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Autophagy-related protein UvAtg14 contributes to mycelial growth, asexual reproduction, virulence and cell stress response in rice false smut fungus *Ustilaginoidea virens*

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Abstract

Autophagy is an evolutionarily conserved degradation process that degrades damaged proteins to maintain homeostasis and to protect cells against stress. In this study, we identified and characterized a critical autophagy-related protein, UvAtg14, in *Ustilaginoidea virens*, which is the ortholog of MoAtg14 in rice blast fungus *Magnaporthe oryzea*. UvAtg14 is co-localized with UvAtg8 (an autophagy marker protein) and is highly expressed at 1–3 days post-inoculation. Deletion of the *UvATG14* gene blocked GFP-UvAtg8 trafficking and autophagic digestion and significantly reduced mycelial growth, asexual reproduction, and virulence of *U. virens*. *UvATG14* deletion mutants also exhibited increased sensitivity to various abiotic stresses. Our findings indicate that UvAtg14 is a key autophagic protein and contributes to mycelial growth, conidia production, and pathogenicity in *U. virens*.

Keywords: *Ustilaginoidea virens*, Autophagy, UvAtg14, Mycelial growth, Conidium, Chlamydospore, Pathogenicity

Background

Rice false smut (RFS) disease, caused by the ascomycete fungal pathogen *Ustilaginoidea virens*, has become one of the most devastating rice diseases in China (Sun et al. 2020). *U. virens* typically infects flowers and hijacks rice nutrients by producing mycelial pistils and forming false smut balls (Ashizawa et al. 2012; Fan et al. 2015, 2020). However, due to a lack of resistant genes or RFS-resistant rice varieties, RFS is mainly controlled by heavy use of fungicides currently. Therefore, research assessing the pathogenic mechanism of RFS is important for developing new strategies to prevent RFS. Since the release of the genomic sequences of *U. virens* in 2014, several pathogenicity-related genes have been identified via comparative functional genomics and/or insertional mutation, which encode: MAP kinase UvPmk1 and Cyclin-dependent kinase UvCdc2; adenylate cyclase UvAc1 and phosphodiesterase UvPdeH in the cAMP pathway; the cell stress response-related protein UvWhi2; the transcription regulatory zinc finger protein UvMsn2; and cysteine-rich effector SCRE2 (Zhang et al. 2014; Fang et al. 2019; Guo et al. 2019; Sun et al. 2020; Tang et al. 2020; Xu et al. 2021; Shuai et al. 2022). However, there are only a small number of pathogenicity-related genes that have been verified as pathogenic genes compared to other phytopathogenic fungi.

Autophagy is an evolutionarily conserved degradation process that maintains homeostasis during eukaryotic development (Levine and Klionsky 2004; Liu et al. 2016; Zhu et al. 2019). It engulfs damaged proteins or

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organelles into autophagosome and transports them to vacuoles or lysosomes for degradation and recycling (Feng et al. 2014). The molecular basis of autophagy has been intensively studied in baker's yeast. More than forty autophagy-related genes (ATGs) have been clarified, and these ATG proteins are divided into five functional groups: (1) Atg1/ULK kinase complex; (2) Atg12-Atg5-Atg16 protein conjugation system; (3) Atg8 lipid conjugation system; (4) Atg9 membrane protein recycling system; and (5) the class III phosphatidylinositol 3-kinase (PI3K) complex (Shibutani and Yoshimori 2014; Dikic and Elazar 2018). Evidence collected over the past two decades has demonstrated that the autophagy occurring in plant pathogenic fungi is similar to that in yeast and mammals. In *Magnaporthe oryzae*, the core ATG proteins in five groups have been verified as MoAtg1 in the Atg1 kinase complex, MoAtg8 in the Atg8 lipid conjugation system, and MoAtg14 in the PI3K kinase complex (Liu et al. 2007b, 2017; Deng et al. 2009). Meanwhile, a large number of ATGs were identified in other plant pathogenic fungi, such as *Fusarium graminearum* and *Botrytis cinerea* (Zhu et al. 2019). In *U. virens*, UvAtg8 was confirmed to be essential for autophagy and full pathogenicity, which can also act as a helpful marker for autophagy (Meng et al. 2020).

Atg14, along with Atg6/Vps30, Vps34, and Vps15, constitutes the PI3K type I complex, which is essential for regular autophagic processes, while Atg14 responds to the localization of the PI3K type I complex to preautophagosomal structure (PAS) (Obara et al. 2006; Obara and Ohsumi 2011). In recent years, the autophagic function of Atg14 orthologs has been identified in several animals and fungi, such as Atg14/BARKOR in humans and MoAtg14 in *M. oryzae* (Itakura et al. 2008; Sun et al. 2008; Liu et al. 2017). Atg14 regulates pathogenicity and reproduction in plant pathogenic fungi, such as hemi-biotrophic pathogen *M. oryzae* and necrotrophic pathogen *F. graminearum* (Liu et al. 2017; Lv et al. 2017).

