" program, the Agricultural Sciences and Technologies Innovation Program. We thank Prof. Junbin Huang for providing the HWD-2 strain.

Supplemental information

Table S1. Differently expression genes and GO enrichment analysis between the WT and Δ*Uvwhi2* strains.

Table S2. Primers used in this study.

Fig. S1 Comparative transcriptomic analysis of the WT and Δ*Uvwhi2* strains.

Fig. 1 Identification of UvWhi2 in *Ustilaginoidea virens*. a, The multiple alignment of amino acid sequences of UvWhi2, MrWhi2, BbWhi2, MoWhi2, AvWhi2 and ScWhi2. Amino acids in black and gray represent amino acids identity and fifty percent similarity, respectively. b, Phylogeny analysis of Whi2 orthologs from different fungi was constructed by MEGA 5.0 using the neighbor-joining method. The sequences included UvWhi2 (*U. virens*, KDB15335.1), MrWhi2 (*Metarhizium robertsii*, XM_007820024.1), BbWhi2 (*Beauveria bassiana*, XM_008598486.1), MoWhi2 (*Magnaporthe oryzae*, XP_003714147.1), AvWhi2 (*Aspergillus vadensis*, XM_025709355.1), and ScWhi2 (*Saccharomyces cerevisiae*, NP_014686). c, The transcriptional analysis of *UvWHI2* in the mycelia and inoculated panicles of WanXian 98 (*oryza sativa* L. *indica*) at 3, 5, and 9 dpi. The data represents the mean ± SD from three independent replicates. The data were subject to Duncan's Test and the significant

differences were indicated in the figure with three asterisks (***, $p < 0.001$).

Fig. 2 Targeted gene deletion of *UvWHI2* and complementation assay in *U. virens*. a, Construction strategy for the *UvWHI2* gene deletion mutant in *U. virens*. The coding region of *UvWHI2* was replaced with the *hygromycin phosphotransferase* gene cassette (*HYG*) by homologous recombination. b, The Southern blot assay was used to validate the loss of *UvWHI2* in the Δ*Uvwhi2* deletion mutant. The *Xho* I enzyme was used to digested genomic DNA of the WT and Δ*Uvwhi2* strains. The digested genomic were processed for the Southern blotting with the 1 Kb downstream of *UvWHI2* as probe. c, qRT-PCR analysis of the expression level of *UvWHI2* in the WT, Δ*Uvwhi2*, and Δ*Uvwhi2*-C strains. The data represents the mean ± SD from three independent replicates. The asterisks (***) indicate a P value < 0.001.

Fig. 3 Deletion of *UvWHI2* results in decreased vegetative growth and increased conidiation in *U. virens*. a, Mycelia growth of the WT, Δ*Uvwhi2*, and Δ*Uvwhi2*-C strains on the PSA (potato sucrose agar) medium in dark at 28℃ for 15 d. b, Colony morphology of indicated strains. c, Dry weight of mycelia was measured in the PS medium after 7 d culture. d and e, Knockout of *UvWHI2* enhanced conidiation under nutrient-rich condition. Data represents the mean ± SD from three independent replicates. The data were subject to Duncan's Test and the significant differences were indicated in the figure with three asterisks (***, $p < 0.001$). Scale bar = 5 μm.

Fig. 4 UvWhi2 is required for pathogenesis in *U. virens*. a, Disease symptoms of indicated strain on the rice panicles of WanXian 98 at 21 dpi. b, Statistical analysis of the average number of false smut balls on the inoculated spikelets. Each experiment was performed with three independent biological experiments and more than thirty panicles were inoculated each time. Data were showed as Mean ± SD (n = 3). ***, $p < 0.001$.

Fig. 5 *UvWHI2* contribute to the stress responses to the cell wall, oxidative and osmotic agent in *U. virens*. a, b and c, qRT-PCR analysis of the expression of the genes related to laccase, peroxidase, chitin deposition and hyperosmotic activities, respectively. d, The tested strains grown on the PSA or PSA with 0.03% H_2O_2 (Oxidative stress agent), 0.03% SDS (Sodium dodecyl sulfate), 120 μg/mL CFW (Calcofluor white), 120 μg/mL CR (Congo red), 0.4 M NaCl, or 0.7 M sorbitol. Typical cultures were photographed after 15 d at 28℃. e, Statistical analysis of inhibition rate of tested strains with different stress agents. The diameters of colonies were measured and calculated. Similar results were obtained by three repeated experiments. The error bars represent the standard deviation and the asterisk represents the significant difference compared to the WT strain under the same conditions (*, $p < 0.01$; **, $p < 0.005$; ***, $p < 0.001$).

Fig. 6 UvWhi2 is involved in the regulation of nutrient stresses responses in *U. virens*. a, qRT-PCR analysis of the genes related to sugar synthesis. b, The growth of tested strains on the SD (synthetic dropout medium), SD-G (synthetic dropout medium without glucose), and SD-N (synthetic dropout medium without nitrogen) medium. Typical cultures were photographed after culturing for 15 d at 28℃. The diameters of colonies were measured to calculate the inhibition rate. c, Statistical analysis of tested strains grown on the SD-G and SD-N medium at 15 d. Similar results were obtained by three repeated experiments. ** or ***, $p < 0.005$ or $p < 0.001$.

Fig. 7 Conidial germination of the Δ*Uvwhi2* mutant on the nutrient limited surface and rice sheath surface. a-c, Conidial germination of *U. virens* on the PSA and agarose plates and the rice sheath. The conidia were inoculated on the indicated surface at 28 °C for 3 d. Scale bar = 3 μm.

Boeckstaens M, Llinares E, Van Vooren P, Marini A, M, (2014). The TORC1 effector kinase Npr1 fine tunes the inherent activity of the Mep2 ammonium transport protein. *Nat Commun* 5: 3101. https://doi.org/10.1038/ncomms4101

Chen X, Wang G, Zhang Y, Dayhoff-Brannigan M, Diny NL, Zhao M et al, (2018). Whi2 is a conserved negative regulator of TORC1 in response to low amino acids. *PLoS Genet* 14, (8): e1007592. https://doi.org/10.1371/journal.pgen.1007592

Chen XY, Hai D, Tang JT, Liu H, Huang JB, Luo CX et al, (2020). UvCom1 is an important regulator required for development and infection in the rice false smut fungus *Ustilaginoidea virens*. *Phytopathology* 110, (2): 483-93. https://doi.org/10.1094/PHYTO-05-19-0179-R

Fan J, Guo XY, Huang F, Li Y, Liu YF, Li L et al, (2014). Epiphytic colonization of *Ustilaginoidea virens* on biotic and abiotic surfaces implies the widespread presence of primary inoculum for rice false smut disease. *Plant Pathology* 63: 937-45. https://doi.org/10.1111/ppa.12167

Fan J, Yang J, Wang YQ, Li GB, Li Y, Huang F et al, (2016). Current understanding on *Villosiclava virens*, a unique flower-infecting fungus causing rice false smut disease. *Mol Plant Pathol* 17, (9): 1321-30. https://doi.org/10.1111/mpp.12362

Fang A, Gao H, Zhang N, Zheng X, Qiu S, Li Y et al, (2019). A novel effector gene *SCRE2* contributes to full virulence of *Ustilaginoidea virens* to rice. *Front Microbiol* 10: 845. https://doi.org/10.3389/fmicb.2019.00845

Guo W, Gao Y, Yu Z, Xiao Y, Zhang Z, Zhang H, (2019). The adenylate cyclase UvAc1 and phosphodiesterase UvPdeH control the intracellular cAMP level, development, and pathogenicity of the rice false smut fungus *Ustilaginoidea virens*. *Fungal Genet Biol* 129: 65-73. https://doi.org/10.1016/j.fgb.2019.04.017

Harata K, Nishiuchi T, Kubo Y, (2016). Colletotrichum orbiculare WHI2, a yeast stress-response regulator homolog, controls the biotrophic stage of hemibiotrophic infection through TOR signaling. *Mol Plant Microbe Interact* 29, (6): 468-83. https://doi.org/10.1094/MPMI-02-16-0030-R

Kaida D, Yashiroda H, Tohe A, Kikuchi Y, (2002). Yeast Whi2 and Psr1-phosphatase form a complex and regulate STRE-mediated gene expression. *Genes Cells* 7, (6): 543-52. https://doi.org/10.1046/j.1365-2443.2002.00538.x

Leadsham JE, Miller K, Ayscough KR, Colombo S, Martegani E, Sudbery P et al, (2009). Whi2p links nutritional sensing to actin-dependent Ras-cAMP-PKA regulation and apoptosis in yeast. *J Cell Sci* 122, (Pt 5): 706-15. https://doi.org/10.1242/jcs.042424

Li M, Liu X, Liu Z, Sun Y, Liu M, Wang X et al, (2016). Glycoside hydrolase MoGls2 controls asexual/sexual development, cell wall integrity and infectious growth in the rice blast fungus. *PLoS One* 11, (9): e0162243. https://doi.org/10.1371/journal.pone.0162243

Liang Y, Han Y, Wang C, Jiang C, Xu JR, (2018). Targeted deletion of the *USTA* and

UvSLT2 genes efficiently in *Ustilaginoidea virens* with the CRISPR-Cas9 system. *Front Plant Sci* 9: 699. 10.3389/fpls.2018.00699

Lin X, Bian Y, Mou R, Cao Z, Cao Z, Zhu Z et al, (2018). Isolation, identification, and characterization of *Ustilaginoidea virens* from rice false smut balls with high ustilotoxin production potential. *J Basic Microbiol* 58, (8): 670-8. https://doi.org/10.1002/jobm.201800167

Lu S, Sun W, Meng J, Wang A, Wang X, Tian J et al, (2015). Bioactive bis-naphtho-gamma-pyrones from rice false smut pathogen *Ustilaginoidea virens*. *J Agric Food Chem* 63, (13): 3501-8. https://doi.org/10.1021/acs.jafc.5b00694

Lv B, Zheng L, Liu H, Tang J, Hsiang T, Huang J, (2016). Use of random T-DNA mutagenesis in identification of gene *UvPRO1*, a regulator of conidiation, stress response, and virulence in *Ustilaginoidea virens*. *Front Microbiol* 7: 2086. https://doi.org/10.3389/fmicb.2016.02086

Marsikova J, Pavlickova M, Wilkinson D, Vachova L, Hlavacek O, Hatakova L et al, (2020). The Whi2p-Psr1p/Psr2p complex regulates interference competition and expansion of cells with competitive advantage in yeast colonies. *Proc Natl Acad Sci U S A* 117, (26): 15123-31. https://doi.org/10.1073/pnas.1922076117

Martin JE, Zheng YW, Mark AJ, Nicholas Smirnoff, Nicholas JT, (2007). Generation of reactive oxygen species by fungal NADPH oxidases is required for rice blast disease. *Proc Natl Acad, U S A* 104, (28): 11772-7. https://doi.org/10.1073/pnas.0700574104

Mendl N, Occhipinti A, Muller M, Wild P, Dikic I, Reichert AS, (2011). Mitophagy in yeast is independent of mitochondrial fission and requires the stress response gene *WHI2*. *Journal of Cell Science* 124, (8): 1339-50. https://doi.org/10.1242/jcs.076406

Meng S, Xiong M, Jane SJ, Wang CC, Qiu JH, Shi HB et al, (2020). UvAtg8-mediated autophagy regulates fungal growth, stress responses, conidiation, and pathogenesis in *Ustilaginoidea virens*. *Rice* 13: 56-69. https://doi.org/10.1186/s12284-020-00418-z

Muller M, Reichert AS, (2011). Mitophagy, mitochondrial dynamics and the general stress response in yeast. *Biochem Soc Trans* 39, (5): 1514-9. https://doi.org/10.1042/BST0391514

