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The role of effector Ssc1 in *Sclerotinia Sclerotiorum* and pathogenicity of *Botrytis cinerea* in the early infection stages

Li Xiaolong[1#], Ren Aizhi[1#], Shang Na[1,2], Guan Mengjiao[1], Zhang Jialin[1], Wang Lijun[1], Sun Shulei[3*] and Zhao Peibao[1,2*]

[1]Liaocheng University, Liaocheng, 252000, China.
[2]Liaocheng Academy of Agricultural Sciences, Liaocheng, 252000, China.
[3]University of California San Diego, San Diego, CA92121, USA.

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In this experiment, the action mechanism of the gene *Ssc*1 was studied in the process of interaction with plants through heterologous expression, subcellular localization and fluorescent PCR technology. It was found that the gene *Ssc*1 could enhance the pathogenicity of *Botrytis cinerea* through heterologous expression. Fusing the promoter, SP and CTP of *Ssc*1 with GFP and expressing in tobacco, it was found that the fusion protein could be secreted into plant cells and located in chloroplasts. Trypan blue staining and fluorescence detection found that in the early stage after inoculation and in the areas outside of the scab, GFP fluorescence could be detected in the tobacco leaves despite the trypan blue staining being negative. Additionally, it was proved by quantitative PCR that the gene *Ssc*1 was highly expressed in the early infection stages. Taken together, these results indicated that the effector *Ssc*1 was an important pathogenic factor, which could locate in chloroplasts and mainly play the role in the early stage during the interaction between *S. sclerotiorum* and plants.

Key words: *Sclerotinia sclerotiorum*; effector; chorismate mutase; heterologous expression; subcellular localization.

INTRODUCTION

Sclerotinia sclerotiorum (Lib. de Bary) is an important necrotrophic phytopathogenic fungus with a wide host range of more than 400 plants, including rape, sunflower, legumes, *Cucurbitaceae* and *Solanaceae* (Boland and Hall, 1994; Derbyshire, 2022), with infection mechanism similar to that of *Botrytis cinerea* (Pers.) (Amselem et al., 2011). *S. sclerotiorum* is intensely destructive to its host and oxalic acid and cell wall-degrading enzymes play important roles in its infection process (Bateman and Beer, 1965). In addition to cell wall-degrading enzymes, oxalic acid and other traditional pathogenic factors, researchers have found that secretory effector play an

*Corresponding authors. E-mail: zhaopeibao@163.com, shuleisun@gmail.com. Tel: 86-13326352519.

#Co- first author

important role in the interaction of *S. sclerotiorum* with plants (Hancock, 1966; Riou et al., 1991; Kim et al., 2011). For example, Guyon et al. (2014) identified 78 potential effector candidates by secretome analysis andanalyzed the expression patterns of 16 secretory proteins in different plants, Lyu et al. (2016) demonstrated that a small molecular effector protein, SsSSVP1, could be beneficial to self-infection by affecting plant energy metabolism. Wang Xin-yu et al. (2009) found that the effector protein *Sspg1* could interact with IPG-1, a small protein in plants, playing an important role in early pathogenic stage. Zhu et al. (2013) found that *SSITL*, a secretory effector protein, could inhibit the disease resistance mediated by jasmonic acid in the early stages of infection. Fan et al. (2021) Found that a novel effector protein SsERP1 inhibits plant ethylene signaling to promote *Sclerotinia sclerotiorum* infection.

Chorismate mutase catalyzes the conversion of chorismate to prephenic acid, providing precursors for the synthesis of phenylalanine and tyrosine (Andrew, 2003). Although chorismate mutase is ubiquitous in microorganisms and plants and is required for the synthesis of some essential amino acids, not all plant pathogenic microorganisms have this enzyme (Romero et al., 1995). Moreover, the *Cmu1* gene, which is homologous to chorismate mutase, has been shown to be closely related to pathogenicity in the biotrophic pathogenic fungi *Ustilago maydis*. *Cmu1* was also found to influence the salicylic acid (SA) level and then weaken the disease resistance signal (Armin et al., 2011). A novel chorismate mutase from *Erysiphe quercicola* performs dual functions of synthesizing amino acids and inhibiting plant salicylic acid synthesis (He et al., 2021). For nematodes, in addition to providing nutritional needs, CM also provides some help for its parasitic life (Lander et al., 2020).

Previous studies (Dickman Marty Lab.) discovered that the effector *Ssc1*, which is homologous to chorismate mutase, is related to the pathogenicity of *S. sclerotiorum* through deletion mutation (unpublished), but the mechanism is not clear.