However, the UvAtg14 has not been identified and characterized in *U. virens,* which is considered a biotrophic pathogen (Sun et al. 2020). In this study, we identified UvAtg14 in rice false smut fungus *U. virens*, which is an ortholog of Atg14 in *M. oryzae*, and revealed its function in autophagy and pathogenicity. Our results demonstrated that the deletion of *UvATG14* completely blocked autophagy and reduced mycelial growth, virulence, asexual reproduction, and tolerance to osmotic stress, cell well stress, and oxidative stress. UvAtg14 is co-localized with UvAtg8, the ortholog that is regarded as a component of PAS in filamentous fungi (Dong et al. 2009; Liu et al. 2017).

Results

Identification of UvAtg14 in *U. virens*

To identify the Atg14 homologous protein in *U. virens*, the amino acid sequence of MoAtg14 (GenBank accession no. XP_003716183.1) in *M. oryzae* was used as a query for the BLAST search in *U. virens* genome assembly (https://www.ncbi.nlm.nih.gov/assembly/GCA_000687475.2). A protein (GenBank accession no. XP_042996141.1) was identified as the ortholog of MoAtg14 (with 46.41% identity) and was named UvAtg14. Sequence analysis revealed that UvAtg14 contains a coiled-coil domain in the N-terminal, which is similar to Atg14 in both *Saccharomyces cerevisiae* and *M. oryzae* (Fig. 1a). Phylogenetic analysis of Atg14 protein in filamentous fungi showed that UvAtg14 is most similar to two Atg14 proteins in *Trichoderma* (Fig. 1b).

Subcellular localization and expression pattern of UvAtg14

To determine the location of UvAtg14 protein in *U. virens*, we transformed the fusion expression vector

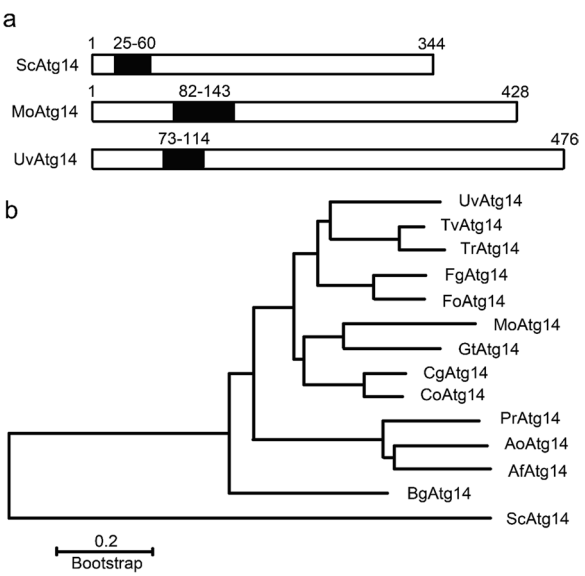

Fig. 1 UvAtg14 is an ortholog of Atg14. **a** The coiled-coil domains of ScAtg14 in *S. cerevisiae*, MoAtg14 in *M. oryzae,* and UvAtg14 in *U. virens*. The boxes in black indicate the domains. **b** The phylogenetic tree of fungal Atg14 orthologs constructed using the neighbor-joining method and MEGA 7.0 software. UvAtg14, ScAtg14 in *S. cerevisiae*, MoAtg14 in *M. oryzae,* TvAtg14 in *Trichoderma virens*, TrAtg14 in *T. reesei*, FgAtg14 in *F. graminearum*, FoAtg14 in *F. odoratissimum*, GtAtg14 in *Gaeumannomyces tritici*, CgAtg14 in *Colletotrichum gloeosporioides*, CoAtg14 in *C. orbiculare*, PrAtg14 in *Penicillium roqueforti*, AoAtg14 in *Aspergillus oryzae*, AfAtg14 in *A. fumigatus*, and BgAtg14 in *Blumeria graminis* were included in the phylogenetic tree. GenBank accession no. (TvAtg14, XP_013959553; TrAtg14, XP_006966865; FgAtg14, XP_011316371; FoAtg14, EMT61395; GgAtg14, XP_009224438; CgAtg14, EQB48915; CoAtg14, ENH80301; PrAtg14, CDM36188; AoAtg14, BAE65502; AfAtg14, XP_747209; BgAtg14, EPQ63265)

UvAtg14-mCherry and GFP-UvAtg8 into the wild-type strain JT209 of *U. virens*. The red and green signals produced by UvAtg14-mCherry and GFP-UvAtg8, respectively were detected in mycelia and appeared as punctuate dots. The merged images showed that the spots of UvAtg14-mCherry could overlap with those of GFP-UvAtg8 and suggested that UvAtg14 is co-located with UvAtg8 while it functions (Fig. 2a).

The expression pattern of *UvATG14* during the infection process was determined by qPCR assays. Compared with that in vegetative hyphae and conidia, the expression level of UvATG14 dramatically increased more than six fold at 1–3 days post-inoculation (dpi) and subsequently decreased at 5 dpi (Fig. 2b).

Deletion and complementation of *UvATG14* in *U. virens*

To reveal the biological function of UvAtg14, the *UvATG14* deletion mutants were generated. Five hundred and seventy-six hygromycin B-resistant transformants were obtained, among which three (Δ*UvATG14*-130, Δ*UvATG14*-516, and Δ*UvATG14*-517) were identified to be *UvATG14* deletion mutants by the PCR method. These *UvATG14* deletion mutants were further confirmed by RT-PCR and DNA sequencing. To confirm that the phenotypic differences observed in the *ΔUvATG14* mutants were all associated with the gene replacement event, the complementary strains were generated by transforming the full-length gene copy of *UvATG14* into Δ*UvATG14*-130 mutant. The transformants c*UvATG14*-130–1 and c*UvATG14*-130–2 were selected and confirmed as *UvATG14* complementary strains by RT-PCR and DNA sequencing for further testing (Additional file 1: Figure S1).