Qiu JH, Meng S, Deng YZ, Huang SW, Kou YJ, (2019). *Ustilaginoidea virens*: a fungus infects rice flower and threats world rice production. *Rice Science* 26, (4): 199-206. https://doi.org/10.1016/j.rsci.2018.10.007

Robert X, Gouet P, (2014). Deciphering key features in protein structures with the new ENDscript server. *Nucleic Acids Res* 42, (Web Server issue): W320-4. https://doi.org/10.1093/nar/gku316

Sadeh A, Movshovich N, Volokh M, Gheber L, Aharoni A, (2011). Fine-tuning of the Msn2/4-mediated yeast stress responses as revealed by systematic deletion of Msn2/4 partners. *Mol Biol Cell* 22, (17): 3127-38. https://doi.org/10.1091/mbc.E10-12-1007

561 Song W, Dou X, Qi Z, Wang Q, Zhang X, Zhang H et al, (2010). R-SNARE homolog
562 MoSec22 is required for conidiogenesis, cell wall integrity, and pathogenesis of
563 *Magnaporthe oryzae*. *PLoS One* 5, (10): e13193.
564 https://doi.org/10.1371/journal.pone.0013193
565 Sun W, Fan J, Fang A, Li Y, Tariqjaveed M, Li D et al, (2020). *Ustilaginoidea virens*:
566 insights into an emerging rice pathogen. *Annu Rev Phytopathol*.
567 https://doi.org/10.1146/annurev-phyto-010820-012908
568 Sun WB, Wang A, Xu D, Wang WX, Meng JJ, Dai JG et al, (2017). New ustilaginoidins
569 from rice false smut balls caused by *Villosiclava virens* and their phytotoxic and
570 cytotoxic activities. *J Agric Food Chem* 65, (25).
571 https://doi.org/10.1021/acs.jafc.7b01791
572 Tang J, Bai J, Chen X, Zheng L, Liu H, Huang J, (2019). Two protein kinases UvPmk1
573 and UvCdc2 with significant functions in conidiation, stress response and
574 pathogenicity of rice false smut fungus *Ustilaginoidea virens*. *Curr Genet*.
575 https://doi.org/10.1007/s00294-019-01029-y
576 Teng X, Yau E, Sing C, Hardwick JM, (2018). Whi2 signals low leucine availability to
577 halt yeast growth and cell death. *FEMS Yeast Res* 18, (8).
578 https://doi.org/10.1093/femsyr/foy095
579 Timpano H, Chan Ho Tong L, Gautier V, Lalucque H, Silar P, (2016). The *PaPsr1* and
580 *PaWhi2* genes are members of the regulatory network that connect stationary
581 phase to mycelium differentiation and reproduction in *Podospora anserina*.
582 *Fungal Genet Biol* 94: 1-10. https://doi.org/10.1016/j.fgb.2016.06.006
583 Wang X, Wang J, Lai D, Wang W, Dai J, Zhou L et al, (2017). Ustiloxin G, a new
584 cyclopeptide mycotoxin from rice false smut balls. *Toxins (Basel)* 9, (2).
585 https://doi.org/10.3390/toxins9020054
586 Wang Y, Wang F, Xie S, Liu Y, Qu J, Huang J et al, (2019). Development of rice
587 conidiation media for *Ustilaginoidea virens*. *PLoS One* 14, (10): e0217667.
588 https://doi.org/10.1371/journal.pone.0217667
589 Xie S, Wang Y, Wei W, Li C, Liu Y, Qu J et al, (2019). The Bax inhibitor UvBI-1, a
590 negative regulator of mycelial growth and conidiation, mediates stress response
591 and is critical for pathogenicity of the rice false smut fungus *Ustilaginoidea*
592 *virens*. *Curr Genet* 65, (5): 1185-97. 10.1007/s00294-019-00970-2
593 Xiong M, Meng S, Qiu JH, Shi HB, Shen XL, Kou YJ, (2020). Putative phosphatase
594 UvPsr1 is required for mycelial growth, conidiation, stress response and
595 pathogenicity in *Ustilaginonidea virens*. *Rice Science* 27, (6): 529-36.
596 https://doi.org/10.1016/j.rsci.2020.09.009
597 Yu J, Yu M, Song T, Cao H, Pan X, Yong M et al, (2019). A homeobox transcription
598 factor UvHox2 regulates chlamydospore formation, conidiogenesis, and
599 pathogenicity in *Ustilaginoidea virens*. *Front Microbiol* 10: 1071.
600 https://doi.org/10.3389/fmicb.2019.01071
601 Yu M, Yu J, Hu J, Huang L, Wang Y, Yin X et al, (2015). Identification of pathogenicity-
602 related genes in the rice pathogen *Ustilaginoidea virens* through random

603 insertional mutagenesis. *Fungal Genet Biol* 76: 10-9.
604 https://doi.org/10.1016/j.fgb.2015.01.004
605 Zheng D, Wang Y, Han Y, Xu JR, Wang C, (2016). *UvHOG1* is important for hyphal
606 growth and stress responses in the rice false smut fungus *Ustilaginoidea virens*.
607 *Sci Rep* 6: 24824. https://doi.org/10.1038/srep24824
608 Zheng MT, Ding H, Huang L, Wang YH, Yu MN, Zheng R et al, (2017). Low-affinity
609 iron transport protein Uvt3277 is important for pathogenesis in the rice false
610 smut fungus *Ustilaginoidea virens*. *Curr Genet* 63, (1): 131-44.
611 https://doi.org/10.1007/s00294-016-0620-4
612
613

Figures

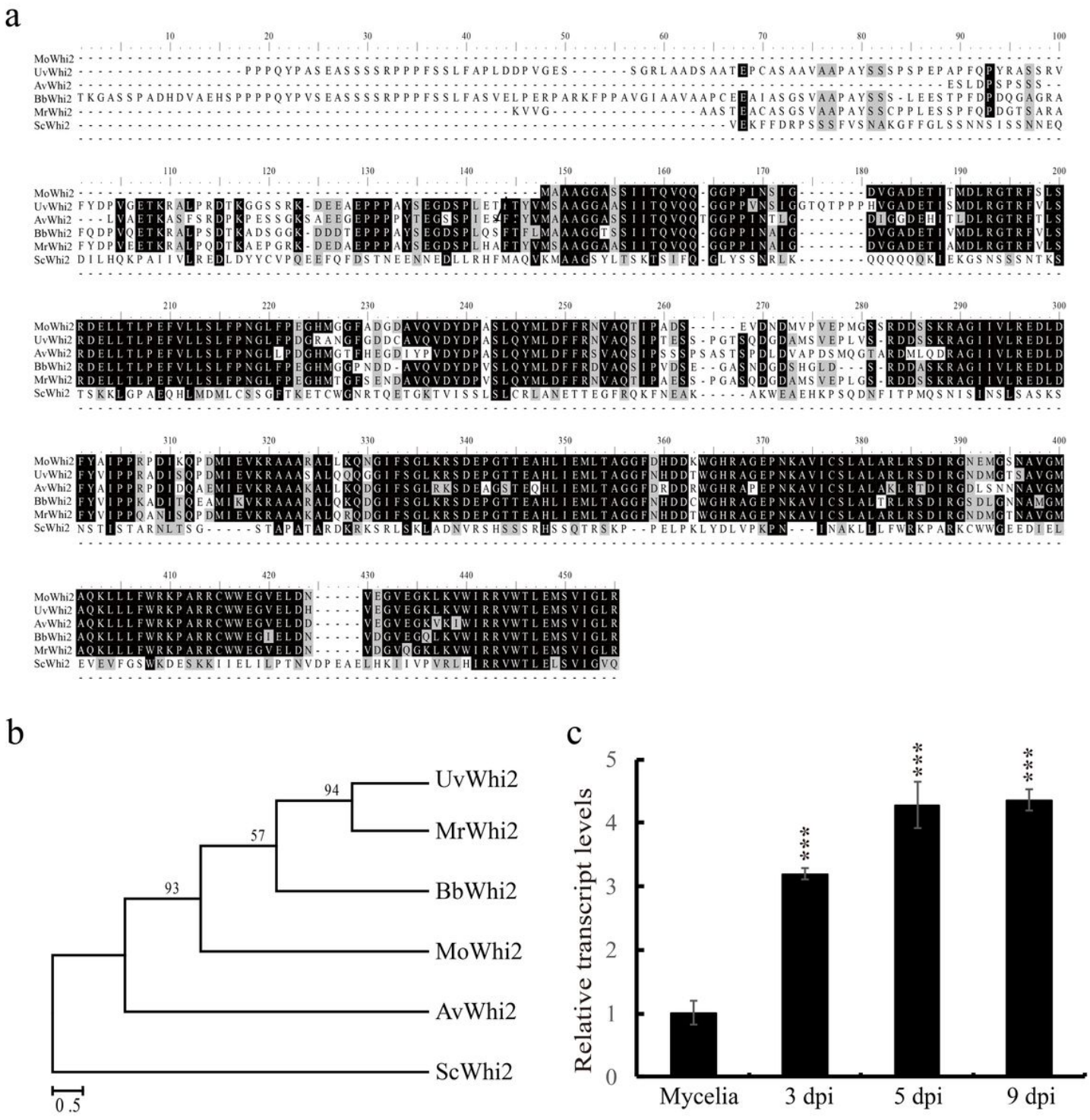

Figure 1

Identification of UvWhi2 in Ustilaginoidea virens. a, The multiple alignment of amino acid sequences of UvWhi2, MrWhi2, BbWhi2, MoWhi2, AvWhi2 and ScWhi2. Amino acids in black and gray represent amino acids identity and fifty percent similarity, respectively. b, Phylogeny analysis of Whi2 orthologs from different fungi was constructed by MEGA 5.0 using the neighbor-joining method. The sequences included

UvWhi2 (U . virens, KDB15335.1), MrWhi2 (Metarhizium robertsii, XM_007820024.1), BbWhi2 (Beauveria bassiana, XM_008598486.1), MoWhi2 (Magnaporthe oryzae, XP_003714147.1), AvWhi2 (Aspergillus vadensis, XM_025709355.1), and ScWhi2 (Saccharomyces cerevisiae, NP_014686). c, The transcriptional analysis of UvWHI2 in the mycelia and inoculated panicles of WanXian 98 (oryza sativa L. indica) at 3, 5, and 9 dpi. The data represents the mean ± SD from three independent replicates. The data were subject to Duncan's Test and the significant differences were indicated in the figure with three asterisks (***, $p < 0.001$).