Thus, in order to further explore the action mechanism of the gene *Ssc*1, the secretory, subcellular localization, and specific expression at different infection stages were analyzed in this experiments. The results showed that the gene *Ssc*1 could enhance the pathogenicity of *B. cinerea* through heterologous expression. Also it was found that *Ssc*1 proteins could be secreted into the host cells and colocalized with the chloroplast. Finally, through GFP fluorescence detection, trypan blue staining and quantitative PCR, it was proved that the gene *Ssc*1 were highly expressed in the early infection stages. All these indicated that the gene *Ssc*1 was an important pathogenicity factor and mainly play the role in the early stage during the interaction between *S. sclerotiorum* and plants.

MATERIALS AND METHODS

Materials and primers

S. sclerotiorum 1980, *B. cinerea* T_4 and the vector pCX62, pBluntNAT-GFP1-1 were obtained from Prof. Dickman Marty of Texas A&M University. The *Escherichia coli* derivative DH5α was used for cloning purposes. *S. sclerotiorum* 1980 and *B. cinerea* T_4 were routinely cultured on potato dextrose agar (PDA) at 25°C. All chemicals used were of analytical grade. All the primers used in this experiment are listed in Table 1.

Molecular techniques and sequence analysis of the *Ssc*1 gene

Fungal RNA and DNA were extracted using the TRIzol or cetyl trimethylammonium bromide (CTAB) protocol. The plasmid DNA was isolated using the plasmid kit (OMIGA) according to the protocol. Phylogenetic tree generation, and DNA and protein sequence alignment and analysis were conducted using DNAman7.0 software (Lynnon Biosoft). Primers were designed using Primer Premier 5.0 (Premier). Fungi were transformed using the method of restriction enzyme-mediated integration (REMI) based on the same method described previously (Zhao et al., 2010; 2011).

Cloning of the *Ssc*1 gene and heterologous expression in *B. cinerea*

The DNA fragment of the *Ssc*1 gene and its promoter was cloned using the primers SCP1 and SCP2, after which the expression vector pCX62-*Ssc*1 was constructed by inserting the fragment into pCX62. They were then digested by *Xho* I and *Hin*d III, respectively, and then transformed into the protoplast of *B. cinerea* T_4 strains as mentioned above (Zhao et al., 2010; 2011). Transformants were selected on PDA plates using hygromycin B at 250 ug/mL, and then the integration of gene *Ssc*1 into the genome of *B. cinerea* and its normal expression were confirmed by PCR and RT-qPCR, respectively. Finally, the transformants were inoculated onto tobacco using the methods of *in vitro* leaf inoculation with mycelial wafer and spraying with conidial suspension as described previously (Zhao et al., 2018; Xu et al., 2011). The culture dishes and nutrient plates were placed in a 25°C, 85% relative humidity, 14 h light /10 h dark cycle phytotron. The lesions were studied from 12 h, and the disease severity was determined by calculating the disease index. The disease grades were as follows: grade 0: no symptoms; grade 1: small infection spots identified on 1–2 leaves; grade 2: small infection spots identified on 3–5 leaves; grade 3: 1–2 leaves began rotting; grade 4: 3–4 leaves began to rot; grade 5: the whole plant has begun to rot.

Cloning and confirming the promoter of *Ssc*1

The promoter element and the *cis* element were analyzed using the online software Promoter 5.0. The DNA sequence of the promoter was cloned by PCR using the XS1-1 and XS1-2 primers, after which the GFP fusion vector was constructed and transformed in the protoplast of *S. sclerotiorum.* The GFP fluorescence in the hyphae of the transformant was detected by confocal fluorescence microscope.

Analysis of the secretory and subcellular localization of the effector

The signal peptide and localization peptide were predicted using

Table 1. All primers used in this experiment.

Name	Sequence	Location	Using and the size of expected fragment
SCP1	CCGCTCGAGCGCAAGGAGGATCCTAATAG	-733	promoter + *Ssc*1(1216bp)
SCP2	CCCAAGCTTTTAAGAAGAAATCGCCCAAAC	483	promoter + *Ssc*1
XS1-1	GCAAGGAGGATCCTAATAGAATC	-733	Promoter(732bp)
XS1-2	TCCCCCCGGGGTTGGGTGATTGAAG	-1	promoter
XS1-3	TCCCCCCGGGGGAGATGGCGAGAGGAG	60	Promoter+SP(793bp)
XS1-4	TCCCCCCGGGGCATGTTGTTCCATTAGGAAGG	138	promoter+SP+CTP(871bp)
XS1-5	GCATGTTGTTCCATTAGGAAGG	138	mutation screening
HPH1	ATGAAAAAGCCTGAACTC	hph	Transformant screening(1000bp)
HPH2	CTATTCCTTTGCCCTCGG	hph	Transformant screening
Ssc1-1	ATGAAATTCACCACCATTTC	1	*Ssc*1
Ssc1-2	TTAAGAAGAAATCGCCCAAAC	483	*Ssc*1
Ssc1-3	CCCCTCCTACACCCTTCCT	154	Real-time PCR(178bp)
Ssc1-4	GGCACATTCACATCACCCA	332	Real-time PCR
b-t1	TTGGATTTGCTCCTTTGACCAG	b-tubulin	Real-time PCR(104bp)
b-t2	AGCGGCCATCATGTTCTTAGG	b-tubulin	Real-time PCR

