



Sclerotinia sclerotiorum

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ABSTRACT

Sclerotinia sclerotiorum

38 . G (G) A 15 .

8,44 . A (V238A) SsOs1

S. sclerotiorum 17 .

S. sclerotiorum 24 .

Neurospora crassa

34 . A DCF Bo-

trytis cinerea 11,35 .

A DCF 10, DCF

17,24,37,39,40,48 . *S. sclerotiorum*, 14–17 ,

DCF fi S.

(D) ()

(Sshk, SsYpd, Sssk1) A (Sssk2, SsPbs, SsHog)

2. Materials and methods

2.1. Isolates of *S. sclerotiorum*

fi (G2, G3, G4, G5) 2011 48 . A S.

G 4 C

85.5, 94.8 78.3, 47 .

75% 3 , 1% 30 ,

fi 30 48 . (DA)

23 C 23 C 2

S. sclerotiorum (5 . F) S.

2- - DA .

F DA 0.25 μ / . A , fi 23 C 3 ,

2.2. Fungicide

(96.2% , C) ,

C . 1000 μ /

4 C .

2.3. Determination of glycerol content in mycelia

G 14,45 . B fl ,

(630 . : 1

C 4 0.05 / 3.5 0.05 /

50 10

fi 0, 0.0025, 0.003, 0.004, 0.005, 100

0.006, 0.008, 0.01 / .

12 , fi

. A 630

. DA 0.25 μ /

2.1.

DA 20

50

80 C 15 . A

8500 10 ,

630

2.4. Determination of oxalic acid content

D

. 12 . (III)

510

: 2 F C₃ (0.5 /) ,

20 C (C 50 , 2), 1.2

50 fl . D (5 /) (0, 0.1, 0.2, 0.4 0.8)

(2 /)

10 25 . fi

510 25 C 30 .

250- fl 100 (DB).

F DB 0.25 μ / . A 23 C 3

175 1500 10 ,

510

2.5. Determination of cell membrane permeability

14 . 2.3.

(0.3) 50

30 . E

(BA E900, BA E , C) 0, 5, 10,

20, 40, 60, 80, 120 160 . A 160 ,

10 , fi

: (%) = C / F

× 100.

2.6. Determination of peroxidase and polyphenol oxidase activity

A 0.35 -
 2.3 0.1 -
 - 0.8 0.05
 (5.5 D 6.8).
 12000 , 4 C 20 .
 D
 D . 14 . 1.5 ,
 0.04 0.475 0.2% -
 50
 470
 0.5 0.3% 2 2
 fi
 fi
 0.69 0.05 (6.8) 0.75
 0.02 30 C 2 . A
 398

2.7. Cloning and sequencing of genes involved in two-component HK system and MAP kinase cascades

G D A C AB ()
 B) 33 . A
 S. sclerotiorum 1 ,
 (Sssk1), (Sshk),
 (SsHog), A (SsPbs), A (Sssk2)
 5.0 (5.0, B , A , CA),
 1. A fi
 D A, 1-5™ 2 -F
 400 μ (E B , B ,
 C). A fi 2 98 C, 35
 : 98 C 10 , 55 C 15 ,
 E 72 C 1 , fi 72 C 10 D A
 (B - , C1000). A fi
 (C) 1%
 fi E . A G E (EGA),
 GE - E (),
 E C , .
 , CA) D A (6.0, C ,
 / -) (:// . . . / /
 / D A

Table 1

	(5'-3')	
F1	A GGGGGACAC ACGA AGC C	Sshk
1	AG ACAGG CC GCAAG GG	
-F	A GGC GAA CG AGAG	SsHog
-F	GACCA CCACCAGA GGGCG	
-	A GACAGA AA CC CAA AGA CG	SsPbs
-	CA CAAACCC GC CC C CA	
2-F	A GGAGCG CCACA A G	SsSk2
2-	AC GAGAGC CCAC A CGC G	
1-F	GACGA AGGCGA C CAAAACACGA	SsSk1
1-	A CCCACAACCACC CA A CG	
-F	A G CC C CCAC C ACC C	SsYpd
-	G GGCA AAAAC C CAAG	
β -F	GA GC CC GACCAG	β-
β -	AGCGCCA CA G C AGG	
-F	AAGGG AGGGAAG AAGGCAA C	Sshk
-	G A AGGGCCAG GG AG G A G	

2.8. Quantitative RT-PCR

A (, C -
 , CA, A). F - D A
 () . A - C
 2 - 5 μ C (B -) .
 (B - , , CA, A), 1 μ F™ E G
 0.5 μ (10) (1),
 3.0 μ
 95 C 30 , 40 95 C 5 , 60 C
 10 . : 10
 55 C 95 C 0.5 C
 Sshk β-
 2^{-ΔΔC}
 25 .