UvAtg14 is essential for autophagy in *U. virens*

To identify the function of UvAtg14 in autophagy, the autophagic marker GFP-UvAtg8 was used in trafficking assays (Meng et al. 2020). In wild-type strain JT209 and *UvATG14* complementary strains, the GFP signal was weak and did not accumulate under regular culture conditions. However, the GFP signal increased and accumulated in the vacuoles after further inducing autophagy under nitrogen-starving conditions in SD-N medium for 6 h. In contrast, the GFP signal was not observed in the vacuoles of the *UvATG14* deletion mutants, but accumulated in the cytoplasm in the SD-N medium (Fig. 3a). This indicates that the *UvATG14* deletion mutant lost the ability to transport GFP-UvAtg8 to the vacuoles under autophagy-inducing conditions. To further monitor the autophagic degradation in these *U. virens* strains, a Western blot assay was performed to detect GFP-UvAtg8 fusion protein and free GFP that was derived from digested GFP-UvAtg8. In *UvATG14* deletion mutants, the free GFP bands were completely undetectable under both nutrient-rich and nitrogen-starved conditions (Fig. 3b, c). These results indicate that UvAtg14 is essential for autophagy in *U. virens*.

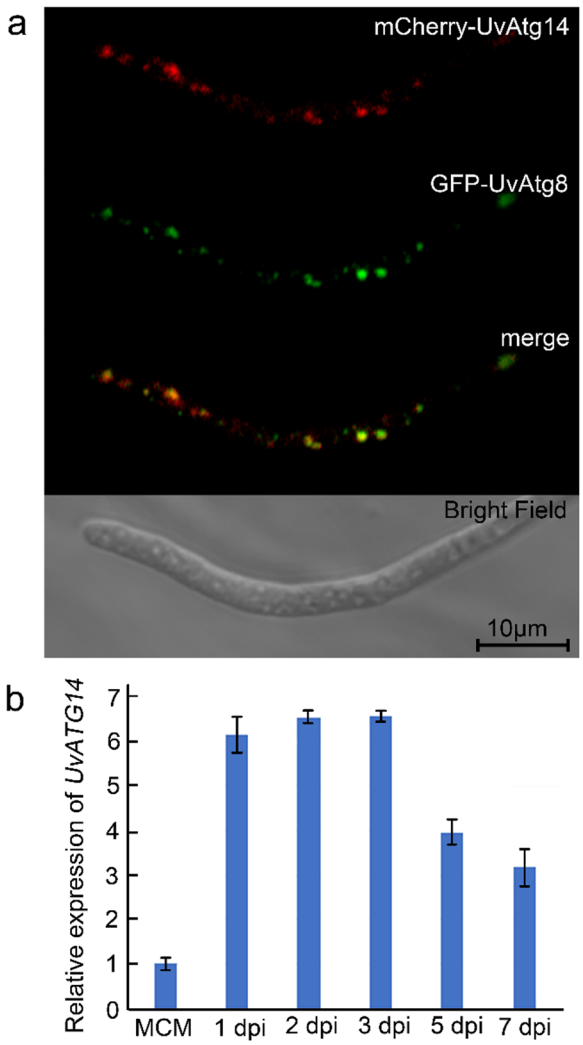

Fig. 2 Subcellular localization and expression pattern of UvAtg14. **a** Fluorescent microscope observation of UvAtg14 in mycelia, with mCherry and GFP signals indicating the distribution of UvAtg14 and UvAtg8, respectively. **b** Expression pattern of *UvATG14* during the infection process. The comparative expression level of *UvATG14* was determined by qPCR. A house-keeping gene, *β-tubulin*, was employed as the reference gene. MCM: a mixture of conidia and blended mycelia. dpi, days post-inoculation. Three replicates were performed and values are expressed as mean ± standard deviation

UvAtg14 contributes to mycelial growth, asexual reproduction, and pathogenicity

To characterize the biological function of UvAtg14 in *U. virens*, we determined the mycelial growth rate, conidia production, and virulence capacity of the