Source: Authors

online bioinformatics software, including the SignalP 4.1 Server, PredictNLS and ChloroP 1.1 Server. The fragments containing the promoter+signal peptide (SP) and the promoter+signal peptide (SP) +localization peptide (CTP) were then cloned by PCR using the primer pairs XS1-1/XS1-3 and XS1-1/XS1-4, respectively. Nextly, the GFP fusion vector was constructed and transformed into the protoplast of *S. sclerotiorum* as mentioned above. When inoculating tobacco with the transformant, the fusion protein localization was detected through confocal fluorescence microscope.

Detecting the secretory time and tissue specificity of the *Ssc*1 effector through trypan blue staining

At different times after inoculation with the transformant, in which the GFP fusion protein could be expressed, the leaves of tobacco were dyed using trypan blue and then analyzed by confocal fluorescence microscope.

Detection of transcriptional differences of Ssc1 gene at different stages and locations

Following inoculation on tobacco with the wild type of *S. sclerotiorum*, the mycelia were collected from the areas of the early stage of inoculation site, within the scab and outside the scab, after which RT-PCR of the *Ssc*1 gene was conducted using the primer set *Ssc1*-1/*Ssc1*-2. Additionally, RT-qPCR of the gene *Ssc*1 was performed according to the manufacturer's suggestions, primers were designed according to the sequence of the gene (Table1), and tubulin was used as reference gene for fluorescent qPCR.

RESULTS

The gene *Ssc*1 could heterologously enhance the pathogenicity of *B. cinerea*

A 1216 bp DNA fragment containing the *Ssc*1 gene and its promoter was obtained (Supplementary data 1) by PCR using the primers SCP1 and SCP2 (Table1). The fragment was inserted into the vector pCX62 and transformed into the protoplast of the pathogenic fungus *B. cinerea* T_4 strains. The gene was confirmed to have been integrated into the genome of *B. cinerea* and could be expressed normally by PCR and RT-PCR (Supplementary 2). The pathogenicity of the transformant 3-6-1 was enhanced comparing with that of the wild type following inoculation of tobacco leaves (Figure 1 and Table 2).

The promoter of *Ssc*1 was obtained

The promoter element was analyzed using bioinformatics software, which revealed the presence of a TATA box and a CAAT box in the upstream portion of the gene. The promoter was predicted as shown below.

Promoter predictions for seq 0–2000 bp:
Start End Score
1888 1938 0.98
Promoter Sequence (transcription start shown in bold):
CTCCTTTCTGGTGGCTGTCATATAAGTACGCTCCCAA
CCTCAATGTTCAA

A 733-bp DNA fragment upstream of ATG was cloned by PCR using the XS1-1 and XS1-2 primers (the sequence is shown in Supplementary 2), after which the GFP fusion vector was constructed based on the pBluntNAT-GFP plasmid according to the roadmap (Supplementary 3) and transformed into the protoplast of *S. sclerotiorum*. Some transformants in which the GFP fluorescence was detected through confocal fluorescence microscopy were

Table 2. Disease severity in nutritious pots.

Time	Strains	Total plants	Disease incidence (%)	Disease index	F test	
					0.05	0.01
3dpi	WT(T4)	30	33.3	7.4	ns	ns
	Ssc1+	30	83.3	42.7	**	**
	CK	30	3.3	2.0		
5dpi	WT(T4)	30	50.0	14.7	ns	ns
	Ssc1+	30	100	60.7	**	**
	CK	30	6.7	4.7		

(**: greatly significant difference; *: significant difference; ns: no significant difference. CK, inoculated with water)
Source: Authors

Figure 1. Comparison the pathogenicity of the transformant 3-6-1 (*Ssc*1+) with that of wild-type.
When inoculating on the tobacco leaves, the pathogenicity of the transformate 3-6-1 (*Ssc*1+) was enhanced than that of the wild-type *B. cinerea* T_4 strain. (a):Spraying with conidia suspension; (b): in vitro leaf inoculation with mycelial wafer.
Source: Authors

then obtained (Supplementary 4). Taken together, these findings indicated that the 733 bp DNA fragment upstream of the ATG codon could act as the promoter.

The effector could be secreted and colocalized with the chloroplasts of tobacco

Online bioinformatics software revealed a predicted signal peptide (SP) with 20 amino acids and a chloroplast target peptide (CTP) with 26 amino acids in the gene sequence of *Ssc*1 (Supplementary 5).

