Survival factor 1 contributes to the oxidative stress response and is required for full virulence of *Sclerotinia sclerotiorum*

YANG YU1,2, JIAO DU1, YABO WANG1, MENGYAO ZHANG1, ZHIQIANG HUANG1, JUNSONG CAI1, ANFEI FANG1, YUHENG YANG1, LING QING1, CHAO WEI BI1 AND JIASEN CHENG1,2,*

1College of Plant Protection, Southwest University, Chongqing City, 400715, P R China  
2State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan City, 430070, P R China

**SUMMARY**

*Sclerotinia sclerotiorum* is a devastating necrotrophic fungal pathogen that infects over 400 species of plants worldwide. Reactive oxygen species (ROS) modulations are critical for the pathogenic development of *S. sclerotiorum*. The fungus applies enzymatic and non-enzymatic antioxidants to cope with the oxidative stress during the infection processes. Survival factor 1 was identified and characterized to promote survival under conditions of oxidative stress in *Saccharomyces cerevisiae*. In this research, a gene named SsSvf1 was predicted to encode a survival factor 1 homologue in *S. sclerotiorum*. SsSvf1 transcripts showed high expression levels in hyphae under oxidative stress. Silencing of SsSvf1 resulted in increased sensitivity to oxidative stress in culture and increased levels of intracellular ROS. Transcripts of SsSvf1 showed a dramatic increase during the initial stage of infection and the gene-silenced strains displayed reduced virulence on oilseed rape and *Arabidopsis thaliana*. Inhibition of plant ROS production partially restores virulence of SsSvf1 gene-silenced strains. SsSvf1 gene-silenced strains exhibited normal oxalate production, but were impaired in compound appressorium formation and cell wall integrity. The results suggest that SsSvf1 is involved in coping with ROS during fungal-host interactions and plays a crucial role in the pathogenicity of *S. sclerotiorum*.

**Keywords:** oxidative stress, *Sclerotinia sclerotiorum*, Survival factor 1, virulence.

**INTRODUCTION**

*Sclerotinia sclerotiorum* (Lib.) de Bary is one of the most devastating fungal pathogens with a worldwide distribution. This pathogen can infect more than 400 plant species and lead to significant losses in many cultivated crops including oilseed, sunflower, soybean and the common bean (Boland and Hall, 1994; Bolton et al., 2006; Purdy, 1979).

*S. sclerotiorum* has been considered a model necrotrophic fungal pathogen, which kills host tissue via the secretion of oxalic acid (OA) (Cessna et al., 2000; Favaron et al., 2004; Kim et al., 2008; Williams et al., 2011) and cell wall degrading enzymes (Martel et al., 1996; Poussereau et al., 2001; Riou et al., 1991; Yajima et al., 2009; Yu et al., 2016; Zuppini et al., 2005). Recent evidence indicates that this fungus secretes effector proteins to suppress host defence in the initial stage of infection (Guyon et al., 2014; Lyu et al., 2016; Yang et al., 2018; Zhu et al., 2013). These strategies are mainly applied by biotrophic and hemibiotrophic fungal pathogens. In addition, biotrophic growth at the leading edge of fungal colonization was suggested for *S. sclerotiorum* (Kabbage et al., 2015). These studies indicate that the pathogenesis of *S. sclerotiorum* is more complex than we thought and more evidence is needed to detail the underlying molecular mechanism.

Rapid generation of reactive oxygen species (ROS) including hydrogen peroxide (H$_2$O$_2$), the superoxide anion (O$_2^-$), and hydroxyl radical (OH•) is an early resistance response in many plant/pathogen interactions (Lamb and Dixon, 1997). Such oxidative bursts have direct and powerful antimicrobial activity including inhibition of the spore germination of a number of fungal pathogens (Mousavi and Robson, 2004; Peng and Kuc, 1992). In response, fungal pathogens apply specific enzymes and non-enzyme-mediated antioxidant mechanisms to handle ROS (Aguirre et al., 2006). Several studies have shown that ROS modulation is critical for the hyphae of *S. sclerotiorum* to successfully colonize host plant tissue (Kim et al., 2011; Williams et al., 2011; Yarden et al., 2014); however, evidence for the molecular mechanisms of ROS detoxification and tolerance in *S. sclerotiorum* are still sparse.