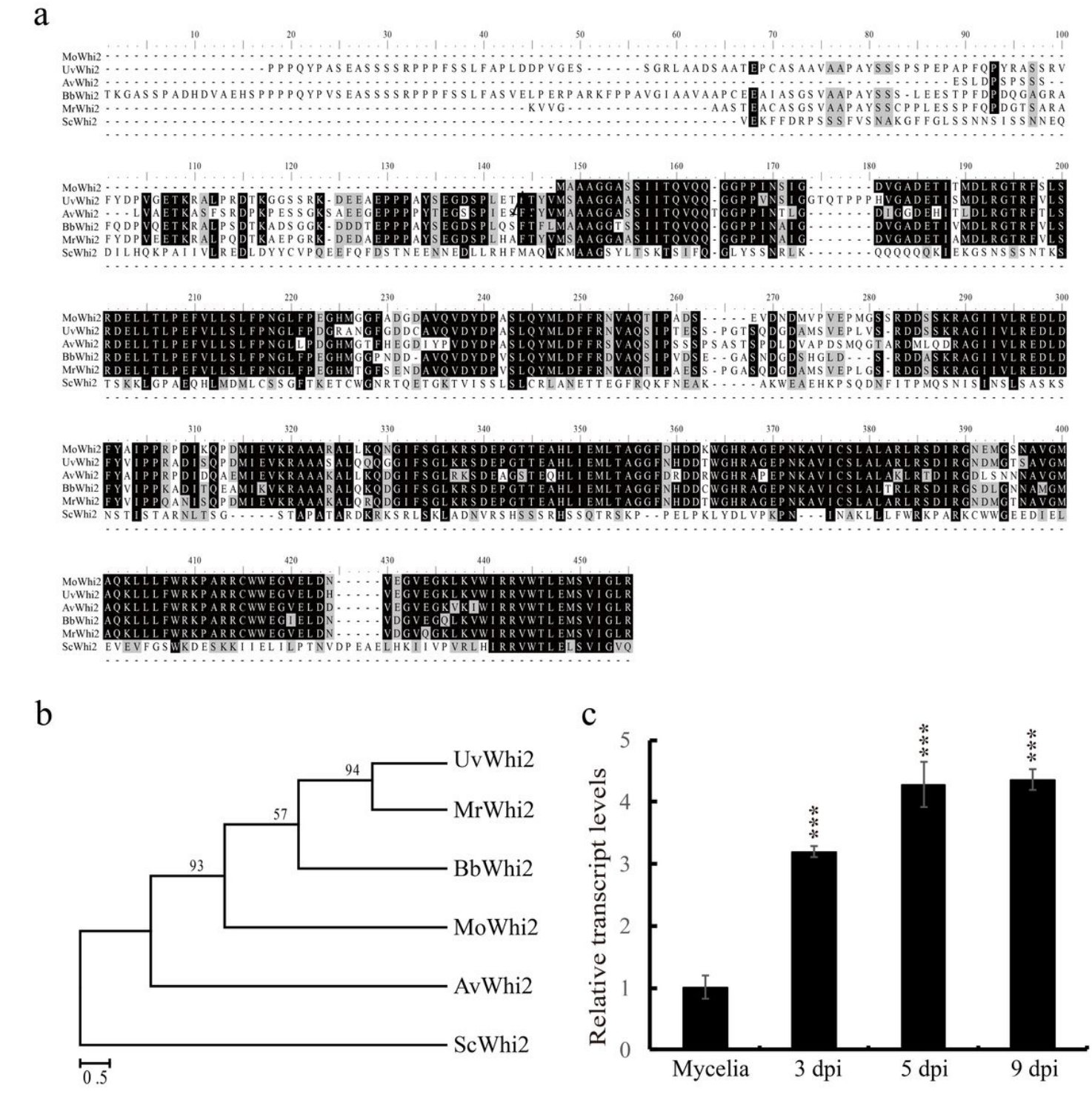

Figure 1

Identification of UvWhi2 in Ustilaginoidea virens. a, The multiple alignment of amino acid sequences of UvWhi2, MrWhi2, BbWhi2, MoWhi2, AvWhi2 and ScWhi2. Amino acids in black and gray represent amino acids identity and fifty percent similarity, respectively. b, Phylogeny analysis of Whi2 orthologs from different fungi was constructed by MEGA 5.0 using the neighbor-joining method. The sequences included UvWhi2 (U . virens, KDB15335.1), MrWhi2 (Metarhizium robertsii, XM_007820024.1), BbWhi2 (Beauveria bassiana, XM_008598486.1), MoWhi2 (Magnaporthe oryzae, XP_003714147.1), AvWhi2 (Aspergillus vadensis, XM_025709355.1), and ScWhi2 (Saccharomyces cerevisiae, NP_014686). c, The transcriptional analysis of UvWHI2 in the mycelia and inoculated panicles of WanXian 98 (oryza sativa L. indica) at 3, 5, and 9 dpi. The data represents the mean ± SD from three independent replicates. The data were subject to Duncan's Test and the significant differences were indicated in the figure with three asterisks (***, $p < 0.001$).

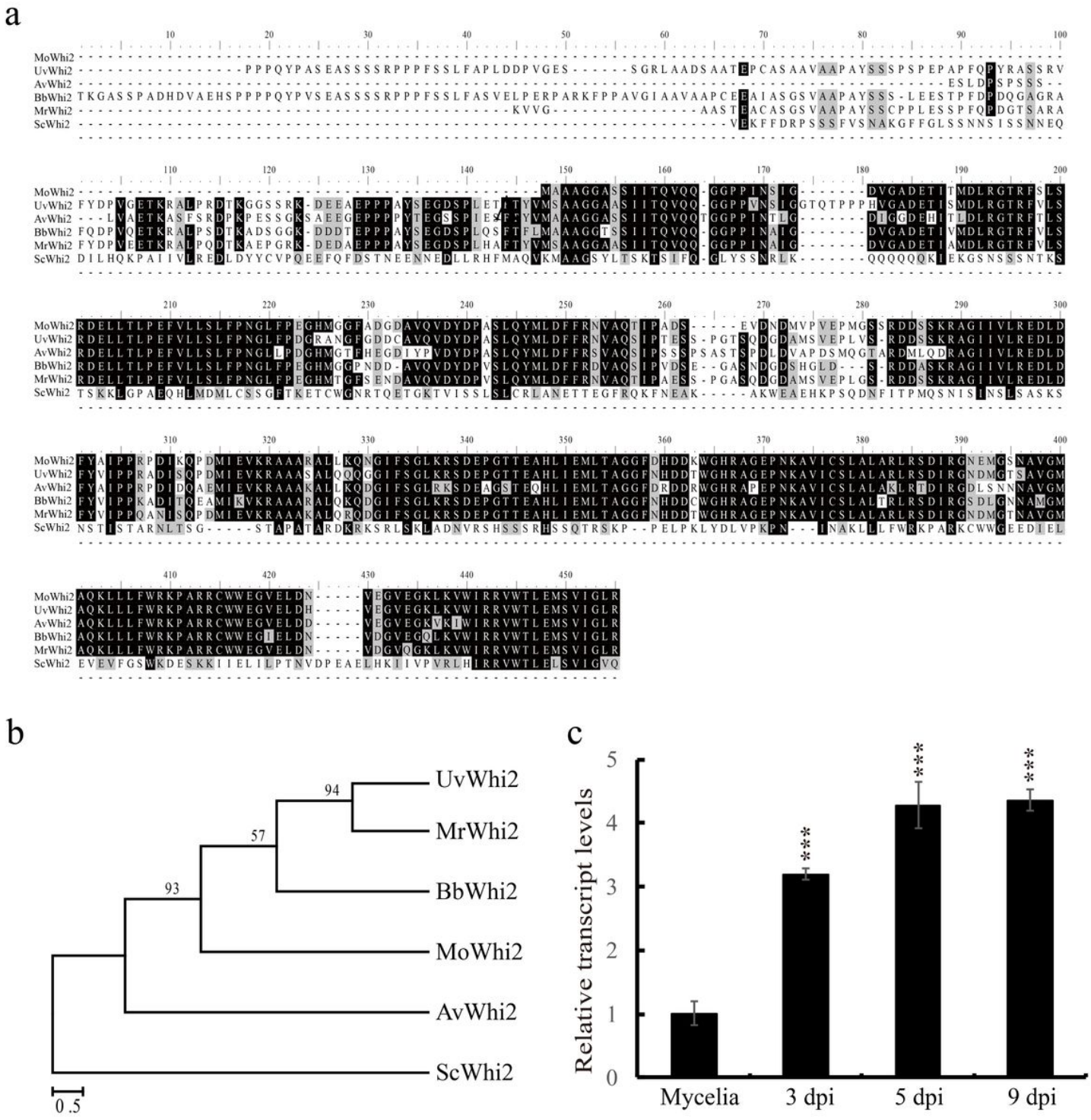

Figure 1

Identification of UvWhi2 in Ustilaginoidea virens. a, The multiple alignment of amino acid sequences of UvWhi2, MrWhi2, BbWhi2, MoWhi2, AvWhi2 and ScWhi2. Amino acids in black and gray represent amino acids identity and fifty percent similarity, respectively. b, Phylogeny analysis of Whi2 orthologs from different fungi was constructed by MEGA 5.0 using the neighbor-joining method. The sequences included UvWhi2 (U . virens, KDB15335.1), MrWhi2 (Metarhizium robertsii, XM_007820024.1), BbWhi2 (Beauveria bassiana, XM_008598486.1), MoWhi2 (Magnaporthe oryzae, XP_003714147.1), AvWhi2 (Aspergillus

vadensis, XM_025709355.1), and ScWhi2 (Saccharomyces cerevisiae, NP_014686). c, The transcriptional analysis of UvWHI2 in the mycelia and inoculated panicles of WanXian 98 (oryza sativa L. indica) at 3, 5, and 9 dpi. The data represents the mean ± SD from three independent replicates. The data were subject to Duncan's Test and the significant differences were indicated in the figure with three asterisks (***, $p < 0.001$).

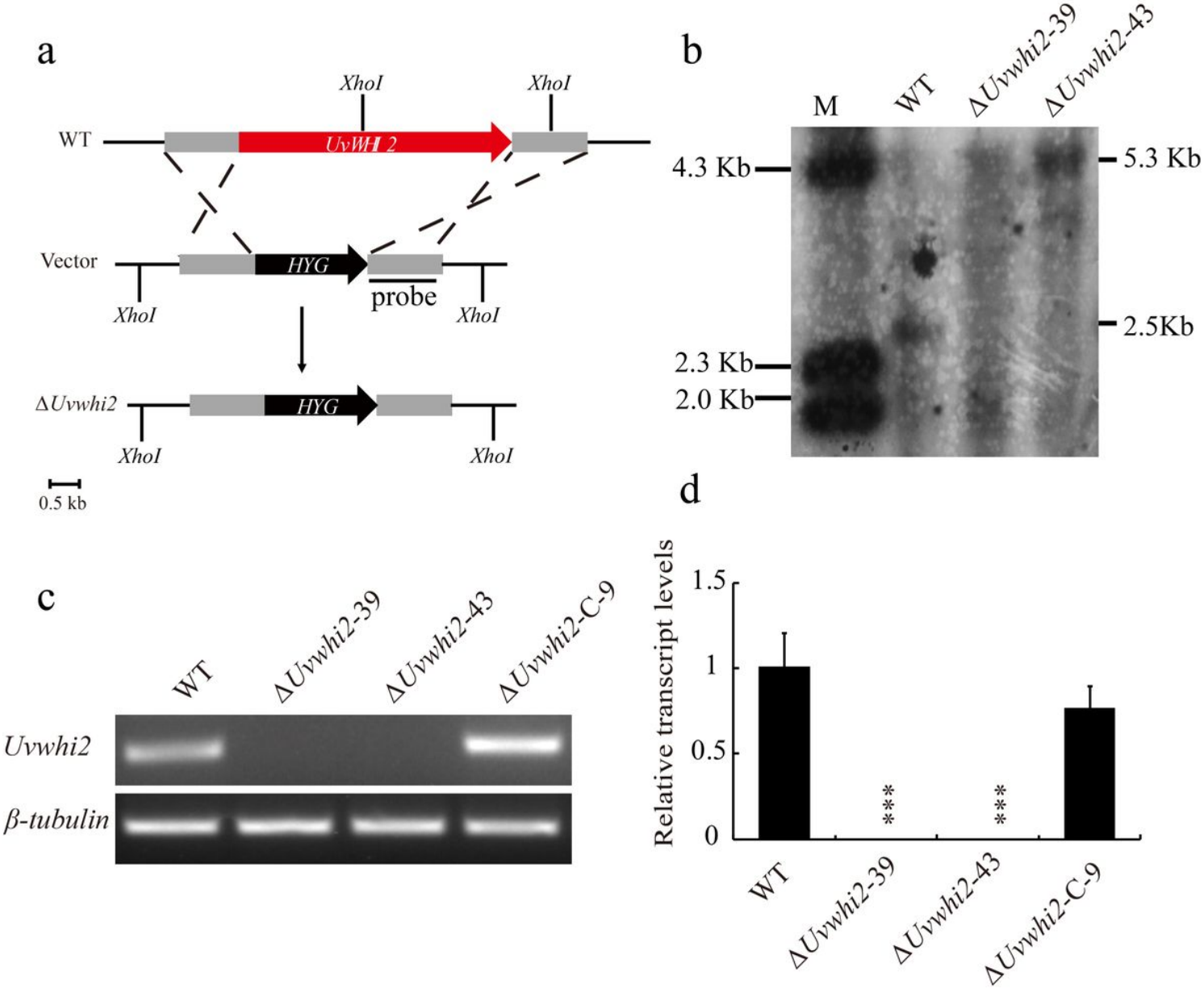

Figure 2

Targeted gene deletion of UvWHI2 and complementation assay in U. virens. a, Construction strategy for the UvWHI2 gene deletion mutant in U. virens. The coding region of UvWHI2 was replaced with the hygromycin phosphotransferase gene cassette (HYG) by homologous recombination. b, The Southern blot assay was used to validate the loss of UvWHI2 in the ΔUvwhi2 deletion mutant. The Xho I enzyme was used to digested genomic DNA of the WT and ΔUvwhi2 strains. The digested genomic were processed for the Southern blotting with the 1 Kb downstream of UvWHI2 as probe. c, qRT-PCR analysis