Two PCR products that contained promoter+SP and promoter+SP+CTP were cloned by PCR using the primer pairs XS1-1/XS1-3 and XS1-1/XS1-4, respectively. The GFP fusion vector was then constructed according to the roadmap (Supplementary 3), after which it was transformed into the protoplast of *S. sclerotiorum* as mentioned above. A few transformants were obtained, and the recombinant vector was confirmed to have been integrated into the genome by PCR using the primers of HPH1 and HPH2 by PCR in the transformant 3-7-5 and transformant 4-2-8 (Supplementary 6).

Finally, inoculation on the tobacco leaves with transformant 3-7-5, in which GFP was fused with promoter+SP+CTP, revealed that the GFP fusion protein

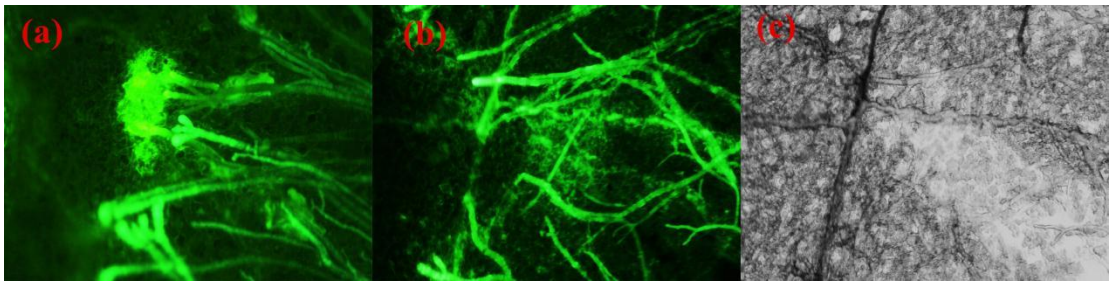

Figure 2. The expression and secretory of the GFP fusion protein in transformant 3-7-5.
When the transformant 3-7-5 in which the GFP fusion vector have been integrated into the genome was inoculated on tobacco, it had been found that the fusion protein could be expressed and secreted into the plant tissue, (a) (b): Fungi hyphae inoculating on tobacco; (c): the result in bright field of vision.
Source: Authors

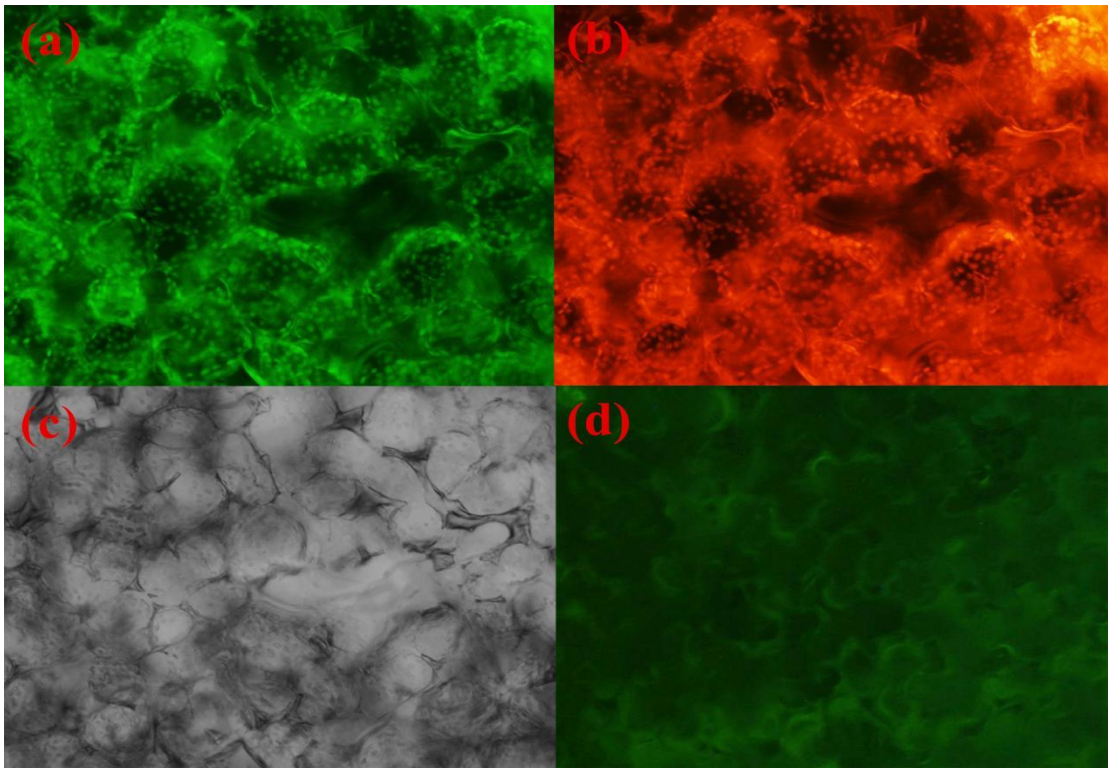

Figure 3. The subcellular localization of the GFP Fusion protein in transformant 3-7-5.
After inoculating the transformant 3-7-5 for 24h, the fluorescence of GFP were colocalized with the Chloroplast. (a): The fluorescence of GFP in the Chloroplast; (b): The autofluorescence of Chloroplast; (c): the result in bright field of vision; (d): The fluorescence of the leaves which not be inoculated with transformant (CK).
Source: Authors