The Survival factor 1 (SVF1) gene was first identified in *Saccharomyces cerevisiae* in a screen for mutations that could be functionally complemented by exogenous expression of the human anti-apoptotic gene Bcl-x (Brace et al., 2005; Vander Heiden et al., 2002). However, SVF1 and Bcl-x have distinct roles in regulating cell survival (Brace et al., 2005). *S. cerevisiae* cells...
lacking Svf1 protein showed hypersensitivity to direct chemical precursors of ROS, suggesting that Svf1 is necessary for survival under oxidative stress (Brace et al., 2005). Deeper research has shown that Svf1-mediated cell survival under conditions of oxidative stress by affecting the sphingolipid metabolism in S. cerevisiae (Brace et al., 2007). To date, the role of Svf1 in the oxidative stress response and pathogenicity of filamentous fungal pathogens has remained unknown.

Here, a gene in S. sclerotiorum (SS1G_01919) named SsSvf1 (Sclerotinia sclerotiorum Survival factor 1) was predicted to encode a yeast Svf1 homologous protein. The function of SsSvf1 was determined via a reverse genetic approach, and its role in oxidative stress response and pathogenicity was investigated. The research may help clarify the function of Svf1 in fungal plant pathogens and the pathogenicity of S. sclerotiorum in more detail.

RESULTS

SsSvf1 encodes a survival factor-1 homologue in S. sclerotiorum

The S. sclerotiorum SsSvf1 gene consists of four exons and three introns, and encodes a protein with 381 amino acids. Conserved Domain Database (CDD) analysis of the protein sequence revealed that a Svf-like domain was predicted at amino acid position T52–I380 (Marchler-Bauer et al., 2017). Alignment of amino acid sequences of the N-terminal and C-terminal of the Svf1 domains in SsSvf1 and yeast Svf1 exhibit great similarity (Brace et al., 2005) (Fig. 1). BLASTP searches using the amino acid sequence of SsSvf1 as a query showed that the homologous sequences are widely present in fungi including some important plant pathogens such as Botrytis cinerea (XP_001548941), Gibberella zeae (XP_011323561) and Colletotrichum higginsianum (XP_018161001).

**SsSvf1 is required for response to oxidative stress**

The expression of SsSvf1 under oxidative stress conditions was analysed to explore the role of the SsSvf1 gene in response to oxidative stress of S. sclerotiorum. As shown in Fig. 2A, the expression level of SsSvf1 was much higher in hyphae treated with H₂O₂ (5 mM and 10 mM). To determine the function of SsSvf1, a gene-silencing vector was constructed based on pSilent-1 (Nakayashiki et al., 2005) as described in methods. The vector was linearized and transformed into the wild-type strain of S. sclerotiorum via PEG (polyethylene glycol) methods (Rollins, 2003). Several transformants were obtained, and silencing of SsSvf1 in the transformants was evaluated by real-time Reverse Transcription-Polymerase Chain Reaction (RT-PCR) (Fig. S1). The expression levels of SsSvf1 in SiSvf1-230 and SiSvf1-213 were 15% and 2% of that in the wild-type
Survival factor 1 in *S. sclerotiorum* strain, respectively. Thus, these two strains were chosen for deeper research.

The hyphal growth of the wild-type and *SsSvf1* gene-silenced strains on potato dextrose agar (PDA) containing 0 mM to 10 mM H₂O₂ were compared. The results showed that these two gene-silenced strains displayed wild-type levels of susceptibility to 2.5 mM H₂O₂, while being more sensitive at higher H₂O₂ concentrations (Fig. 2B). *SiSvf1-230* and *SiSvf1-213* were also more sensitive to menadione, a chemical inducer of oxidative stress, than the wild-type strain (Fig. 3). The results indicated that *SsSvf1* was required for managing oxidative stress in *S. sclerotiorum*.