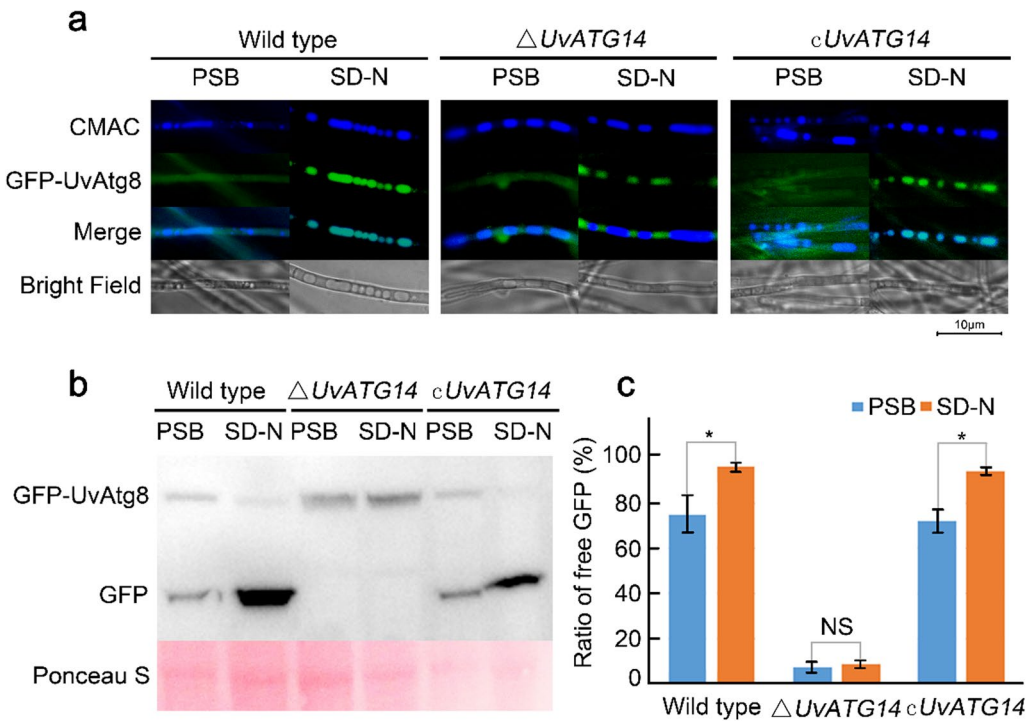

Fig. 3 UvAtg14 is essential for autophagy in *U. virens*. **a** Subcellular localization of GFP-UvAtg8 in wild-type strain JT209, *UvATG14* deletion mutant and complementary strain under normal (PSB) and nitrogen-starvation (SD-N) conditions. Vacuoles were stained with CMAC (7-amino-4-chlorome thylcoumarin). **b** Detection of proteolysis of GFP-UvAtg8 by Western blot. A main band of total protein stained by Ponceau S was used as a control. **c** The percentage of free to total GFP. Three replicates were performed and values are expressed as mean ± standard deviation. *, the significant difference at the 0.05 level (*t*-test) . NS, no significant difference

wild-type strain JT209, *UvATG14* deletion mutants, and complementary strains. The diameter of the colonies was measured after cultured on PSA, SD, and SD-N for 20 days. Compared with the wild-type strain, the colonies of *UvATG14* deletion mutants were slightly smaller and showed sparse hyphae in PSA and SD media. However, the mycelial growth rate of the *UvATG14* deletion mutants on the SD-N medium significantly decreased, and few hyphae were observed (Fig. 4a, d). Meanwhile, the conidial yield of UvATG14 deletion mutants was approximately one-third that of the wild-type strain (Fig. 4e). To further evaluate how UvAtg14 affects virulence, a mixture of mycelia and conidia was injected into rice spikelets at the booting stage. The RFS incidence was detected at 30 dpi. Compared to the wild-type strain, the number of RFS balls caused by *UvATG14* deletion mutants dramatically reduced (Fig. 4b, f). Additionally, the chlamydospore layer (in the color of yellow to dark green) produced by *UvATG14* deletion mutants was significantly thinner than that produced by the wild-type strain (Fig. 4c). All defection in mycelial growth, asexual reproduction, and virulence of *UvATG14* deletion mutants could be restored in *UvATG14* complementary strains (Fig. 4). These results demonstrate that UvAtg14 contributes to asexual reproduction and pathogenicity.

UvAtg14 is involved in various abiotic stresses

Since autophagy typically participates in stress responses (Yin et al. 2020), the role of UvAtg14 in various abiotic stresses was also determined by inoculating *U. virens* strain/mutants on a medium with different stress elements, including NaCl and sorbitol (osmotic stress), CR and CFW (cell well stress), SDS (cell membrane stress), and H_2O_2 (oxidative stress). The results demonstrated that the inhibition ratios (IR) of NaCl, sorbitol, CFW, SDS, and H_2O_2 to *UvATG14* deletion mutants were significantly higher than that of the wild-type strain. All defects to these abiotic stresses were recovered in the *UvATG14* complementary strains (Fig. 5a, b). This suggests that UvAtg14 contributes to cell responses to multiple abiotic stresses, including osmotic stress, cell well stress, cell membrane stress, and oxidative stress.

Discussion

Autophagy is a conserved catabolic process that is common in eukaryotes. In recent years, autophagy has been extensively studied in many filamentous fungi, like *M.*