could be expressed, secreted into the host cell, and localized in the chloroplast (Figures 2 to 3). However, the GFP could not be co-localized with the chloroplast when the transformant 4-2-8 was inoculated on tobacco, as the GFP fusion protein had no chloroplast target peptide (Figure 4).

The *Ssc*1 protein were secreted in plant cells in the early infection stage

Following inoculation on tobacco with transformant 3-7-5 for 12 h, GFP fluorescence was detected in the leaves by confocal fluorescence microscopy, despite no scab

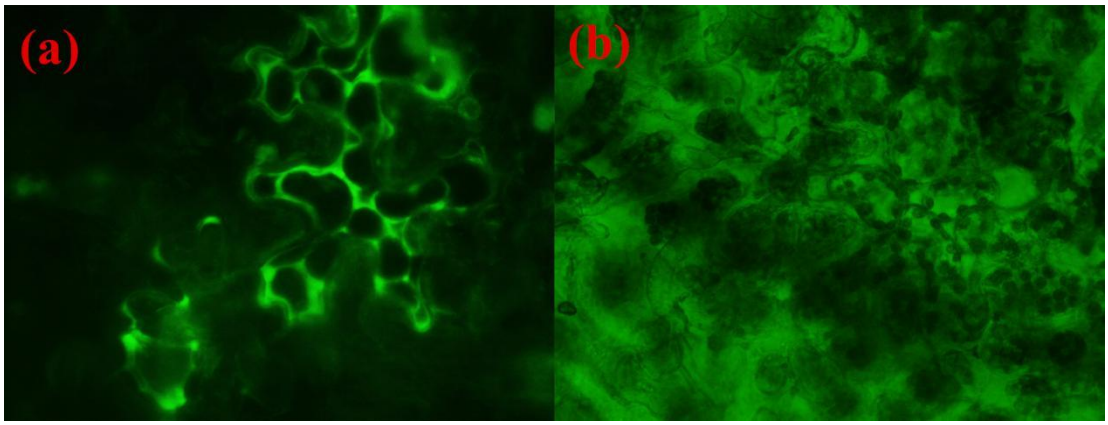

Figure 4. The subcellular localization of the GFP Fusion protein in transformant 4-2-8.
After inoculating the transformant 4-2-8 for 24h, The fluorescence of GFP were not localized in the Chloroplast. (a): The fluorescence of GFP; (b): The autofluorescence of the leaves.
Source: Authors

emerging on the leaf and negative trypan blue staining results (Figure 5). After inoculation for 24 h, a scab began to form, and the leaf tissues of the tobacco and pathogenic hyphae within the scab could be stained by trypan blue, which indicated that the plant cells had died. On the contrary, in the areas outside the scab, although the trypan blue staining of tobacco tissue and the fungal hyphae was negative, GFP fluorescence could be detected (Figure 5). In combination, these findings indicate that although the plant cells were alive in the early infection stage, the fungi had begun to expand; also the effector had begun to be secreted into the plant cells.

The gene *Ssc*1 was highly expressed in the early infection stage

Analysis by RT-PCR and RT-qPCR revealed that the gene *Ssc*1 was highly expressed in the early infection stage and in the areas outside the scab, but its expression was relatively low within the scab, in which plant cells have died (Table 3 and Figure 6).

DISCUSSION

In this study, the gene *Ssc*1 homologous to chorismate mutase in *S. sclerotiorum* was proved to be closely associated with pathogenicity through heterologous expression in *B. cinerea*. The gene encodes a small protein, which could be secreted into plant cells and localized in the chloroplast, playing a role in the early stages of infection.

The shikimate pathway is a fundamental metabolic pathway in plants and microorganisms. The final product of this pathway is chorismate, which is the precursor of many important compounds, including aromatic amino acids (phenylalanine, tryptophan, and tyrosine), salicylic acid (SA), indole-3-acetic acid (IAA) and other secondary metabolites (Strack, 1997). These chorismate-derived compounds (CDCs) play important roles in plant growth, development, defense, and interaction with other organisms (Romero et al., 1995).

Chorismate mutase catalyzes the conversion of chorismate to pre-phenylic acid, providing precursors for the synthesis of phenylalanine and tyrosine (Andrew, 2003). For parasitic pathogenic microorganisms, phenylalanine and tyrosine can be obtained from the host, and thus chorismate mutases may not be necessary for their amino acid metabolism.