*SsSvf1* gene-silenced strains show overproduction of ROS

*Svf1* inhibits ROS generation in *S. cerevisiae* (Brace et al., 2005). To understand whether silencing of *SsSvf1* would lead to altered ROS generation, we detected ROS production during hyphal growth in *SsSvf1* gene-silenced strains. The strains were stained with nitroblue tetrazolium (NBT), which specifically detects superoxide. More dark-blue formazan precipitates were seen in the hyphae of *SiSvf1-230* and *SiSvf1-213*, which indicated that *SsSvf1* gene-silenced strains produce more superoxide than the wild-type strain (Fig. 4A). The ROS production in the hyphae tips was then quantified via mean pixel intensity. The results showed that *SiSvf1-230* and *SiSvf1-213* exhibited greatly increased superoxide production (Fig. 4B).

*SsSvf1* gene-silenced strains show reduced virulence and a lower efficiency of appressoria formation

The expression level of *SsSvf1* during wild-type infection was evaluated, and the results indicated that the expression of *SsSvf1* showed a strong increase during the initial stage of...
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infection (Fig. 5A). To carry out functional analysis of the role of SsSvf1 in the pathogenicity of S. sclerotiorum, SsSvf1 gene-silenced strains were inoculated on detached oilseed rape leaves and on Arabidopsis thaliana plants. As shown in Fig. 5B, both gene-silenced strains caused small lesions on the oilseed rape leaves. The smaller lesions were also observed on A. thaliana inoculated with the SsSvf1 gene-silenced strains (Fig. 5B). The results indicate that SsSvf1 is important for the pathogenicity of S. sclerotiorum.

The OA and compound appressoria were key factors in the pathogenesis of S. sclerotiorum infection; thus, the OA accumulation and compound appressorium formation were compared between the wild-type and SsSvf1 gene-silenced strains. Our results showed that SsSvf1 gene-silenced strains secreted similar levels of OA as the wild-type strain (Fig. S2). To assay appressoria formation, both the wild-type and SsSvf1 gene-silenced strains were inoculated on parafilm-overlaid growth medium and on rapeseed leaves. The results showed that the wild-type strain formed complex and frequent appressoria, but SiSvf1-213 rarely produced appressoria on parafilm or rapeseed leaves (Fig. 6A). This indicated that SsSvf1 is associated with compound appressoria formation in S. sclerotiorum. To confirm this, the rapeseed leaves were wounded with a dissecting needle and then inoculated with the wild-type strain and SiSvf1-213. The results showed that SiSvf1-213 caused larger lesions by 48 h post-inoculation on wounded rapeseed leaves than on intact leaves (Fig. 6B). SiSvf1-213 was also inoculated on wounded leaves of A. thaliana and similar phenotypes were observed (Fig. 6C).
Fig. 5  Functional characterization of SsSvf1 in pathogenicity of S. sclerotiorum. (A) Relative expression of SsSvf1 in wild-type hyphae after contact with Arabidopsis thaliana and growing on potato dextrose agar (PDA) plates. The Tub1 gene in each sample was used as an internal control. The relative expression of SsSvf1 in hyphae stage or in hyphae inoculated on plants at 0 h was set as one. Bars indicate standard deviation. (B) Pathogenicity of SsSvf1 gene-silenced strains on detached leaves of rapeseed and on A. thaliana plants. Each strain was investigated with five rapeseed leaves or A. thaliana plants each time. One representative replicate from three experiments is shown.
Inhibition of plant ROS production partially restores virulence of SsSvf1 gene-silenced strain

SsSvf1 gene-silenced strains showed increased sensitivity to ROS, which is an early plant response to pathogen infection. Thus, the virulence of SsSvf1 gene-silenced strain was next tested on oilseed rape leaves with reduced generation of ROS. The rapeseed leaves were sprayed with diphenyleneiodonium (DPI) to inhibit activity of the plant NADPH oxidases, and the leaves were then inoculated with the wild-type strain and SsSvf1-213, respectively.

As shown in Fig. 7, larger lesions were produced by SiSvf1-213 in leaves treated with DPI than in leaves treated with water.