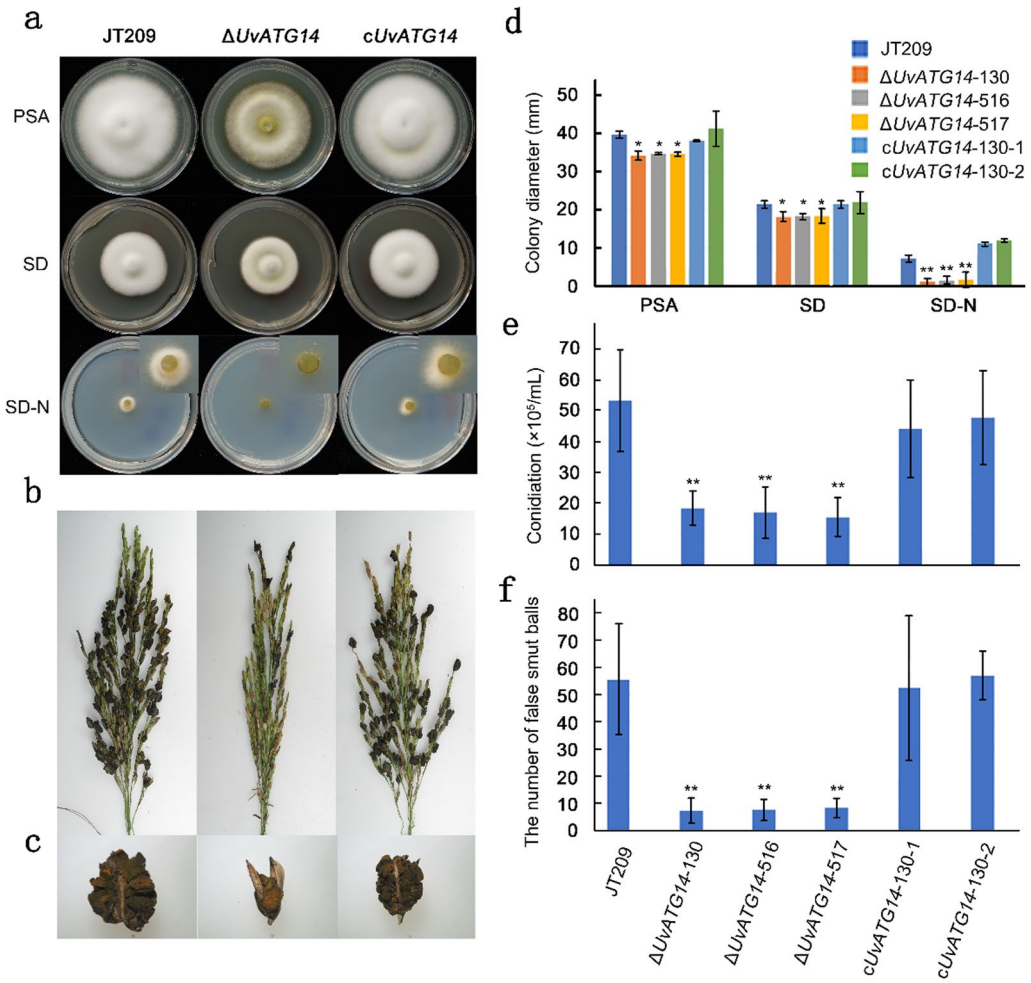

Fig. 4 UvAtg14 contributes to mycelial growth, asexual reproduction, and pathogenicity in *U. virens*. **a** Colonies of wild-type strain JT209, *UvATG14* deletion mutant Δ*UvATG14*-130 and complementary strain *cUvATG14*-130–1 cultured on PSA, SD, and SD-N media at 28ºC in the dark for 20 days. **b** Rice panicles inoculated with JT209, Δ*UvATG14*-130, and c*UvATG14*-130–1 for 30 days. **c** Rice false smut balls generated by JT209, Δ*UvATG14*-130, and c*UvATG14*-130–1. **d** Column diagram showing colony diameter of JT209, *UvATG14* deletion mutants (Δ*UvATG14*-130, Δ*UvATG14*-516, and Δ*UvATG14*-517) and complementary strains (c*UvATG14-130–1*, c*UvATG14*-130–2). **e** Conidiation capacity of *U. virens* strains in **d** incubated in 1%YT broth at 28ºC, 160 r/min, in the dark for 5 days. **f** The number of false smut balls per panicle artificially inoculated with *U. virens* strains in **d**. Three replicates were performed and values are expressed as mean ± standard deviation. ** and *, significant difference at the 0.01 level and 0.05 level (*t*-test) compared to wild-type strain JT209, respectively

oryzae and *F. graminearum*. Most of the key ATG proteins are involved in differentiation, development, and pathogenicity in plant pathogenic fungi (Zhu et al. 2019). However, only UvAtg8 has been verified in *U. virens* (Meng et al. 2020). In this study, we identified a ATG protein UvAtg14 in *U. virens*, the ortholog of Atg14 in *M. oryzae*, and evaluated its function via gene deletion and complementation analysis. Our results indicated that UvAtg14 is critical for autophagy and participates in the regulation of virulence, hyphal growth, production of conidia and chlamydospores, and various stress responses in *U. virens*.

A large number of plant pathogenic fungi need conidia to perform their infection cycle, and conidiation has been confirmed to be impaired in many autophagy-deficient mutants, like *MoATG1, 2, 6, 7, 8, 9, 14* deletion mutants of *M. oryzae* and *FgATG1, 2, 3, 6, 14* deletion mutants of *F. graminearum*. Therefore, conidiation defects are considered to be the cause of reduction/loss of virulence in these ATGs deletion mutants. (Liu et al. 2007a, b, 2016, 2017; Dong et al. 2009; Kershaw and Talbot 2009; Shoji et al. 2014; Lv et al. 2017). In *U. virens*, the deletion of *UvATG14* also reduces the number of conidia, however, when we used the same concentration of conidia in the