However, BLAST searches of the NCBI database revealed that only some pathogenic microorganisms have genes homologous to chorismate mutases, such as nematodes, *U. maydis*, *Erysiphe quercicola*, etc., which have been proved to play a role in the interaction of plant pathogens and plants (Armin et al., 2001; He et al., 2021; Lander et al., 2020).

Armin et al. (2011) found that the *Cmu*1 gene homologous to chorismate mutase was closely related to pathogenicity through affecting the SA level and the resistance signal. It was usually considered that the SA pathway typically plays an important role in the defense against biotrophic pathogens but does not play a major role in resistance to necrotrophic pathogens in plants (Strack, 1997; Govrin and Levine, 2000; Yang et al., 2015). Unlike *U. maydis*, *S. sclerotiorum* is a necrotrophic fungus, as for how this effector play a role in necrotrophic fungus in the process of infection the plant is an interesting question.

Previous studies by the Dickman Lab (unpublished) on the deletion mutation of *Ssc*1 indicated that the *Ssc1* effector was related to pathogenicity. In this study, this

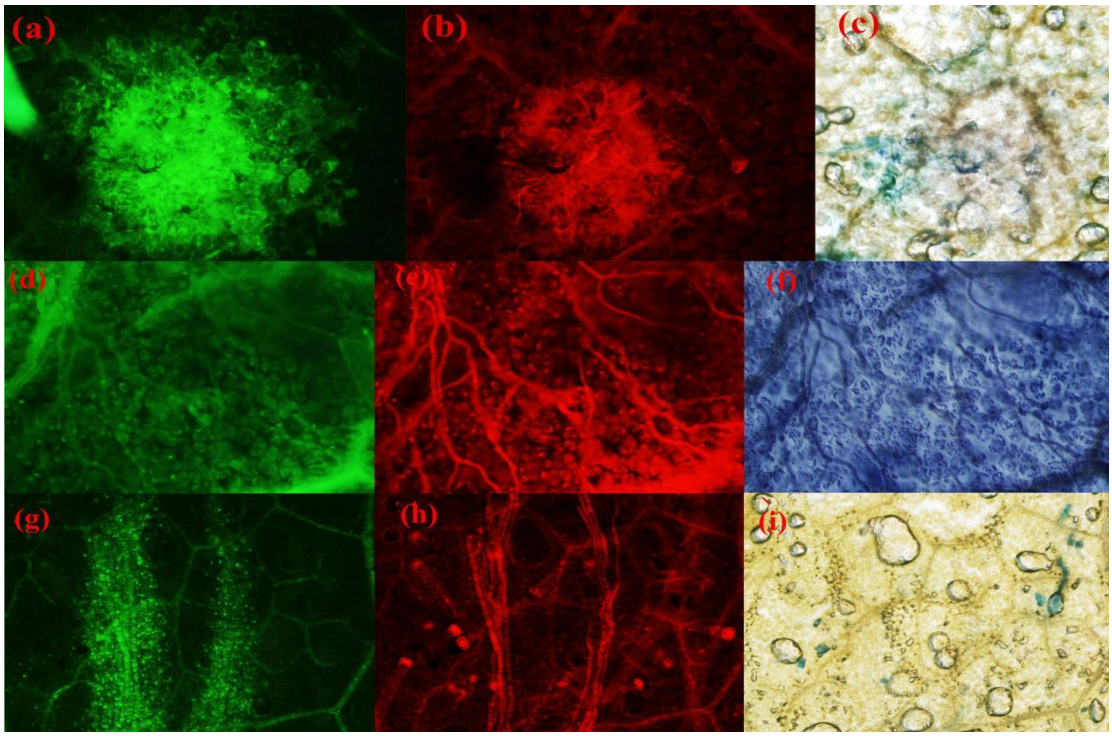

Figure 5. The result of trypan blue staining after inoculating the transformate 3-7-5.
The result of trypan blue staining after inoculating the transformate 3-7-5 for 12h (a): The fluorescence of GFP; (b): The autofluorescence of the plant cells and hyphae; (c): the result of trypan blue staining. The result of trypan blue staining after inoculating the transformate 3-7-5 for 24h(d) (g): The fluorescence of GFP; (e) (h): The autofluorescence of the plant cells and hyphae; (f) (i): The result of trypan blue staining. ((d) (e) (f): within the scab; (g) (h) (i): out of the scab).
Source: Authors

Table 3. The results of RT-qPCR.