SsSvf1 gene-silenced strains are impaired in cell wall integrity of hyphae

The cell wall integrity was compared between the wild-type and SsSvf1 gene-silenced strains. The results showed that SiSvf1-230 and SiSvf1-213 were more sensitive to SDS (sodium dodecyl sulphate) than the wild-type strain, suggesting
a weakened cell wall for SsSvf1 gene-silenced strains (Fig. 8). To evaluate cell wall alteration, the effects of specific cell wall perturbation agents to the SsSvf1 gene-silenced strains were determined. The results showed that SiSvf1-230 and SiSvf1-213 displayed wild-type levels of susceptibility to calcofluor white (CFW) while being more sensitive to Congo red (CR) (Fig. 8). This suggests that SsSvf1 gene-silenced strains were impaired in some aspect of hyphal cell wall integrity. Since the cell wall integrity pathway and hyperosmotic stress response share common functional aspects and are positively coordinated (Alonso-Monge et al., 2001; Rodríguez-Peña et al., 2010), the hyphal growth of SsSvf1 gene-silenced strains under hyperosmotic stress were determined. The inhibition of hyphal growth was significantly greater for SiSvf1-230 and SiSvf1-213 than the wild-type strain when growing on medium with sodium chloride or sorbitol (Fig. 8).

**DISCUSSION**

Fungi use specific enzymatic and non-enzymatic processes to regulate antioxidant response (Aguirre et al., 2006). To date, only a few proteins have been reported related to the anti-oxidative stress responses in S. sclerotiorum. SsSOD1 encodes a Cu/Zn superoxide dismutase (SOD) in S. sclerotiorum, and the gene-deletion mutant exhibited increased sensitivity to oxidative stress (Xu and Chen, 2013). Our previous report also showed that a putative BAX inhibitor-1 in S. sclerotiorum was involved in withstanding H₂O₂ (Yu et al., 2015). In this study, SsSvf1 is predicted to encode a S. cerevisiae Svf1 homologue in S. sclerotiorum. SsSvf1 shows increased expression under oxidative stress and the gene-silenced strains are more sensitive to H₂O₂ and menadione. To date, Svf1 was only identified and characterized in S. cerevisiae; it was required for survival in response to oxidative stress (Brace et al., 2005). Our data demonstrates the function of SVF1 homologues in filamentous fungi for the first time and suggests an important role of Svf1 in response to oxidative stress in S. sclerotiorum.

Evidence for the molecular mechanism underlying Svf1 function in oxidative stress response is still sparse. S. cerevisiae SVF1 gene expression in mammalian cells increases apoptotic resistance (Brace et al., 2005). Deep research showed that yeast Svf1 regulates cell survival by affecting sphingolipid metabolism (Brace et al., 2007). Sphingolipids are important regulators of apoptosis in plant and animal cells and some sphingolipid metabolites may promote cell survival following induction of death by various treatments (Alden et al., 2011; Maceyka et al., 2002). In this research, the relationship between SsSvf1 and sphingosine metabolism in S. sclerotiorum is unclear. The sequences of SsSvf1 were used in a search for potential structural homologues via the HHpred server (Söding et al., 2005; Zimmermann et al., 2018). The top-ranked hit is a putative lipocalin in *Nitrosomonas europaea* with an E-value of 4.94e-24 and a probability score of 99.91. A search with WoLF PSORT server showed that SsSvf1 is

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**Fig. 7**  Pathogenicity of SsSvf1 gene-silenced strains restored by diphenyleneiodonium (DPI) treatment. (A) Pathogenicity assay. The rapeseed leaves were inoculated with mycelium plugs of wild-type strain and SiSvf1-213 after treatment with or without DPI (5 μM) over three independent experiments. Photographs were taken at 48 hpi and the figure shows representative photographs. (B) The size of the expending lesions. Bars indicate standard deviation. Asterisks denote significant differences (one-way analysis of variance [ANOVA]): * P < 0.05.
most likely located in the cytoplasm, suggesting a role for SsSvf1 in lipid metabolism in the cytoplasm. It is interesting to note that lipocalin has been reported to be a protective factor against H$_2$O$_2$ toxicity (Roudkenar et al., 2008).