2.9. Data analysis

VA t-
 D, , (,
 , .17.0, , C ,) . G
 E (.2010, C , -
 , VA).

3. Results

3.1. Glycerol content

fi (P = 0.065)
 (F . 1).
 (P < 0.01)
 30.3%, 86.1%, 27.3%
 13.9%, 19.1%, 15.5%
 G2, G3, G5

3.2. Cell membrane permeability and oxalic acid content

fi (P = 0.853)
 ;

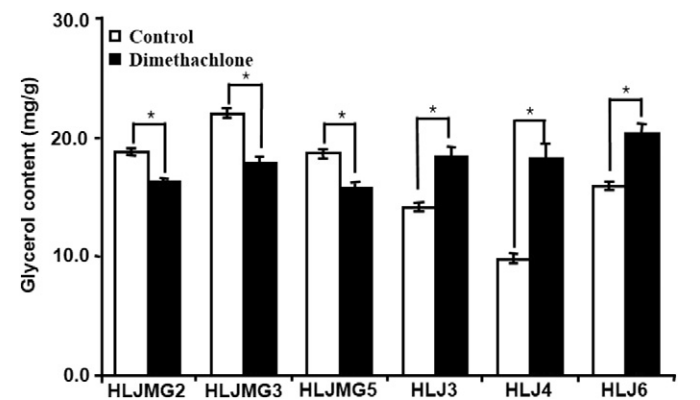


Fig. 1. Glycerol content (mg/g) in Sclerotinia sclerotiorum. **** (p < 0.0001), * (p < 0.05), V (p < 0.001).

C (P < 0.05) F (P < 0.05) 0.25 μ / (F .2), (P < 0.05)

3.3. Peroxidase and polyphenol oxidase activity

D (P < 0.05) DA (P < 0.05) (F .3). D

3.4. Newly identified point mutations in the Sshk and SsPbs genes

Sshk: A AC , 232 fi 90- GG GA (F .4). 1087 Sshk

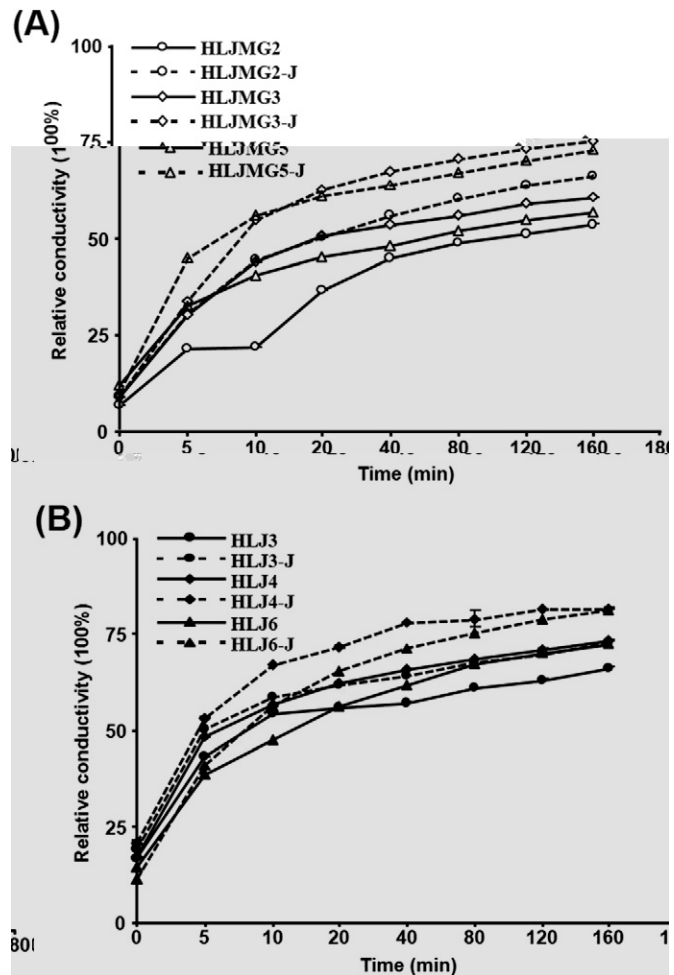


Fig. 2. Sclerotinia sclerotiorum. (A) (B) (E)