of the expression level of UvWHI2 in the WT, ΔUvwhi2, and ΔUvwhi2-C strains. The data represents the mean ± SD from three independent replicates. The asterisks (***) indicate a P value < 0.001.

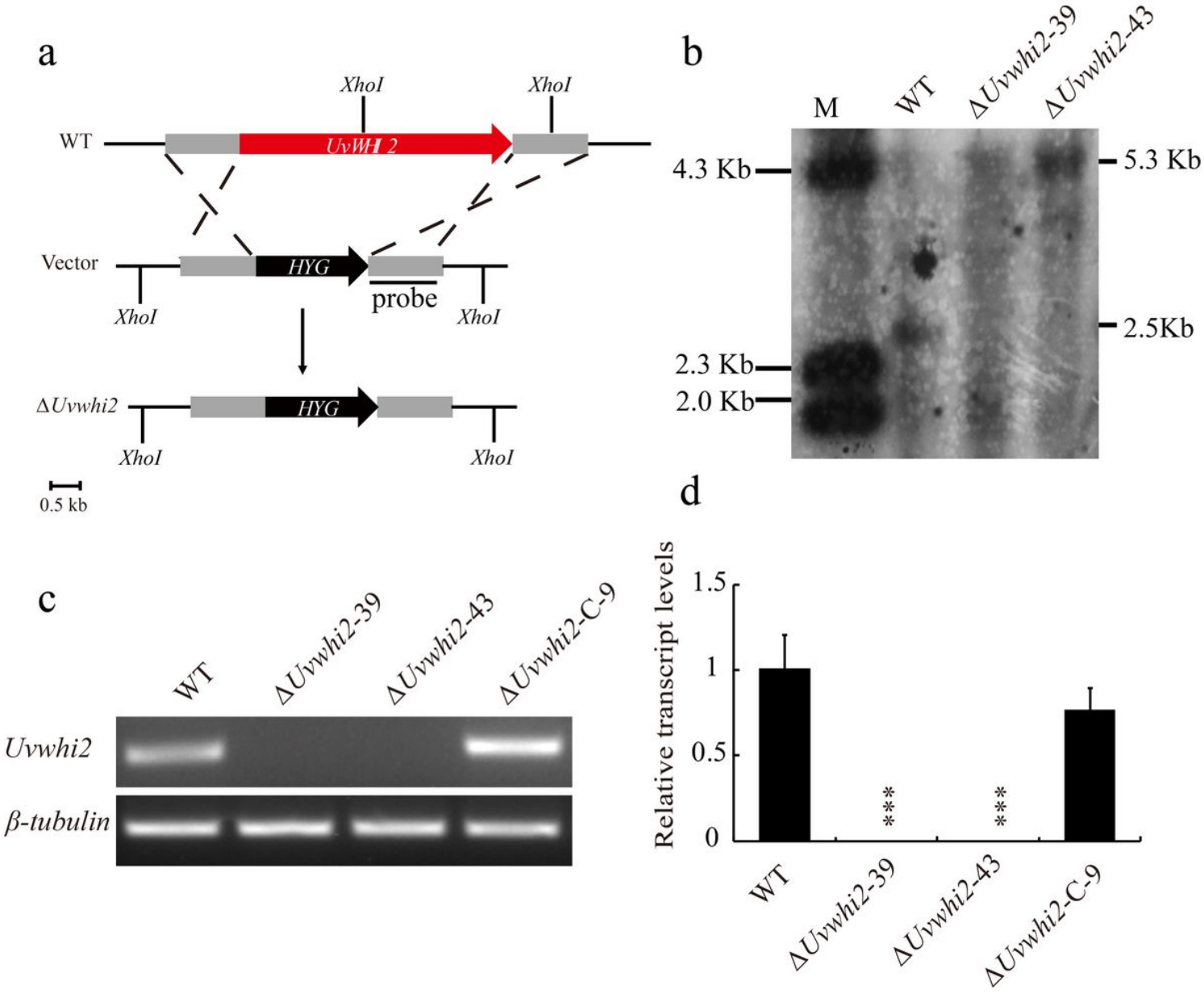

Figure 2

Targeted gene deletion of UvWHI2 and complementation assay in U. virens. a, Construction strategy for the UvWHI2 gene deletion mutant in U. virens. The coding region of UvWHI2 was replaced with the hygromycin phosphotransferase gene cassette (HYG) by homologous recombination. b, The Southern blot assay was used to validate the loss of UvWHI2 in the ΔUvwhi2 deletion mutant. The Xho I enzyme was used to digested genomic DNA of the WT and ΔUvwhi2 strains. The digested genomic were processed for the Southern blotting with the 1 Kb downstream of UvWHI2 as probe. c, qRT-PCR analysis of the expression level of UvWHI2 in the WT, ΔUvwhi2, and ΔUvwhi2-C strains. The data represents the mean ± SD from three independent replicates. The asterisks (***) indicate a P value < 0.001.

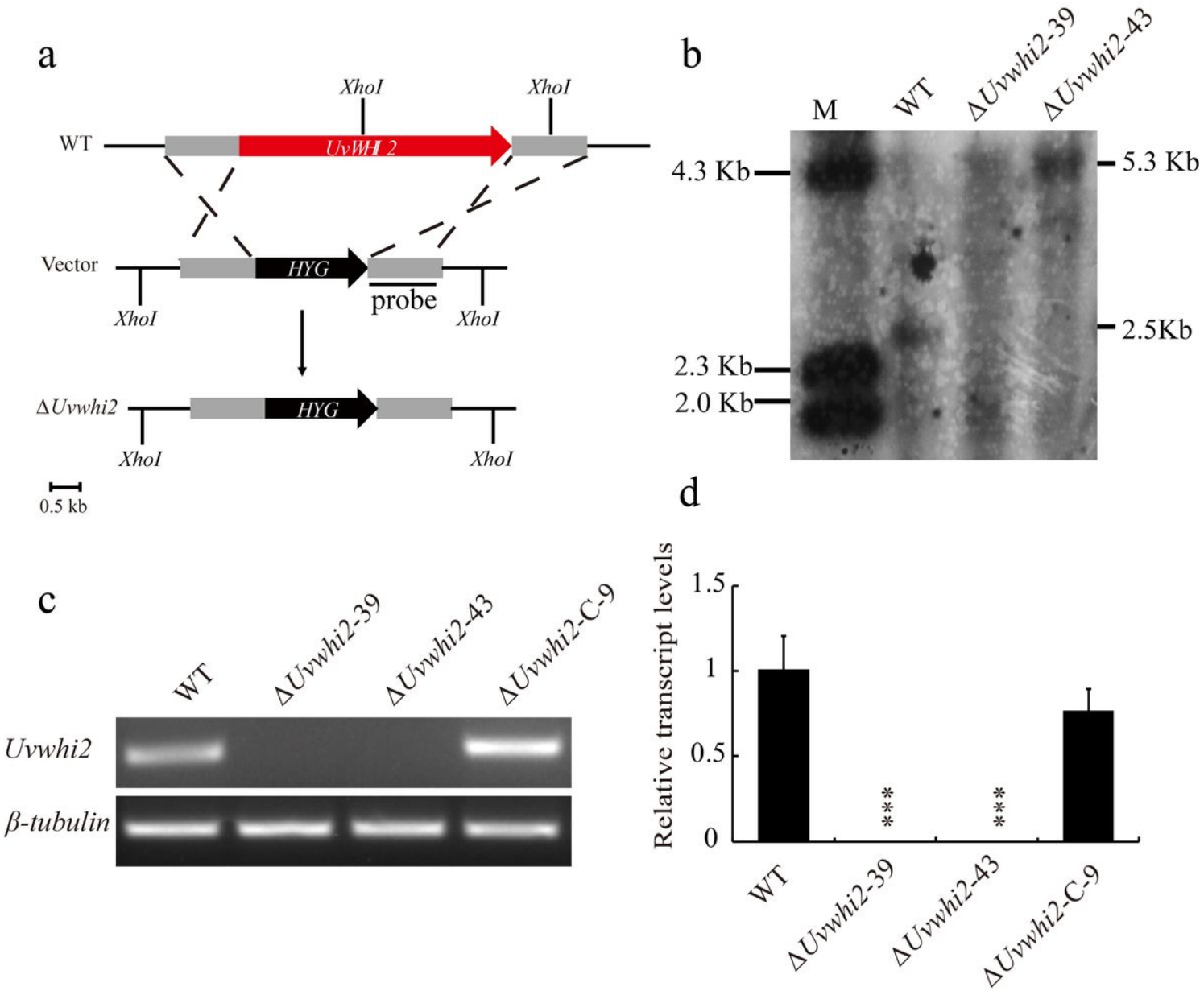

Figure 2

Targeted gene deletion of UvWHI2 and complementation assay in U. virens. a, Construction strategy for the UvWHI2 gene deletion mutant in U. virens. The coding region of UvWHI2 was replaced with the hygromycin phosphotransferase gene cassette (HYG) by homologous recombination. b, The Southern blot assay was used to validate the loss of UvWHI2 in the ΔUvwhi2 deletion mutant. The Xho I enzyme was used to digested genomic DNA of the WT and ΔUvwhi2 strains. The digested genomic were processed for the Southern blotting with the 1 Kb downstream of UvWHI2 as probe. c, qRT-PCR analysis of the expression level of UvWHI2 in the WT, ΔUvwhi2, and ΔUvwhi2-C strains. The data represents the mean ± SD from three independent replicates. The asterisks (***) indicate a P value < 0.001.