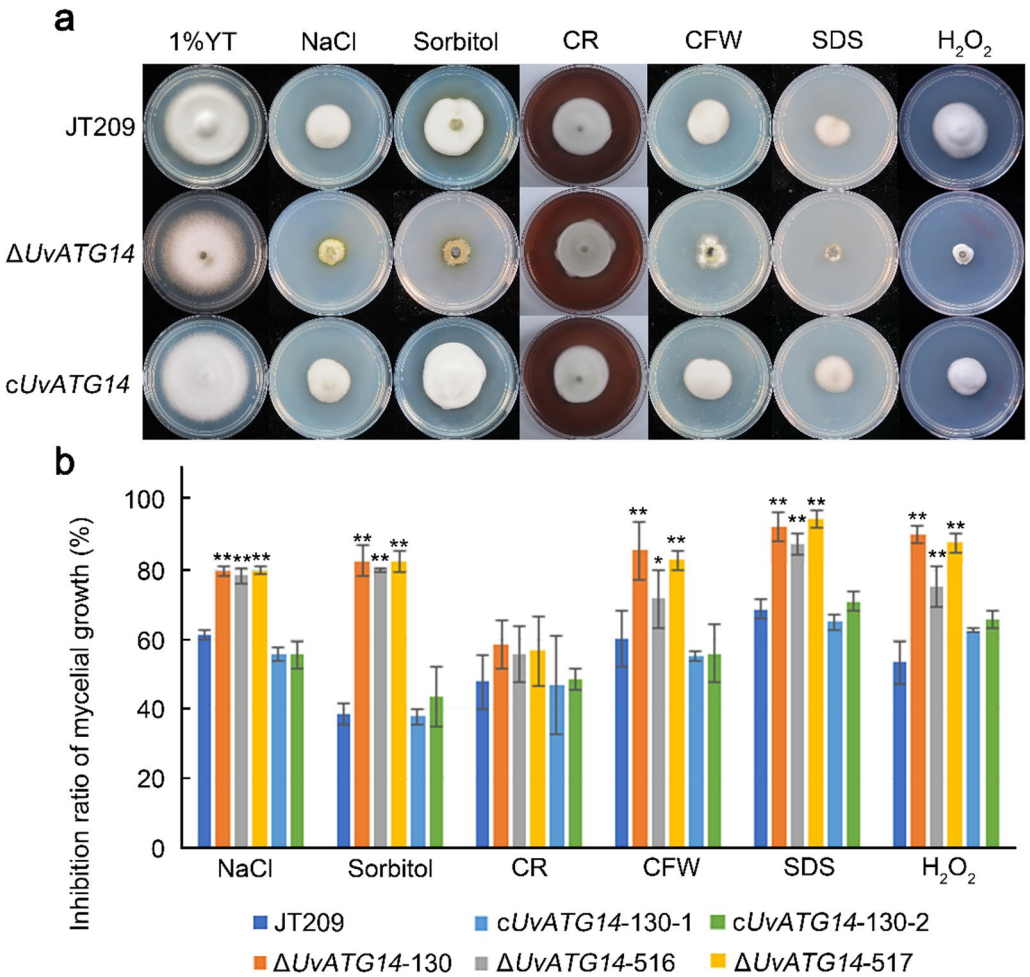

Fig. 5 UvAtg14 is involved in abiotic stress responses in *U. virens*. **a** Colonies of the wild-type strain JT209, *UvATG14* deletion mutant Δ*UvATG14*-130, and complementary strain c*UvATG14*-130–1 that were cultured on 1%YT medium supplemented with different stress elements at 28ºC in the dark for 16 days. **b** Inhibition ratio of mycelial growth of the indicated strains exposed to different stresses. Three replicates were performed and values are expressed as mean ± standard deviation. ** and *, the significant difference at the 0.01 level and 0.05 level (*t*-test) compared to the wild-type strain JT209, respectively

inoculation assay, the virulence of *UvATG14* deletion mutants still decreased. This indicates that the decreased pathogenicity in *UvATG14* deletion mutants is not due to conidiation defects in *U. virens*. Moreover, the conidia production of Atg14 deletion mutants in *M. oryzae* and *F. graminearum* decreased more than 20-fold compared to their starting strains, but the conidia production of *UvATG14* deletion mutants decreased only two-fold (Liu et al. 2017; Lv et al. 2017). This suggests that the regulatory mechanism of conidiation by UvAtg14 in *U. virens* could be different, which requires further study. As a biotrophic fungus, *U. virens* produces a mass of mycelia in rice florets for nitrogen acquisition. However, before it colonizes in these rice florets, *U. virens* may undergo a period of nutritional deficiency when hypha spread over the outer surface of the spikelets and extend into the inner spikelets through the gap between the lemma and palea (Ashizawa et al. 2012; Fan et al. 2015, 2020). *UvATG14* was highly expressed in spikelets at 3 dpi. In this period, *U. virens* conidia germinate and begin to extend into the inner spikelets (Hu et al. 2013; Song et al. 2016; Sun et al. 2020). Autophagy is triggered by starvation stress, which can degrade damaged proteins and organelles to synthesize new molecules or provide energy (Klionsky et al. 2021). In plant pathogenic fungi, autophagy is also involved in cell differentiation, development, and responses to nutrient starvation (Liu et al. 2017; Zhu et al. 2019; Meng et al. 2020). In *UvATG14* deletion mutants, autophagy was totally blocked, mycelial growth decreased more under nutrient-starvation

conditions in the SD-N medium than in the full nutrient medium, and mycelia were more sensitive to reactive oxygen species (ROS) that plays an important role in plant defense to various pathogens. This indicates that *UvATG14* deletion mutants had more difficulty in establishing infections under a nutrient-starved condition, leading to decreased pathogenicity to rice plants.