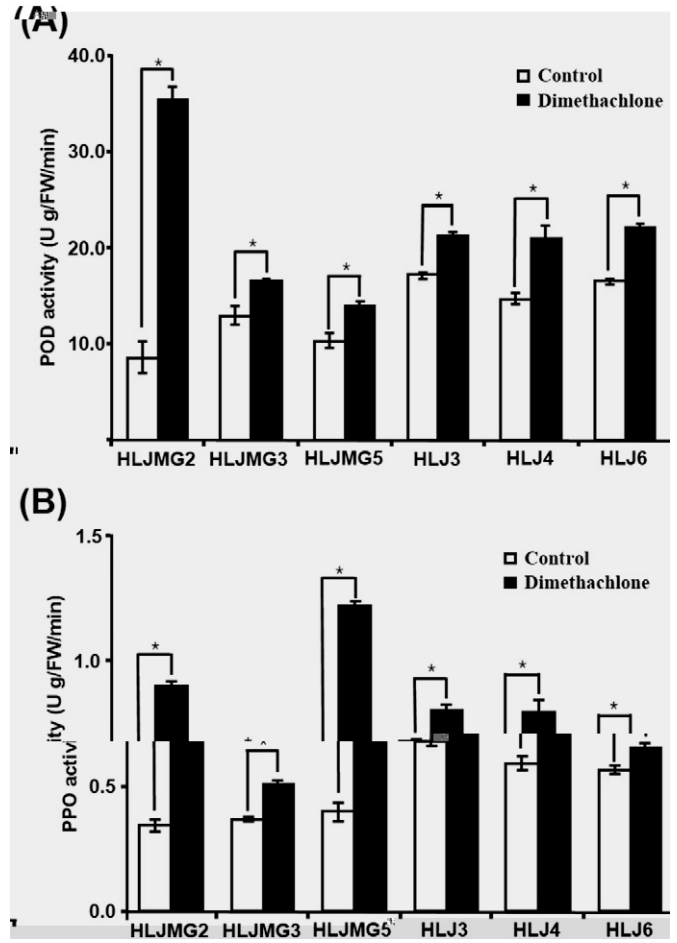


Fig. 3. (D) (A) (B) Sclerotinia sclerotiorum. (E) (α = 0.05). V

SsPbs 96 SsYpd (324C), Sssk1 (A759G), Sssk2 (C48 , A3447 1736G), SsHog

3.5. Transcription levels of the Sshk gene

(P < 0.05) Sshk fi (P < 0.05) (F .5). F Sshk

4. Discussion

fi S. sclerotiorum, B. cinerea S. sclerotiorum, fi

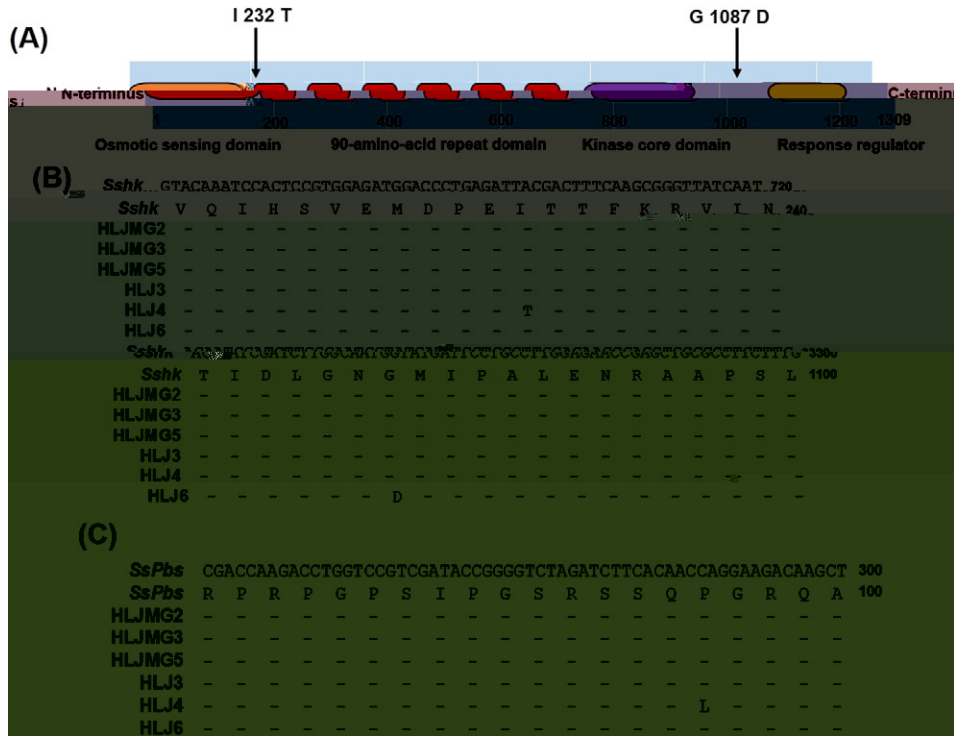


Fig. 4. (A) Schematic of Sshk protein structure and domains. (B) Sequence alignment of Sshk (720 aa) with HLJMG2, HLJMG3, HLJMG5, HLJ3, HLJ4, and HLJ6. (C) Sequence alignment of Sshk (1100 aa) with SsPbs (300 aa) and HLJMG2, HLJMG3, HLJMG5, HLJ3, HLJ4, and HLJ6.