Sample	tubulin Ct	choris Ct	ΔCt	ΔΔCt	2-ΔΔCt
Inoculating *S. sclerotiorum* for 2h	28.02	28.41	0.39	0.00	1.00
Outside of the scab	27.65	28.25	0.6	0.29	0.86
Within the scab	27.58	31.61	4.03	3.64	0.08 aA

Lower-case letters stand for the significant difference at the level of 5%; capital letter stand for the highly significant difference at the level of 1%).
Source: Authors

gene was found to enhance the pathogenicity of the pathogen *B. cinerea* through heterologous expression. These findings suggested that the gene is a pathogenic factor rather than a gene necessary for amino acid metabolism. Both *B. cinerea* and *S. sclerotiorum* are necrotrophic pathogens with similar pathogenic mechanisms. Thus, further investigation is needed to determine why only *S. sclerotiorum* retains this important factor related to the interaction between fungi and plants.

In this experiment, within 12 h after inoculation of the transformants 3-7-5 onto tobacco, the mycelia and plant cells could not be stained by trypan blue. Negative trypan blue staining indicated that the plant cells were alive during the early stages of infection. However, GFP fluorescence could be detected in the tobacco leaves despite the trypan blue staining being negative. Through fluorescence detecting, it could be found that the pathogenic hypha had begun to expand near the inoculation point before trypan blue staining positive. It was reported that the necrotrophic pathogens may have a semi-living biotrophic stage, in which stage *S. sclerotiorum* expanded into the plant tissue through its

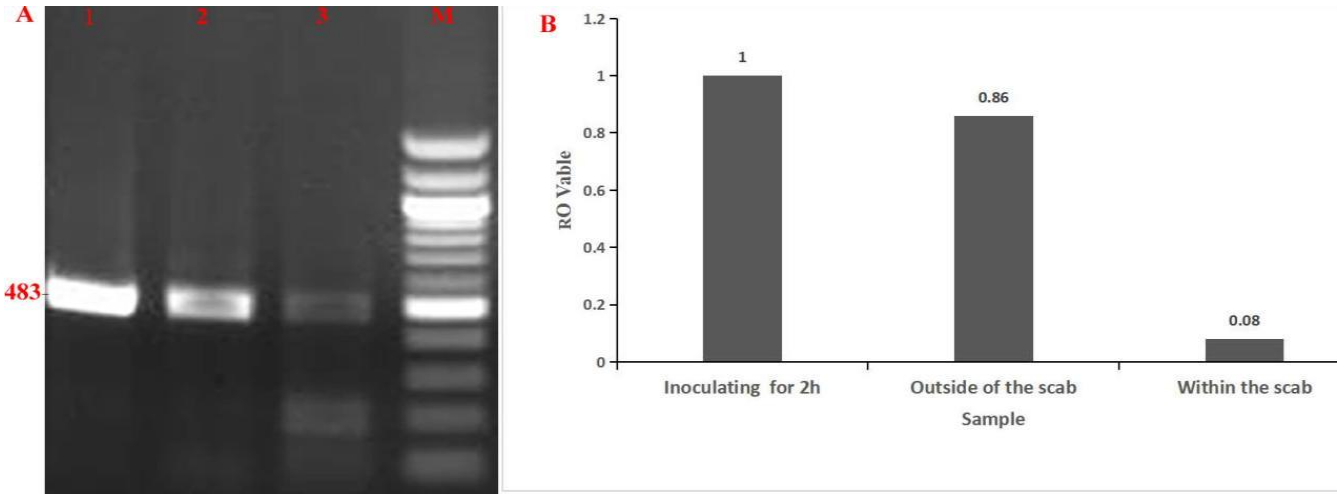

Figure 6. RT-PCR and RT-qPCR of the gene *Ssc*1 in different stage.
(A) The RNA was obtained from the hyphae which 1. when inoculating *S. sclerotiorum* for 12 h; 2 outside of the scab; 3. Within the scab. (B) The RNA used as RT-qPCR were obtained from the leaf tissue which 1. Inoculating *S. sclerotiorum* for 2h; 2. outside of the scab; 3.within the scab.
Source: Authors