To verify the conserved function of Atg14 orthologs in filamentous fungi and budding yeast, we tried to complement the *UvATG14* deletion mutants with *ATG14* orthologs from *M. oryzea* (*MoATG14*) and baker's yeast (*ScATG14*). However, defects in *UvATG14* deletion mutants were recovered in *MoATG14* complementary strains but not in *ScATG14* complementary strains (Additional file 1: Figure S2).

There are two types of PI3K complexes found in yeast and mammals: type I and type II. The type I complex containing Atg14 functions in autophagy, while the type II complex harboring Vps38 instead of Atg14 functions in vacuolar protein sorting (VPS) in yeast and endocytosis in mammalian cells (Obara and Ohsumi 2011; Zhu et al. 2019). It has been reported that Atg14 and Vps38 competitively bind ATG6, which is another core component of the PI3K complex (Zhu et al. 2018, 2019). Using a yeast two-hybrid assay, we confirmed that UvAtg14 can interact with both UvAtg6 and MoAtg6 rather than ScAtg6 (Fig. 6). Along with the results of the *ATG14* complementation assay, this suggests that Atg14 and PI3K complex are conserved in filamentous fungi but differ in budding yeast.

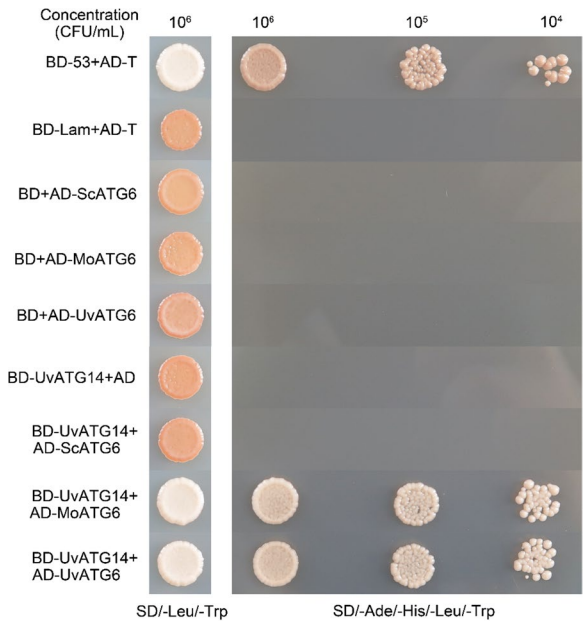

Fig. 6 UvAtg14 interacts with UvAtg6 in *U. virens* and MoAtg6 in *M. oryzea* but not with ScAtg6 in *S. cerevisiae*. Growth phenotypes of yeast cells that were co-transformed with the indicated combinations of bait and prey plasmids and subsequently cultured on SD/-Leu/-Trp and SD/-Ade/-His/-Leu/-Trp media. BD and AD are empty bait and prey vectors, respectively. The yeast cells transformed with the vector pairs BD-53/AD-T and BD-Lam/AD-T were used as the positive and negative control, respectively

Conclusions

Our study demonstrated that UvAtg14, the ortholog of Atg14 in *M. oryzea*, is critical for autophagy and contributes to pathogenicity, mycelial growth, asexual reproduction, and abiotic stress response in *U. virens*. The results of this study provide a better understanding of the important role that autophagy plays during the infection process of *U. virens*.

Methods

Fungal strains, rice variety, and culture condition

A virulent *U. virens* wild-type strain JT209 was used as the starting strain in this study. The PSA (200 g potato extract/L, sucrose 20 g/L, and agar 20 g/L) was used for regular culture at 28 °C in the dark. The samples used for DNA and RNA extraction were collected from mycelia that had been cultured in the PSB (PSA without agar) at 28 °C in the dark. The susceptible rice variety Liangyoupeijiu was used in artificial inoculation experiments to test the virulence of strain/mutants.

Sequence analysis

The sequences of the genes and proteins used in this study were downloaded from the website of the National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/). The protein sequence analysis and domain searching were performed using SMART (http://smart.embl-heidelberg.de/). The protein sequence alignments and phylogenetic analysis were performed using MEGA 7.0 and the neighbor-joining algorithm method.

Reverse transcription PCR and quantitative PCR assays

Mycelia and conidia were collected from 5-day-old cultures on PSB. The rice spikelet samples inoculated with a mixture of mycelia and conidia were collected at 1, 2, 3, 5, and 7 dpi. The OminiPlant RNA Kit (cwbio) and PrimeScript RT reagent Kit with gDNA Eraser (Takara) were used to extract total RNA and synthesize cDNA, respectively. Quantitative PCR (qPCR) was performed with SYBR Premix Ex Taq II (Takara), and the change fold of gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method. The *β-tubulin* gene (NCBI accession number: UV8b_05680) was used as a reference. Three biological

replicates were performed to calculate the mean and the standard deviation. The cDNA from *UvATG14* deletion/complementary strains were analyzed using reverse-transcription PCR (RT-PCR) to detect the expression of *UvATG14*.