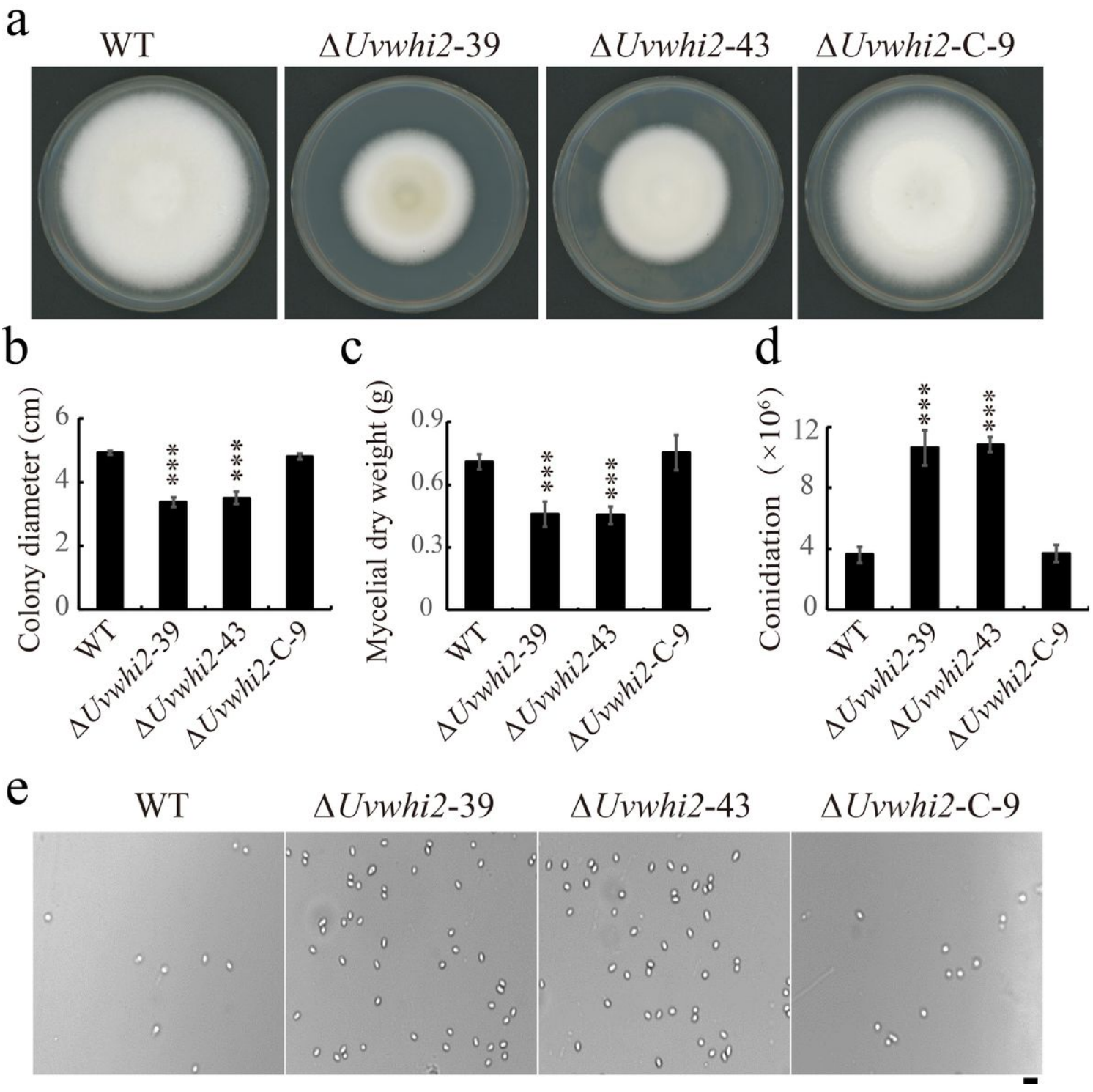

Figure 3

Deletion of UvWHI2 results in decreased vegetative growth and increased conidiation in U. virens. a, Mycelia growth of the WT, ΔUvwhi2, and ΔUvwhi2-C strains on the PSA (potato sucrose agar) medium in dark at 28□ for 15 d. b, Colony morphology of indicated strains. c, Dry weight of mycelia was measured in the PS medium after 7 d culture. d and e, Knockout of UvWHI2 enhanced conidiation under nutrient-rich condition. Data represents the mean ± SD from three independent replicates. The data were subject to

Duncan's Test and the significant differences were indicated in the figure with three asterisks (***, $p < 0.001$). Scale bar = 5 μm.

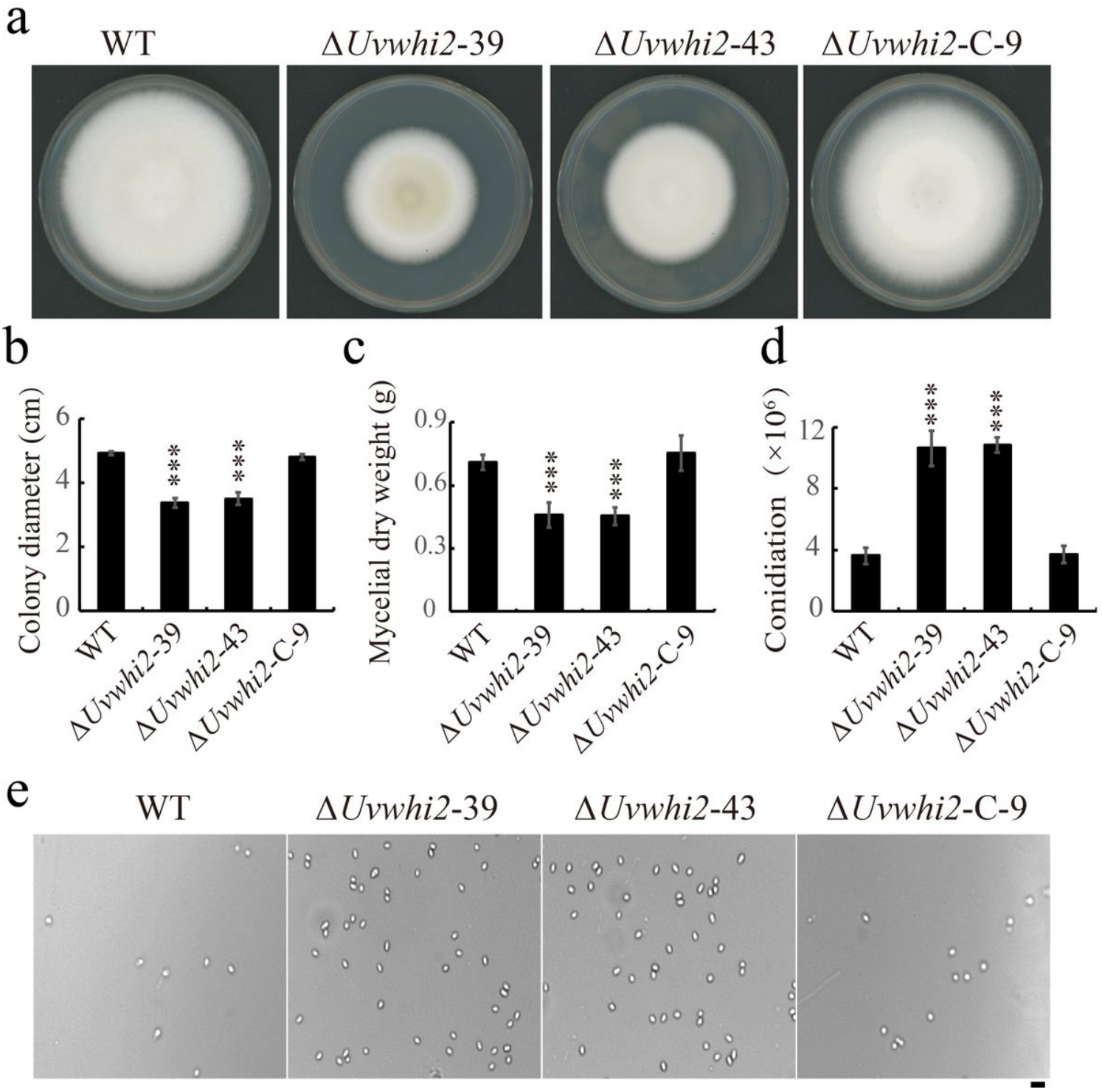

Figure 3

Deletion of UvWHI2 results in decreased vegetative growth and increased conidiation in U. virens. a, Mycelia growth of the WT, ΔUvwhi2, and ΔUvwhi2-C strains on the PSA (potato sucrose agar) medium in dark at 28℃ for 15 d. b, Colony morphology of indicated strains. c, Dry weight of mycelia was measured in the PS medium after 7 d culture. d and e, Knockout of UvWHI2 enhanced conidiation under nutrient-rich

condition. Data represents the mean ± SD from three independent replicates. The data were subject to Duncan's Test and the significant differences were indicated in the figure with three asterisks (***, $p < 0.001$). Scale bar = 5 μm.

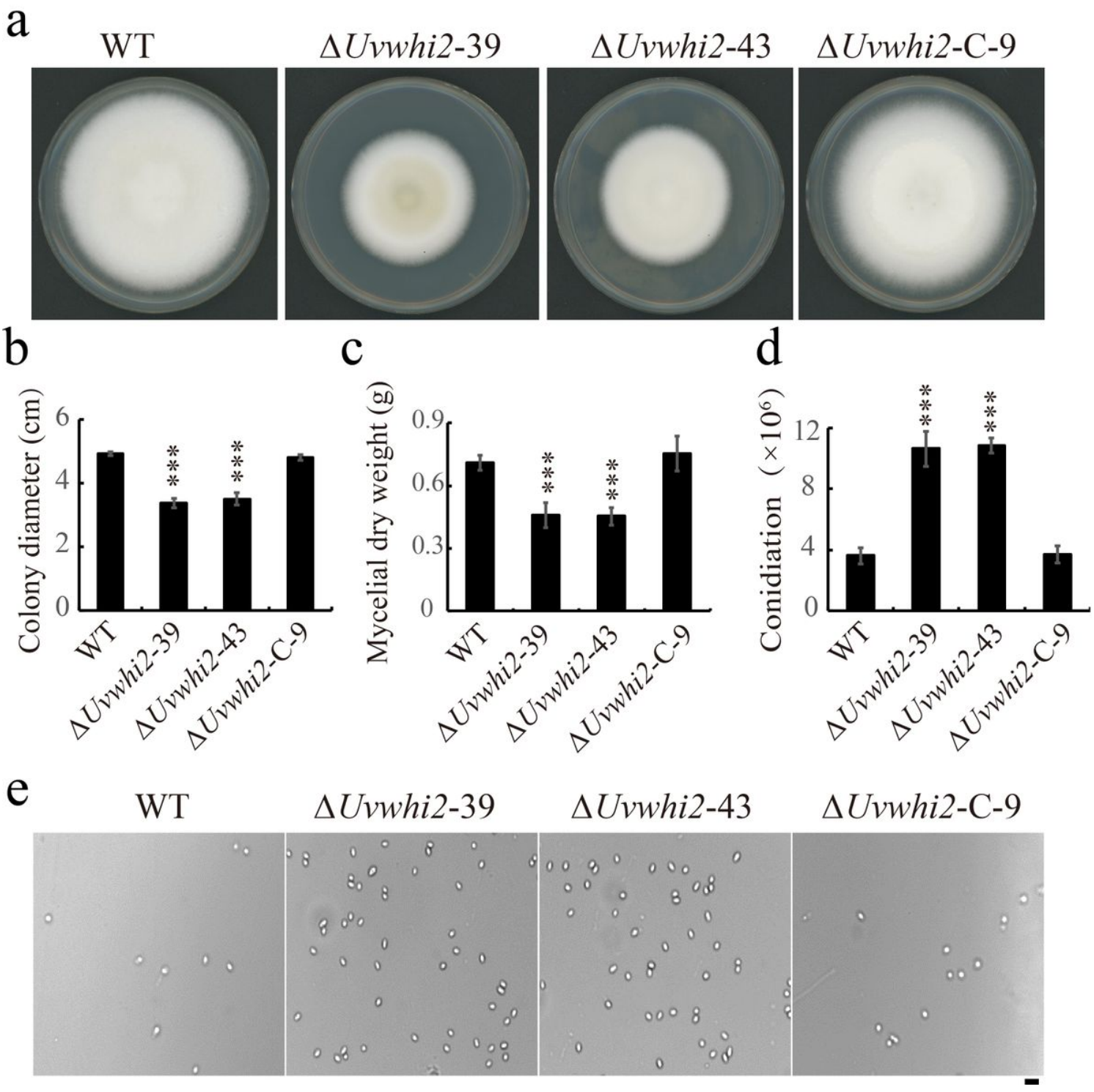

Figure 3

Deletion of UvWHI2 results in decreased vegetative growth and increased conidiation in U. virens. a, Mycelia growth of the WT, ΔUvwhi2, and ΔUvwhi2-C strains on the PSA (potato sucrose agar) medium in dark at 28℃ for 15 d. b, Colony morphology of indicated strains. c, Dry weight of mycelia was measured in

the PS medium after 7 d culture. d and e, Knockout of UvWHI2 enhanced conidiation under nutrient-rich condition. Data represents the mean ± SD from three independent replicates. The data were subject to Duncan's Test and the significant differences were indicated in the figure with three asterisks (***, $p < 0.001$). Scale bar = 5 μm.