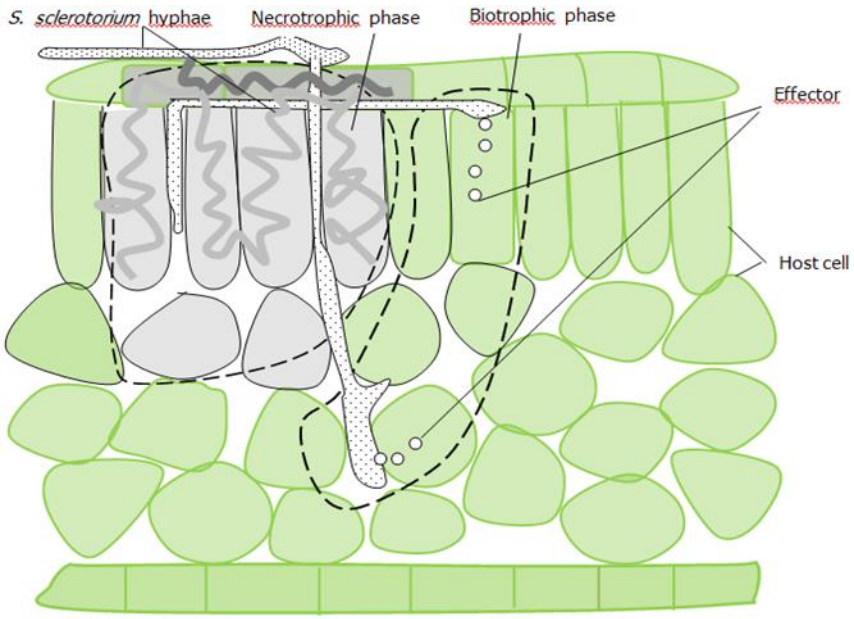

Figure 7. The model of the secretory of *Ssc*1 effector and the infection of the fungus *S. sclerotiorum.*
Source: Authors

adaptation to the plant environment and inhibition of host defense (Mehdi et al., 2015). Effectors produced by the fungus may help its own growth and inhibit host resistance during this semi-living biotrophic stage. It was speculated that the *Ssc*1 effector may influence the SA level and resistance signal by affecting the metabolic pathway of chorismate during this stage, similar to that of the *Cmu*1 gene, and then the mycelium grew and expanded as proposed in Figure 7.

At 24 h after inoculation with the transformant 3-7-5, scabs began to form, and the trypan blue staining of plant cells and pathogenic hypha within the lesion range was

positive, indicating that the cells had died. During this stage, GFP fluorescence was obviously co localized with the chloroplasts of the tobacco, indicating that effector proteins accumulated in chloroplasts (Figure 3). Previous research showed that the response of plants to various stress factors first occurs in the membrane system (Renu et al., 2014). Stress factors, including pathogenic organisms, cause metabolic disorders and accelerate the variation in the biochemical and biophysical structures of membranes, as well as initiate some secondary metabolism related to the stress response (Renu et al., 2014). The chloroplast is an organelle specialized for carrying out photosynthesis in plants, and the Calvin-Benson cycle occurring in the chloroplasts can provide chorismate precursors, such as D-erythritose-4 phosphoric acid and sedum phosphate heptaose (Wang et al., 2006). Perhaps under the pressure of pathogen infection, the chloroplast membrane structure was first disordered, and then the accumulation of some enzymes, including exogenous chorismate mutase, on the imperfect chloroplast affects the secondary metabolism related to plant defense, such as the metabolism related to salicylic acid (SA), IAA and aromatic amino acids (Baier and Dietz, 2005). However, further studies are necessary to determine why the effector protein was located in the chloroplast as well as its role in this organelle.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Short legends for supporting information

Supplementary. 1: The DNA sequence of the promoter and gene *Ssc*1
The full length DNA fragment containing the *Ssc*1 gene and its promoter is 1216 bp.
Supplementary. 2 Detecting the integration of the recombinant vector and the expression of *Ssc*1 in the Transformant 3-6-1 by PCR and RT-PCR
(a) : Detecting the integration of the recombinant vector in the transformant 3-6-1 by PCR using primer pairs Ssc1-1/Ssc1-2 (1) vector; (2): Transformant 3-6-1; (3): WT and HPH1/HPH2 (4) vector; (5): Transformant 3-6-1; (6): WT.
(b) : Detecting the expression of *Ssc*1 in the Transformant 3-6-1 by RT- PCR using the primer pair Ssc1-1/Ssc1-2 (1):Transformant 3-6-1 ; (2):wild-type.
Supplementary. 3 Construction of the GFP fusion vector
The fusion vector was constructed basing on pCX62. Firstly, both pCX62 and pBluntNAT-GFP1 were digested with *Bam*H1 and *Xba*1, then linked by T_4 ligase. Secondly, the vector and PCR fragment which containing (1): promoter +signal peptide (SP)+chloroplast localization peptide(CTP); (2):promoter; (3): promoter +signal peptide(SP) were digested with *Bam*H1 and *Xma*1 respectively, and then linked by T_4 ligase.
Supplementary. 4 Detecting the GFP fluorescence in the hyphae of transformant
Detecting the GFP fluorescence in the transformant in which GFP fusion with the nature promoter of *Ssc*1. (a): The fluorescence of the hyphae; (b): The fluorescence of the hyphae in the leaf of tobacco; (c): the result in bright field of vision.
Supplementary. 5 The predicted SP and CTP in *Ssc*1
Online bioinformatics software revealed a predicted signal peptide (SP) with 20 amino acids and a chloroplast target peptide (CTP) with 26 amino acids in the gene sequence of *Ssc*1.
Supplementary.6 Detection of *hph* gene in the transformant 3-7-5 and the transformant 4-2-8 by PCR using the primers pair HPH1/HPH2
M: DNA Mark DL2000; 1: WT; 2: Transformant 3-7-5; 3: Transformant 4-2-8