Svf1 inhibited ROS generation in S. cerevisiae, and the cells lacking SVF1 have increased levels of ROS (Brace et al., 2005). Our results showed that silencing of SsSvf1 led to overproduction of ROS in the hyphae suggesting that SsSvf1 also regulates ROS generation of S. sclerotiorum. To date, only a few genes have been demonstrated to play important roles in regulating the internal redox environment in S. sclerotiorum. NADPH oxidases (Nox) are a primary source for endogenous generators of ROS (Nauseef, 2008; Takemoto et al., 2007). Silencing of the S. sclerotiorum Nox-encoded gene Ssnox1 expression can impair superoxide production (Kim et al., 2011). The $\gamma$-glutamyl transpeptidase (Ggt) catalyzes the first step in glutathione (GSH) metabolism and recycling; it is an important factor in maintaining cellular redox homeostasis (Lee and Bostock, 2007). Deletion of the Ggt-encoded gene Ss-Ggt1 in S. sclerotiorum resulted in H$_2$O$_2$ being hyper-accumulated in sclerotia (Li et al., 2012). However, more evidence is required to elucidate the mechanism whereby SsSvf1 is involved in regulating ROS generation in S. sclerotiorum, because SsSvf1 exhibits no similarity with any known enzymes.

S. sclerotiorum secretes OA to elicit a host programmed cell death (PCD) response, which provides nutrients that are beneficial for this necrotrophic fungal pathogen (Kim et al., 2008). The PCD response requires ROS generation, the production of which may be stimulated by necrotrophs (Marino et al., 2012). Enhanced ROS generation stimulates the necrosis induced by S. sclerotiorum (Govrin and Levine, 2000). Thus, ROS modulation is critical for successful infection by this fungus. In this research, the virulence of SsSvf1 gene-silenced strains were impaired on different hosts, while showing partial recovery on hosts in which the ROS generation was inhibited. Although DPI was also used to inhibit NADPH oxidases in B. cinerea and S. sclerotiorum, direct evidence that it can inhibit ROS production in these two fungi cannot be found (Kim et al., 2011; Segmüller et al., 2008). Our results suggest that SsSvf1 plays an important role in anti-oxidation during S. sclerotiorum infection. Recent evidence indicates that S. sclerotiorum has a short biotrophic phase during the early stages of infection, and it uses OA to suppress the oxidative burst during initial stages (Kabbage et al., 2015; Williams et al., 2011). The inhibition of ROS generation by DPI can benefit the fungus during compatible interactions, although more evidence is needed.

As multi-cellular infectious structures, compound appressoria play important roles in S. sclerotiorum pathogenesis (Huang et al., 2008; Liang et al., 2015a, b; Xiao et al., 2014). Compound appressoria might help the fungus adhere to and penetrate the host cuticle and also secrete enzymes and toxins (Huang et al., 2008; Jamaux et al., 1995). In S. sclerotiorum, appressorium development might depend on the cAMP-PKA signalling pathway and be associated with OA accumulation (Jurick and Rollins 2007; Liang et al., 2015a, b). In this research, SsSvf1 gene-silenced strains showed impaired activity in appressorial formation, and their virulence was partially restored on wounded leaves of rapeseed, indicated that SsSvf1 was important for the appressoria formation. Svf1 function was only reported in S. cerevisiae, a non-pathogenic and non-appressoria forming fungus that did not form appressorium. Thus, the mechanism of SsSvf1 involved in appressorial formation is still unknown. A closer investigation showed that SsSvf1 gene-silenced strains were more sensitive to some cell wall damaging agents suggesting a

Fig. 8  Sensitivity of SsSvf1 gene-silenced strains to cell wall perturbation agents and hyperosmotic stress. The strains were inoculated on potato dextrose agar (PDA) plates amending 0.02% sodium dodecyl sulphate (SDS), 200 $\mu$M calcofluor white (CFW), 0.4 g/L Congo red (CR), 1 M sorbitol, and 0.4 M NaCl. Percentage inhibition of hyphal growth was calculated at 36 hpi. Bars indicate standard deviation. Asterisks denote significant differences (one-way analysis of variance [ANOVA]): **P < 0.01.
possible defect in the cell wall components of the hyphae for these strains. The combined evidence suggests that the reduced efficiency in appressorial formation is due to the impaired integrity of the hyphae cell wall. Defects in the cell wall components influencing appressorial formation have been reported for many important pathogenic fungi, including *Magnaporthe grisea* (Jeon et al., 2008; Skamnioti et al., 2007; Xu, 2000) and *Colletotrichum graminicola* (Albarouki and Deising 2013).