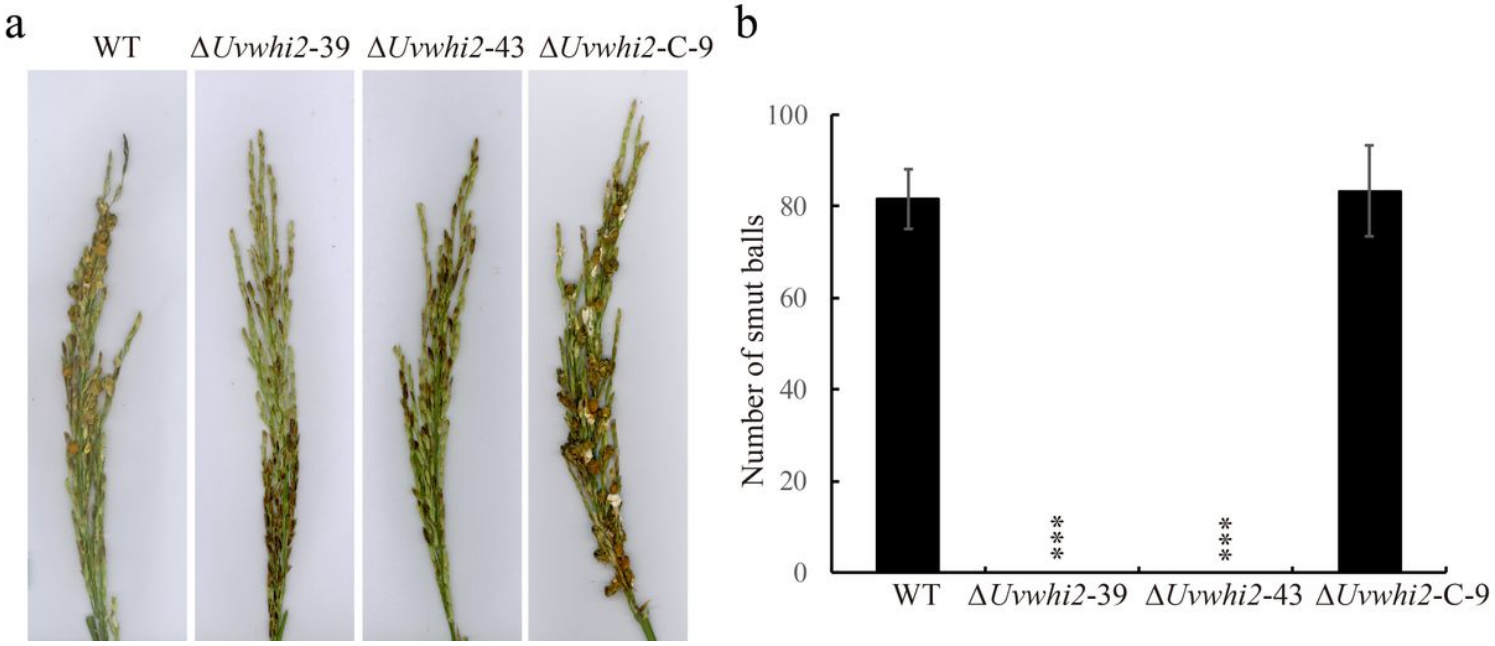

Figure 4

UvWhi2 is required for pathogenesis in U. virens. a, Disease symptoms of indicated strain on the rice panicles of WanXian 98 at 21 dpi. b, Statistical analysis of the average number of false smut balls on the inoculated spikelets. Each experiment was performed with three independent biological experiments and more than thirty panicles were inoculated each time. Data were showed as Mean ± SD (n = 3). ***, $p < 0.001$.

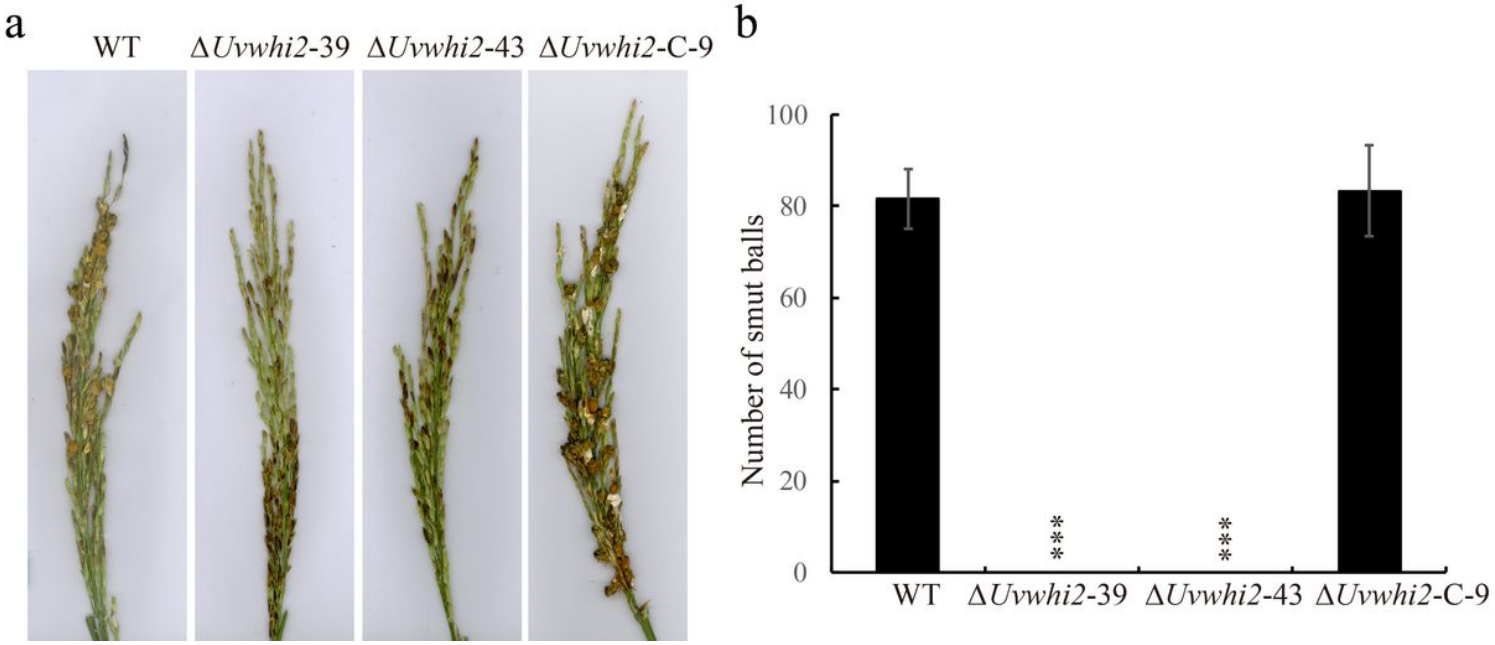

Figure 4

UvWhi2 is required for pathogenesis in U. virens. a, Disease symptoms of indicated strain on the rice panicles of WanXian 98 at 21 dpi. b, Statistical analysis of the average number of false smut balls on the inoculated spikelets. Each experiment was performed with three independent biological experiments and more than thirty panicles were inoculated each time. Data were showed as Mean ± SD (n = 3). ***, p < 0.001.

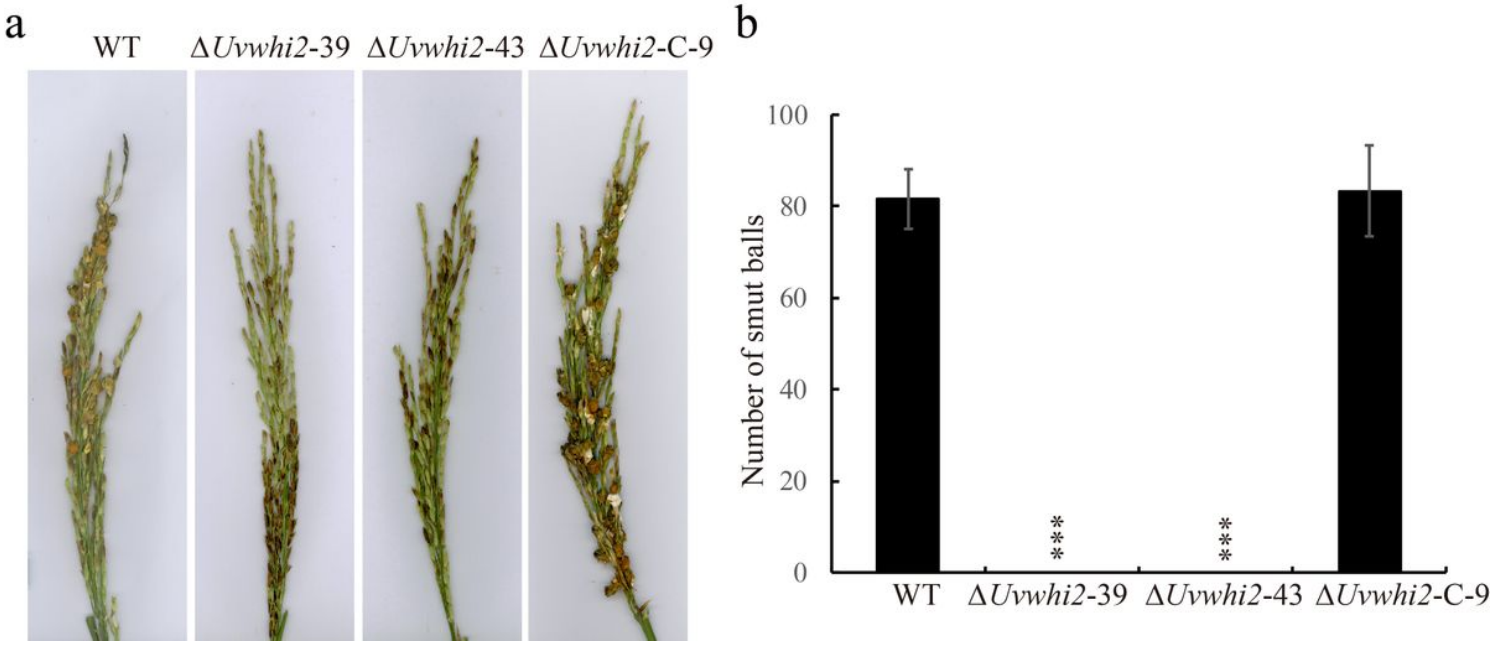

Figure 4

UvWhi2 is required for pathogenesis in U. virens. a, Disease symptoms of indicated strain on the rice panicles of WanXian 98 at 21 dpi. b, Statistical analysis of the average number of false smut balls on the inoculated spikelets. Each experiment was performed with three independent biological experiments and more than thirty panicles were inoculated each time. Data were showed as Mean ± SD (n = 3). ***, p < 0.001.