Generation of *UvATG14* deletion mutants and complementary strains

The *UvATG14* gene deletion vector pD-*UvATG14* was constructed by ligating upstream and downstream flanking sequences of *UvATG14*, and hygromycin resistant gene (*HygB*$^+$) into PMD-19 plasmid. To construct CRISPR (clustered regularly interspaced short palindromic repeats) plasmid pCAS9-*UvATG14*, guide RNA (gRNA) spacers were annealed and inserted into pCAS9-tRp-gRNA plasmid (Arazoe et al. 2015). To generate *UvATG14* deletion mutants, pD-*UvATG14* and pCAS9-*UvATG14* were co-transformed into the wild-type strain JT209 via polyethylene glycol (PEG)-mediated transformation (Zheng et al. 2016). The transformants were cultured on PSA with 100 mg/L hygromycin B. The *UvATG14* deletion mutants were initially screened out by PCR detection of *UvATG14* coding region with primers shown in Additional file 2: Table S1 and further confirmed by DNA sequencing.

For complementation of *UvATG14*, the copy of the *UvATG14* gene containing the native promoter, coding region, and terminator was amplified and inserted into pzp-bar-Ex plasmid. The pzp-*UvATG14* was subsequently transformed into *UvATG14* deletion mutant via *Agrobacterium tumefaciens*-mediated transformation (ATMT), and the transformants were selected on 2% TB3 (yeast extract powder 3 g/L, casein acid hydrolysate 3 g/L, sucrose 20 g/ L, agar 12 g/L) with 0.03% glufosinate ammonium (Lv et al. 2016). The *A. tumefaciens* strain AGL-1 was employed in the ATMT. The *UvATG14* complementary strains were detected by RT-PCR of *UvATG14* coding region and further confirmed by DNA sequencing.

Detection of autophagy

To generate the GFP-UvAtg8 expression vector, the 1 kb upstream flanking of *UvATG8* as a native promoter, GFP, and coding region of *UvATG8* were amplified and inserted into pcam-neo3-tbeta plasmid. The GFP-UvAtg8 expression vector was subsequently transformed into a wild-type *U. virens* strain and mutants via ATMT transformation.

To detect autophagy, the GFP-UvAtg8 strain was shaken in the PSB for 3 days at 28 °C and transferred into starvation broth SD-N (yeast nitrogen base without amino acids 1.7 g/L, glucose 20 g/L) and PSB for 6 h of shaking. The mycelia were collected to observe their GFP signals and perform Western blot with anti-GFP antibodies. The vacuole-staining Blue CMAC (7-amino-4-chloromethylcoumarin) was used as a marker to observe the vacuoles in *U. virens*.

Phenotypic and pathogenicity analysis of *U. virens* strains and mutants

The mycelial growth capacity was determined by measuring the diameter of the colonies cultured on PSA, SD (SD-N add Ammonium sulfate 5 g/L), and SD-N at 28°C in the dark for 20 days. The conidiation capacity was tested by counting the number of conidia in 1%YT broth (yeast extract 1 g/L, tryptone 1 g/L, glucose 10 g/L) at 28°C in the dark for 5 days. For virulence assay, the mixture of mycelia and conidia was broken down with a blender and the rice spikelets were inoculated during the booting stage by leaf sheath injection. The number of false smut balls was counted at 30 dpi. To evaluate the sensitivity of strain/mutants to abiotic stresses, 1%YT media supplemented with 0.4 mol/L NaCl, 0.6 mol/L sorbitol, 500 mg/L congo red (CR), 200 mg/L calcofluor white (CFW), 0.02% sodium dodecyl sulfate (SDS), and 0.02% H_2O_2 were used in the experiments, while the diameter of colonies at 16 dpi was measured and used to calculate the inhibition ratio. Three replicates were performed to calculate the mean and the standard deviation.

Abbreviations

AD: PGADT7; ATG: Autophagy-related gene; ATMT: *Agrobacterium tumefaciens*-mediated transformation; BD: PGBKT7; CFW: Calcofluor white; CMAC: 7-amino-4-chloromethylcoumarin; CR: Congo red; CRISPR: Clustered regularly interspaced short palindromic repeats; dpi: Days post-inoculation; gRNA: Guide RNA; *HygB*$^+$: Hygromycin resistant gene; IR: Inhibition ratio; PAS: Preautophagosomal structure; PEG: Polyethylene glycol; PI3K: Class III phosphatidylinositol 3-kinase; qPCR: Quantitative PCR; RFS: Rice false smut; ROS: Reactive oxygen species; RT-PCR: Reverse transcription-PCR; SDS: Sodium dodecyl sulfate; VPS: Vacuolar protein sorting.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-022-00116-2.

Additional file 1. Figure S1. Deletion and complementation of the *UvATG14* gene in *U. virens*. **Figure S2.** Conserved function of Atg14 orthologs in filamentous fungi and budding yeast.

Additional file 2. Table S1. Primers used in this study.

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Authors' contributions

XH designed the research, performed the experiments, analyzed the data and wrote the manuscript. JY designed the research and wrote the manuscript. XP, HC, MY and TS performed the experiments. ZQ, YD, RZ and DL analyzed the data. YL supervises and wrote the manuscript. All authors read and approved the final manuscript.

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Declarations

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Competing interests
The authors declare that they have no competing interests.

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