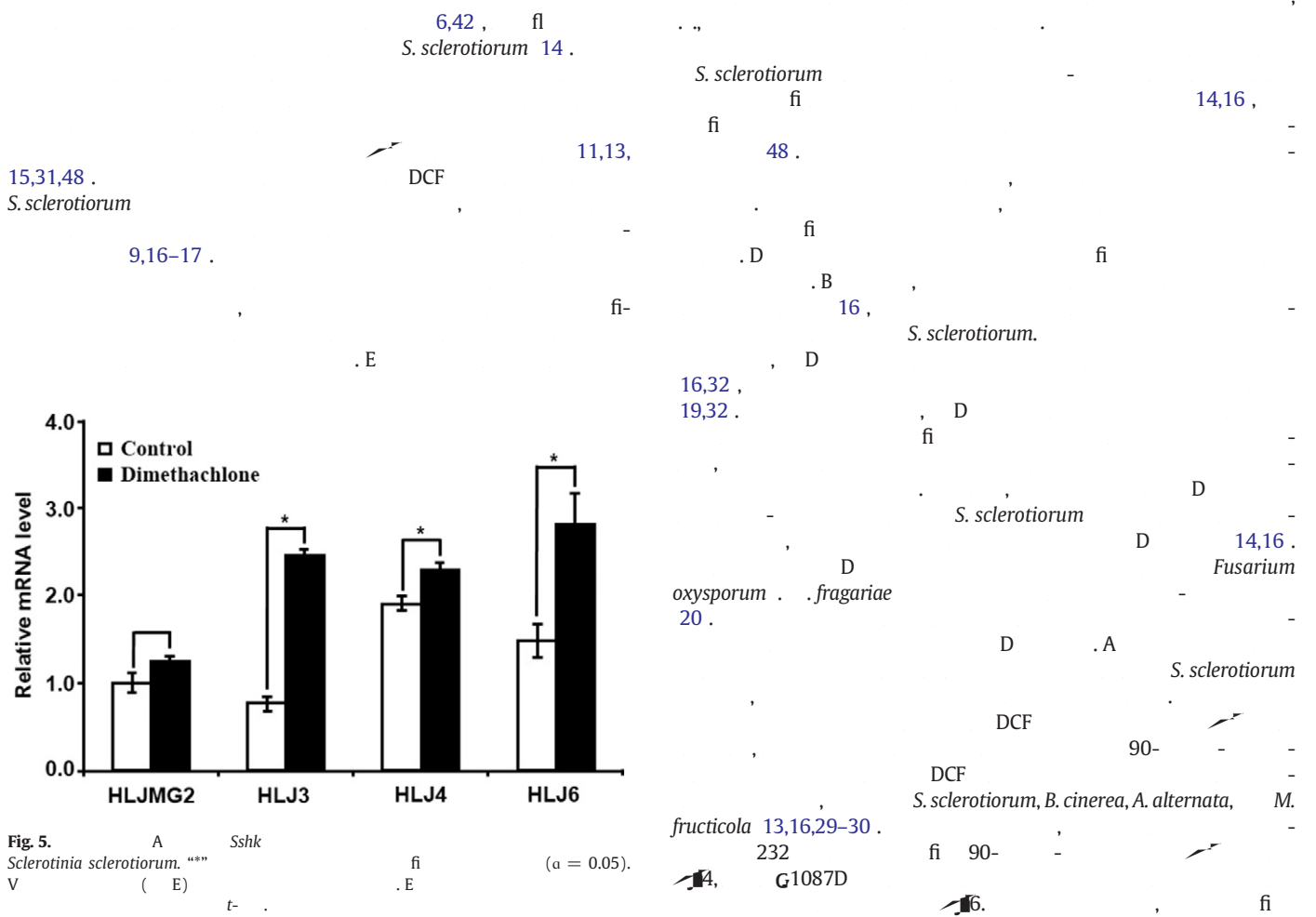


Fig. 5. Relative mRNA levels of Sshk in *Sclerotinia sclerotiorum* under control and dimethachlone treatment. Error bars represent standard deviation. Significance is indicated by asterisks (*). (α = 0.05).

S. sclerotiorum. A
1040
(*Bos1*)
B. cinerea 31.
SsYpd, Sssk1,
. A

(D 1)
Sshk
G2
Sshk . A

sclerotiorum, *A. longipes* *B. cinerea* 17,18,26 . A
V238A
Ssos1
S.
Sshk
S. sclerotiorum.
G1087D) Sshk (232
S. sclerotiorum.
S. sclerotiorum
S.
sclerotiorum - fi Sshk Sshk
16,20,29–31 .

6 C , *Sclerotinia sclerotiorum* C
2008.
7 C , C, B.G. , E C 2 (2003) 1151–1161.
8 C.C , .C. , 117 (1998) 723–731.
9 .C , .C , , Sclerotinia sclerotiorum
Sclerotinia sclerotiorum
10 C , D, C .32 (2010) 534–539.
C.B.
11 C , E.B , D. , A
Alternaria brassicicola, .72 (2009) 1316–1333.
Botrytis cinerea, F G . B .36 (2002) 187
Botryotinia fuckeliana

Competing interests

Acknowledgements

C (31371964).

References