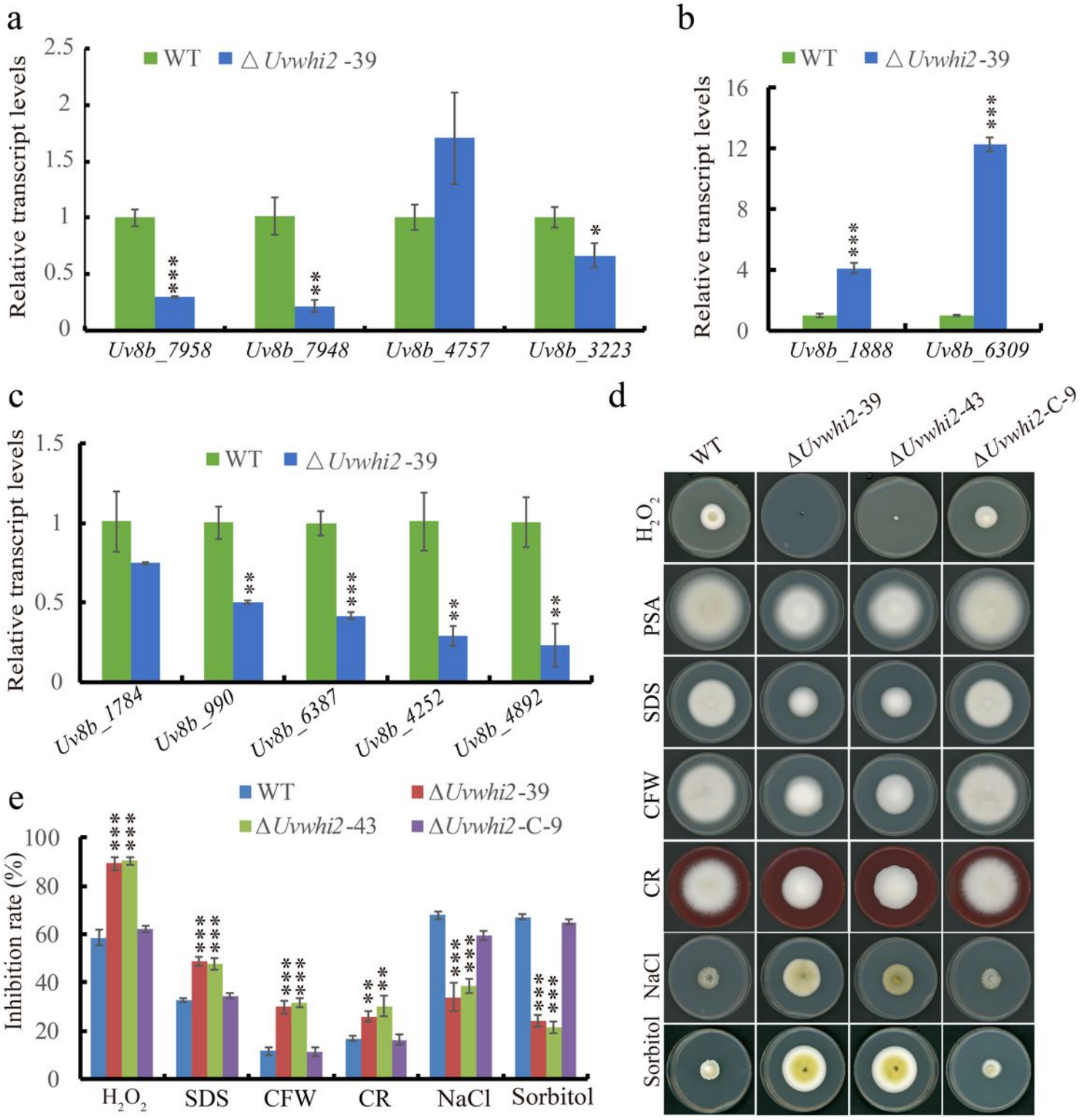

Figure 5

UvWHI2 contribute to the stress responses to the cell wall, oxidative and osmotic agent in U. virens. a, b and c, qRT-PCR analysis of the expression of the genes related to laccase, peroxidase, chitin deposition and hyperosmotic activities, respectively. d, The tested strains grown on the PSA or PSA with 0.03% H2O2 (Oxidative stress agent), 0.03% SDS (Sodium dodecyl sulfate), 120 μg/mL CFW (Calcofluor white), 120 μg/mL CR (Congo red), 0.4 M NaCl, or 0.7 M sorbitol. Typical cultures were photographed after 15 d at

28℃. e, Statistical analysis of inhibition rate of tested strains with different stress agents. The diameters of colonies were measured and calculated. Similar results were obtained by three repeated experiments. The error bars represent the standard deviation and the asterisk represents the significant difference compared to the WT strain under the same conditions (*, p < 0.01; **, p < 0.005; ***, p < 0.001).

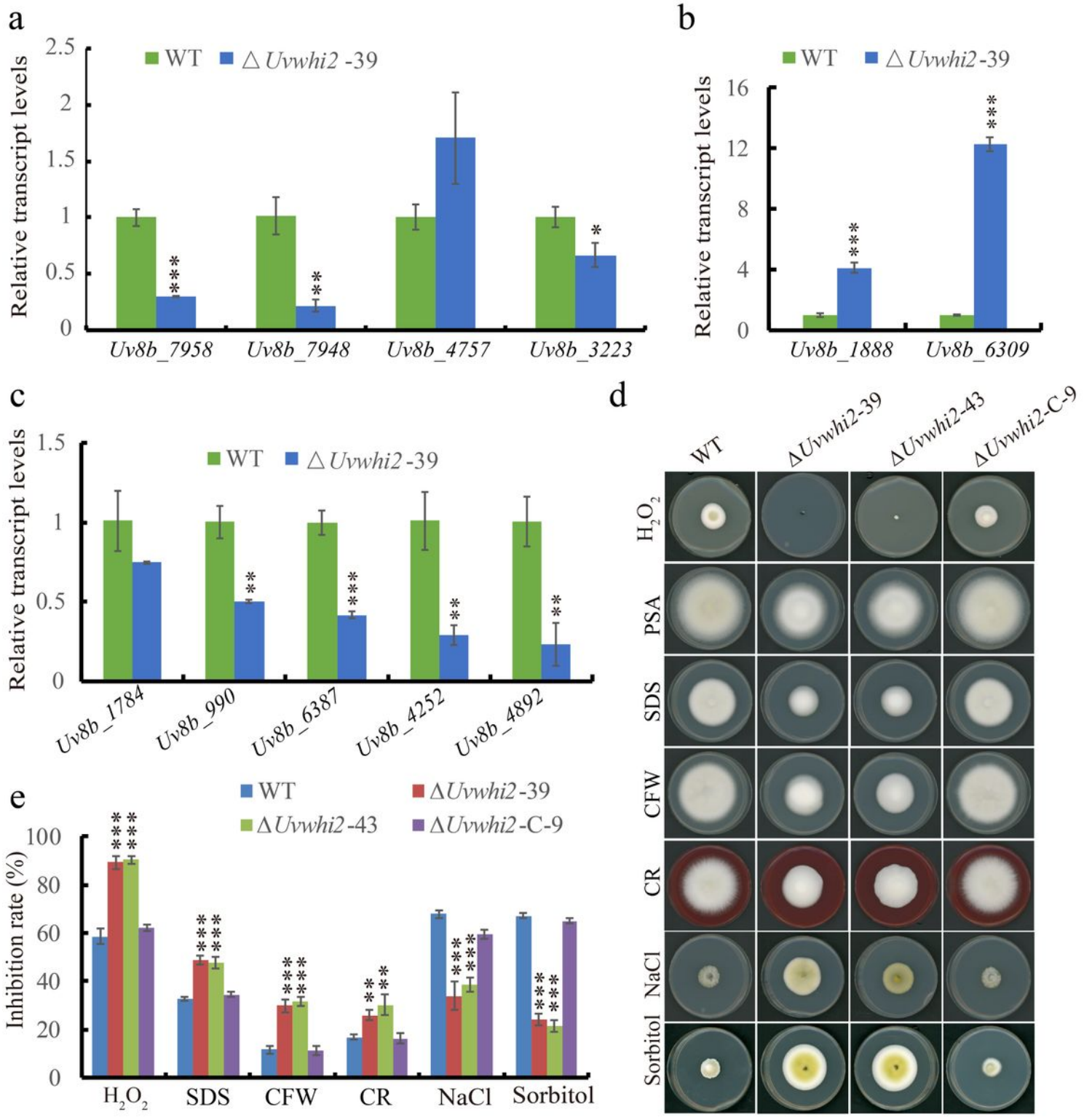

Figure 5

UvWHI2 contribute to the stress responses to the cell wall, oxidative and osmotic agent in U. virens. a, b and c, qRT-PCR analysis of the expression of the genes related to laccase, peroxidase, chitin deposition and hyperosmotic activities, respectively. d, The tested strains grown on the PSA or PSA with 0.03% H2O2 (Oxidative stress agent), 0.03% SDS (Sodium dodecyl sulfate), 120 μg/mL CFW (Calcofluor white), 120 μg/mL CR (Congo red), 0.4 M NaCl, or 0.7 M sorbitol. Typical cultures were photographed after 15 d at 28□. e, Statistical analysis of inhibition rate of tested strains with different stress agents. The diameters of colonies were measured and calculated. Similar results were obtained by three repeated experiments. The error bars represent the standard deviation and the asterisk represents the significant difference compared to the WT strain under the same conditions (*, $p < 0.01$; **, $p < 0.005$; ***, $p < 0.001$).