The molecular mechanism of *SsSvf1* in maintaining the cell wall integrity of hyphae in *S. sclerotiorum* remains unclear. *S. cerevisiae Svf1* affected the localized generation of a pool of phytosphingosine, which might activate the Ypk1p pathway that play a role in the cell wall integrity pathway (Brace et al., 2007; Liu et al., 2005; Schmelzle et al., 2002). Our results showed that the *SsSvf1* gene-silenced strains were highly sensitive to CR but exhibited a similar level of sensitivity to CFW versus the wild-type strain. The CFW inhibits chitin polymer assembly in the cell wall, but CR affects glucan polysaccharide assembly (Daher et al., 2011; Puttikamonkul et al., 2010; Ram and Klis, 2006). The results suggest that the chitin content was likely unaffected in *SsSvf1* gene-silenced strains. Glucan is an important component of the cell wall and is critical for maintaining cell integrity (Ram et al., 1998). However, additional studies on the alteration of glucan in *SsSvf1* gene-silenced strains are needed.

In summary, our results demonstrate that *SsSvf1* encodes a *S. cerevisiae* survival factor 1 homologue in *S. sclerotiorum*. The gene was characterized and found to play a crucial role in the antioxidant response in plant fungal pathogens.

**EXPERIMENTAL PROCEDURES**

**Fungal strains and cultured conditions**

* S. *sclerotiorum* strain 1980 was used as the wild-type strain and was routinely cultured on PDA medium. Transformant strains were cultured on PDA with 100 μg/mL hygromycin B (Calbiochem, San Diego, CA).

**RNA isolation and cDNA synthesis**

Total RNA was extracted from frozen mycelium of the wild-type strain or transformants, or inoculated *A. thaliana* leaf sample with *Rizobol* reagent (Huashun Biogengineering Co, Shanghai, China) according to the manufacturer’s instructions. The total RNA was then treated with DNase I (RNase free) (Takara, Dalian, China) to remove DNA contaminants. Approximately 1 μg of treated RNA was used to synthesize the cDNA with the ReventAid™ First Strand cDNA Synthesis Kit (MBI Fermentas, Flamborough, ON, Canada) following the manufacturer’s instructions.

**Real-time RT-PCR**

To determine the expression levels of *SsSvf1*, real-time RT-PCR using SYBR Green I technology on a CFX96™ Realtime System (BioRad, Hercules, CA, USA) was performed. The housekeeping gene *Tub1* encoding β-tubulin was used as an internal control and amplified with *Rt-tubfp/Rt-tubrp* primers. The primer pair *Rt-Svf1fp/Rt-Svf1rp* was designed according to the cDNA sequence of *SsSvf1*. Real-time RT-PCR was conducted in a 20 μL reaction mixture containing 10 μL of SYBR Green Realtime PCR Master Mix (Toyobo, Osaka, Japan), 4 pmol concentration of each primer, 1 μL of cDNA, and nuclease-free water. The following amplification programme was applied: 95 °C for 2 min (1 cycle), followed by 95 °C for 20 s, 57 °C for 15 s and 72 °C for 20 s (40 cycles). Each sample was analysed in three biological replications, and the average cycle threshold was calculated to evaluate the relative expression. The primers used here are shown in Table 1.

**SsSvf1 gene-silenced vector construction and transformant**

The *SsSvf1* gene-silencing vector was constructed based on plasmid pSilent-1 (Nakayashiki et al., 2005). To amplify the sense and antisense fragments of *SsSvf1*, the primers *SiSvf1Xho I/SiSvf1Hind III* (sense fragment) and *SiSvf1Kpn I/SiSvf1Bgl II* (antisense fragment) were used. The amplified sense and antisense fragments were inserted into the corresponding multiple cloning sites of pSilent-1 to generate the RNA silencing vector pSiSvf1. The vector was then linearized with *Spe I* and used to transform the wild-type strain of *S. sclerotiorum* according to the method of Rollins (2003).