- 1 A , C.A.C , A. , V , E. , B , A.C , C , D , F , E.F , G , A. , A. , B. , B. , B. , B. , C. , C. , V.A , E. , C , E.G.D , C.D. , A.G , C.G , G , C.G , G , A. , C. , E. , B. , A. , D. , C. , D. , Sclerotinia sclerotiorum Botrytis cinerea, G .7 (2011), 1002230.
- 2 D.B , C. , Sclerotinia .23 (2001) 88–98.
- 3 B , D , A , F , D , 2013, .281–310 ().
- 4 G.B , Sclerotinia sclerotiorum, C .16 (1994) 93–108.
- 5 D. , B. , B.D. , Sclerotinia sclerotiorum () B : .7 (2006) 1–16.

36 Sclerotinia sclerotiorum, A. 13 (1997) 32–35.

37 D.A., Fusarium oxysporum, F 1. 11 (2010) 395–407.

38 C. fi, C.

39 (E.), F, (A.) 2010, 523–538. Dic1p, G1- A.

40 (E.), 8, V, F, A. Cochliobolus heterostrophus, C. 2006, 1–6.

41 G., cinerea, A. 19 (2006) 1042–1050. Cercospora zea-maydis, A. 41 (2010) 156–160.

42 Botrytis cinerea, 2007.

43 Sclerotinia sclerotiorum, C, C, G., D. C.

44 A., A., 43 (2014) 307–312.

45 A., D, B. 26 (2001) 369–376.

46 F, 29 (2004) 40–43. Sclerotinia Brassica napus, B. 122 (2003) 19–23.

47 F., D Sclerotinia sclerotiorum, C, A, 2014.

48 F., F., D Sclerotinia sclerotiorum, C, D. 98 (2014) 1221–1226.

49 F., F., A., F Sclerotinia sclerotiorum, C, D. 98 (2014) 568.