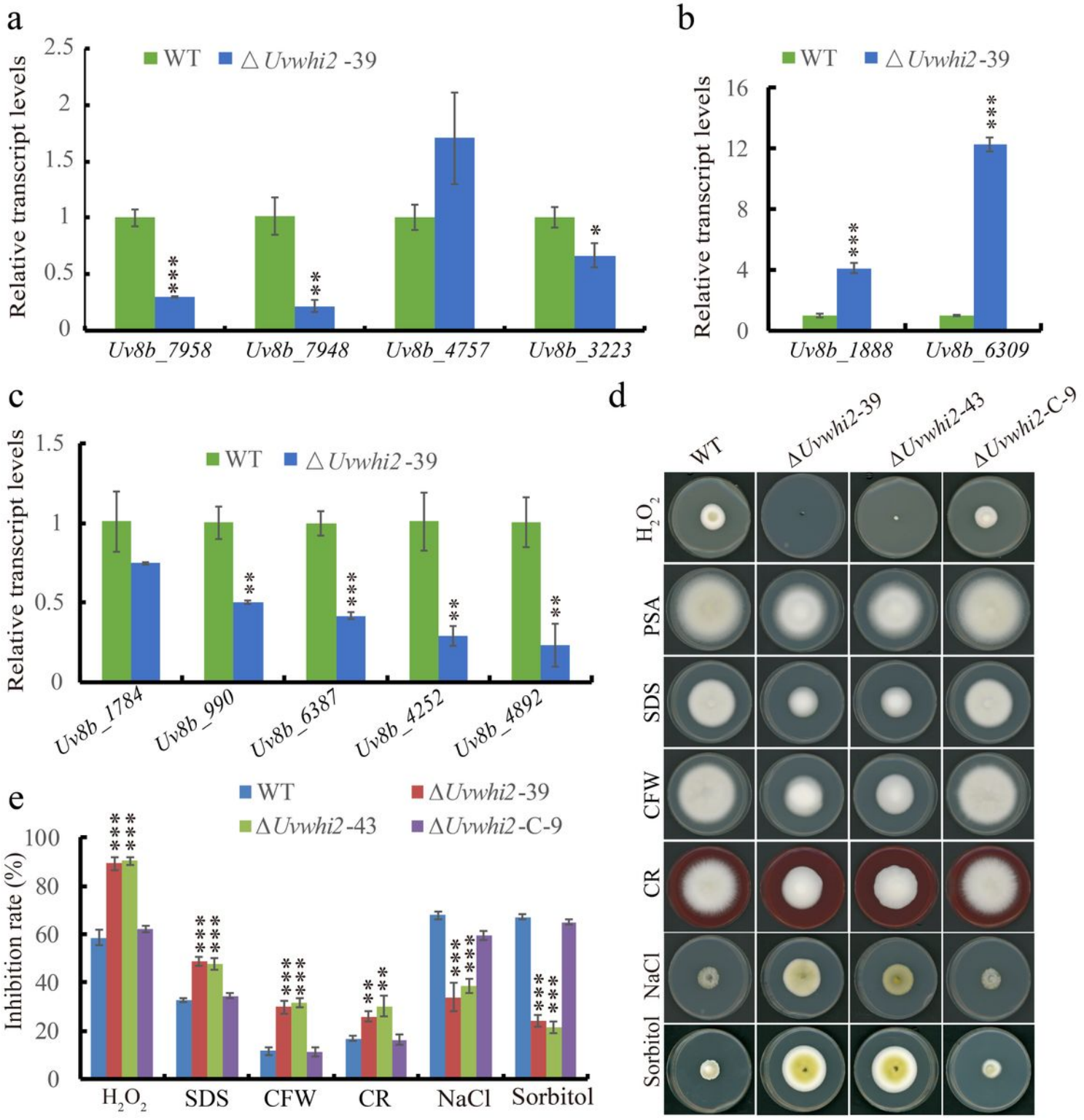

Figure 5

UvWHI2 contribute to the stress responses to the cell wall, oxidative and osmotic agent in U. virens. a, b and c, qRT-PCR analysis of the expression of the genes related to laccase, peroxidase, chitin deposition and hyperosmotic activities, respectively. d, The tested strains grown on the PSA or PSA with 0.03% H2O2 (Oxidative stress agent), 0.03% SDS (Sodium dodecyl sulfate), 120 μg/mL CFW (Calcofluor white), 120 μg/mL CR (Congo red), 0.4 M NaCl, or 0.7 M sorbitol. Typical cultures were photographed after 15 d at

28℃. e, Statistical analysis of inhibition rate of tested strains with different stress agents. The diameters of colonies were measured and calculated. Similar results were obtained by three repeated experiments. The error bars represent the standard deviation and the asterisk represents the significant difference compared to the WT strain under the same conditions (*, p < 0.01; **, p < 0.005; ***, p < 0.001).

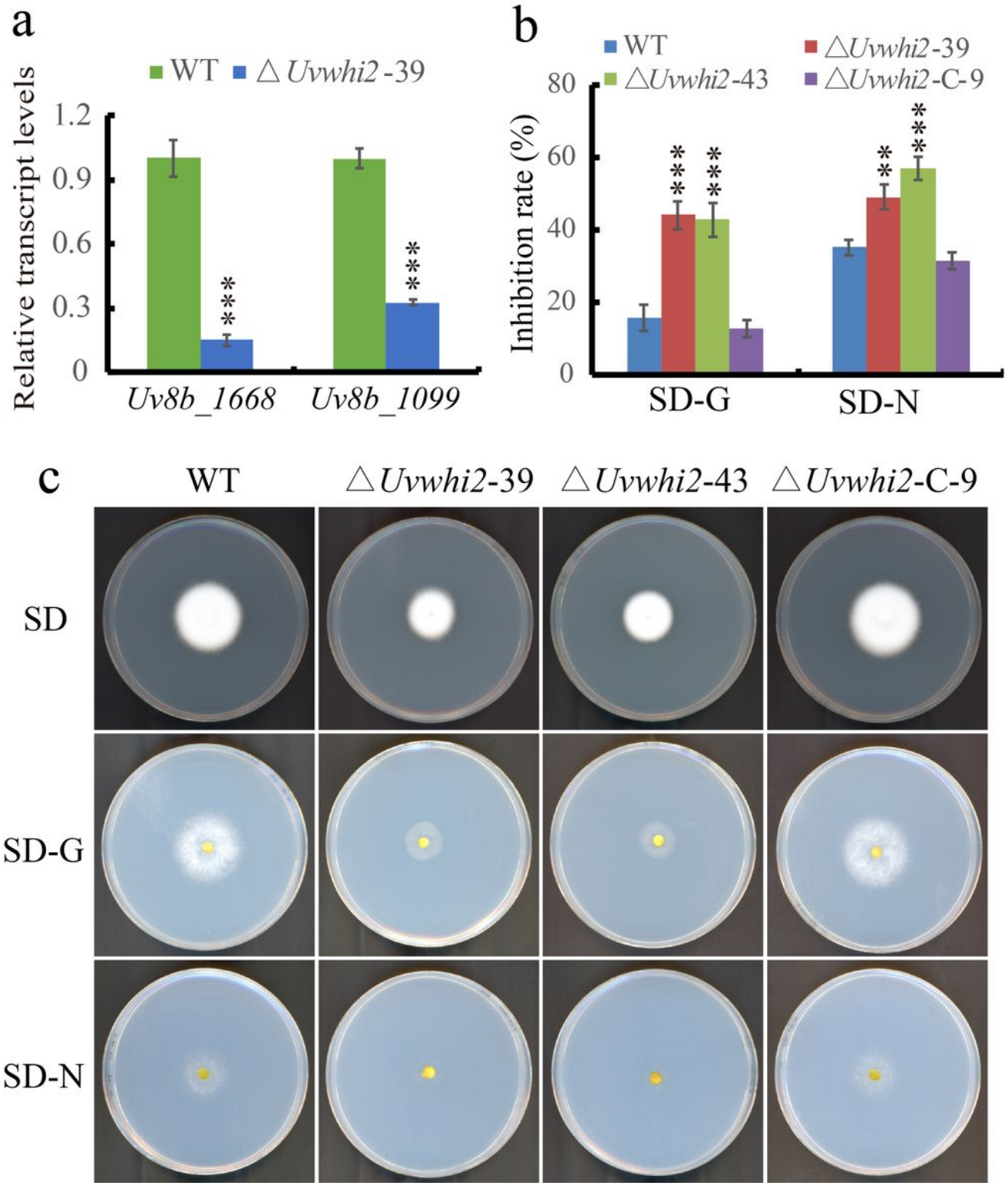

Figure 6

UvWhi2 is involved in the regulation of nutrient stresses responses in U. virens. a, qRT-PCR analysis of the genes related to sugar synthesis. b, The growth of tested strains on the SD (synthetic dropout medium), SD-G (synthetic dropout medium without glucose), and SD-N (synthetic dropout medium without nitrogen) medium. Typical cultures were photographed after culturing for 15 d at 28℃. The diameters of colonies were measured to calculate the inhibition rate. c, Statistical analysis of tested strains grown on the SD-G and SD-N medium at 15 d. Similar results were obtained by three repeated experiments. ** or ***, $p < 0.005$ or $p < 0.001$.

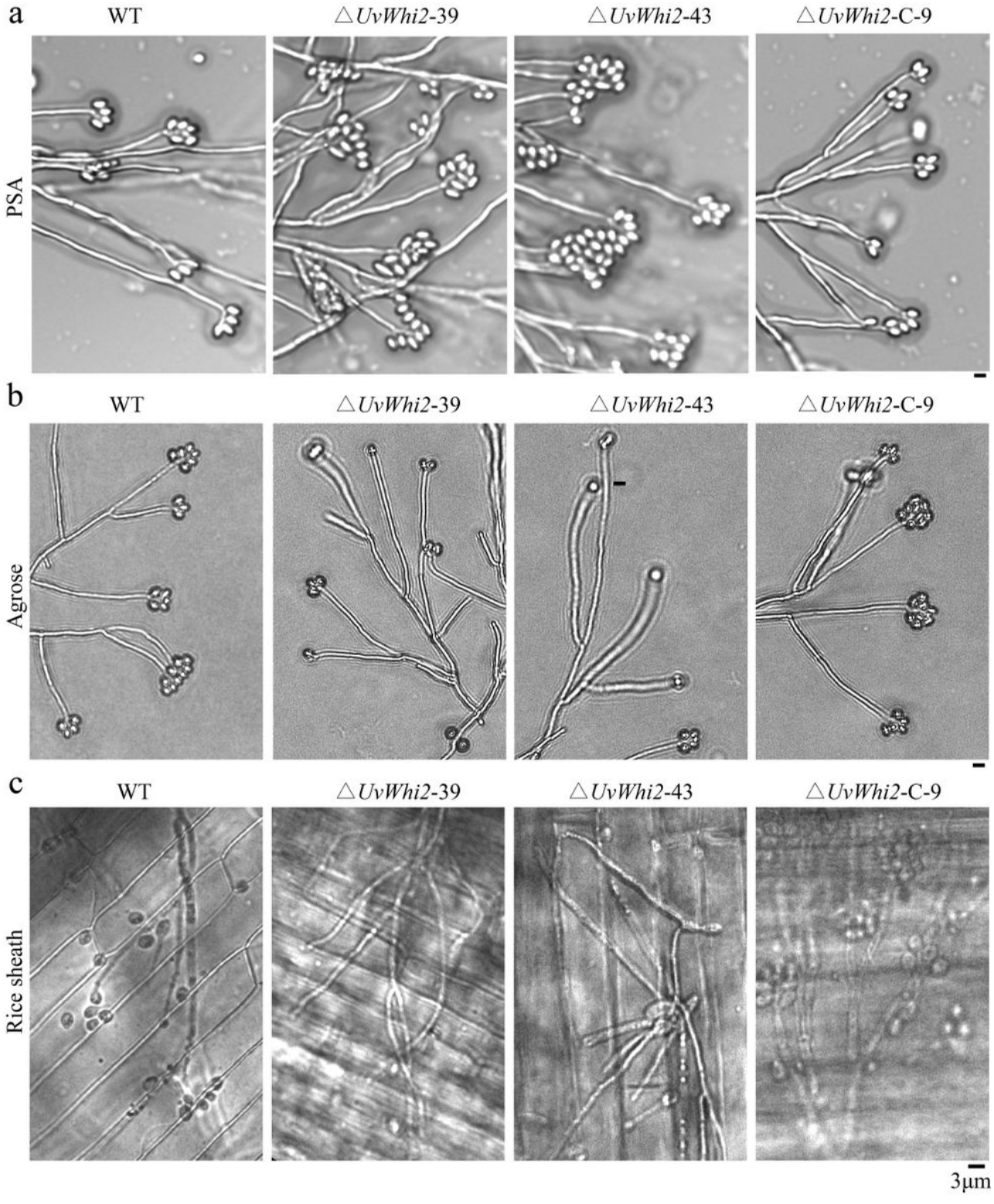

Figure 7

Conidial germination of the ΔUvwhi2 mutant on the nutrient limited surface and rice sheath surface. a-c, Conidial germination of U. virens on the PSA and agarose plates and the rice sheath. The conidia were inoculated on the indicated surface at 28 °C for 3 d. Scale bar = 3 μm.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.doc
- TableS1.doc
- TableS1.doc
- TableS2.xlsx
- TableS2.xlsx
- TableS2.xlsx
- FigS1.pptx
- FigS1.pptx
- FigS1.pptx