**ROS detection assay**

The ROS detection assay was performed according to Kim et al. (2011). The strains were inoculated on PDA plates for 2 days and the plates were then immersed in 0.5 mg/mL NBT (10 mM potassium phosphate buffer, pH 7.5) aqueous solution for 2 h with gentle shaking. The hyphae were observed with light microscopy. ROS accumulation in hyphae tips was

| Primer | Sequences (5’-3’) |
|--------|------------------|
| *Rt-tubfp* | GTAGCCGAAGGGCCTGTGA |
| *Rt-tubrp* | CTTTGGAGTTGAGCAGC |
| *Rt-Svf1fp* | TGTAGAAGACTGAGGCC |
| *Rt-Svf1rp* | GACACTCTTCAGCCTTCATG |
| *SiSvf1Xho I* | CGCTCTAGTCGATGGCGCTCAGAAAATCTAG |
| *SiSvf1Hind III* | CGCAATGTCAGCTGCTGGAATACCC |
| *SiSvf1Kpn I* | CGGGTACTTGCCTGGCTGGGAGAAATCTAG |
| *SiSvf1Bgl II* | GGAAGATCTATGGCGTGAATAAAAATACCGG |
quantified via mean pixel intensity with ImageJ software according to Egan et al. (2007).

**Pathogenicity assay**

*A. thaliana* Columbia-0 and *Brassica napus* Zhongyou 821 were used for the pathogenicity assay according to Yu et al. (2017). To analyse the pathogenicity of the strains on host plants in which ROS production was inhibited, leaves of *B. napus* were sprayed with 5 μM DPI dissolved in water and then were inoculated with mycelia-colonized agar (6 mM in diameter) obtained from the growing colony margins of the strain. The lesions were measured at 48 hpi. In each experiment, each strain was inoculated on five plants or leaves and the experiment was repeated three times.

**OA concentration assays**

The OA concentration assay was performed according to Yu et al. (2017) with minor modifications. The wild-type strain and SiSvf1-213 were cultured on PDA medium for 2 days. Subsequently, four mycelium plugs (6 mM in diameter) of each strain were cultured in 50 mL of potato dextrose broth (PDB) for 3 days with shaking at 150 rpm. OA accumulation was determined by high-performance liquid chromatography according to Zhang et al. (2010). The concentration was expressed as milligrams of OA per gram of dry mycelia. Each strain was repeated three times.

**Compound appressorium assay**

To assay the compound appressorium formation of the wild-type and *SsSvf1* gene-silenced strains, 2-day-old culture plugs (6 mM in diameter) were inoculated onto parafilm and rapeseed leaves. The plugs were removed at 8 hpi for those on parafilm. To observe the appressorium, the parafilm surface were stained with 5% trypan blue for 12 h and then stained with 5% trypan blue for 12 h.

**Cell wall integrity assay**

The sensitivity of the hyphal growth to different cell wall inhibitors were tested to evaluate the cell wall integrity of the wild-type and *SsSvf1* gene-silenced strains. Two-day-old culture plugs (6 mM in diameter) of each strain were inoculated onto PDA amended with 0.02% SDS, 400 μg/mL CR, or 200 μM CFW. The colony diameters were measured after incubation for 36 h to determine the inhibition of hyphal growth; unmodified plates served as the control.

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**SUPPORTING INFORMATION**

Additional supporting information may be found in the online version of this article at the publisher’s web site:

Fig. S1 Expression level of SsSvf1 in different isolates containing pSiSvf1. Tub1 gene in each strain was the internal control. The relative expression of SsSvf1 in the wild-type strain was set as one. Bars indicate standard deviation.

Fig. S2 Oxalic acid (OA) accumulation in wild-type strain and SiSvf1-213. Each strain was cultured in potato dextrose broth (PDB) for 3 days, and the resulting liquid culture was analysed for OA accumulation.