Sclerotinia sclerotiorum

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A B S T R A C T

Sclerotinia sclerotiorum

38 . G
- (G) A 15 . ,
,
( ) . ▼▼
,
- ,
, A
8,44 . A (V238A) *SsOs1*
G -
S. sclerotiorum 17 . -
, , fi 90-
- -
S. sclerotiorum 24 . fl -
Neurospora crassa fi
34 . A
- DCF *Botrytis cinerea* 11,35 .
- A ,
, DCF 10,
17,24,37,39,40,48 . ▼▼ *S. sclerotiorum*, DCF
14–17 ,
DCF fi *S. sclerotiorum* . , -
, -
, (D) ( )
, - -
(*Sshk, SsYpd, Sssk1*) A (*Sssk2, SsPbs, SsHog*)
.
.

2. Materials and methods

2.1. Isolates of S. sclerotiorum

(3, 4 6)
(G2, G3, G5)
fi 2011 48 . A *S. sclerotiorum* , G
. 4 C -
.
3, 4 6 85.5, 94.8 78.3, ,
47 .
75% 3 , 1% -
3 , 30 ,
fi 30 48 .
(DA)
23 C 2 .
23 C . F *S. sclerotiorum* , (5)
2- - - DA .
F , fi
DA 0.25 μ / . A 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, 0.006, 0.008, 0.01 / . 100
12 , fi 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 C_3 (0.5 /),
20 C- C (C 50 , = 2), 1.2
(5 /)
50 fl . D (0, 0.1, 0.2, 0.4 0.8) -
(2 /) , -
fi 25 . fl -
10 25 C 30 . ,
510 .
,
250- fl 100 (DB).
F , fi
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 . - fi . . 41 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 , (*Sshk*), (*Sssk1*), - (*SsYpd*), A (*SsHog*), A (*SsPbs*), A (*Sssk2*) 5.0 (5.0, B , A , CA), 1. A fi 50 D A, 1-5™ 2 -F 400 μ (E B , B , C). A fi : 2 98 C, 35 98 C 10 , 55 C 15 , - 72 C 1 , fi 72 C 10 D A E (B - , C1000). A fi (C) 1% - fi E. . .A. G E (EGA), GE - E ( ), E C ., . , . D A (6.0, C , , CA) (:// . . . / / / -) D A , fi .

2.8. Quantitative RT-PCR

A (, C - , CA, A). F - D A ™ ( ). A - C 2 - C (B —). 5 μ F ™ E G (B - , , CA, A), 1 μ , 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- . $2^{-\Delta\Delta C}$ 25 . .

2.9. Data analysis

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

3. Results

3.1. Glycerol content

fi ($P = 0.065$) (F . 1). , fi ($P < 0.01$) - 30.3%, 86.1%, 27.3% 3, 4, 6, ; fi ($P < 0.01$) 13.9%, 19.1%, 15.5% - G2, G3, G5, .

3.2. Cell membrane permeability and oxalic acid content

fi ($P = 0.853$) - ;

Table 1
.

	(5′–3′)	
F1	A GGGGGACAC ACGA AGC C	*Sshk*
1	AG ACAGG CC GCAAG GG	
-F	A GGC GAA CG AGAG	*SsHog*
-	GACCA CCACCAGA GGGCG	
-F	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	GGA GC CC GACCAG	β-
β -	AGCGGCCA CA G C AGG	
-F	AAGGG AGGGAAG AAGGCAA C	*Sshk*
-	G A AGGGCCAG GG AG G A G	

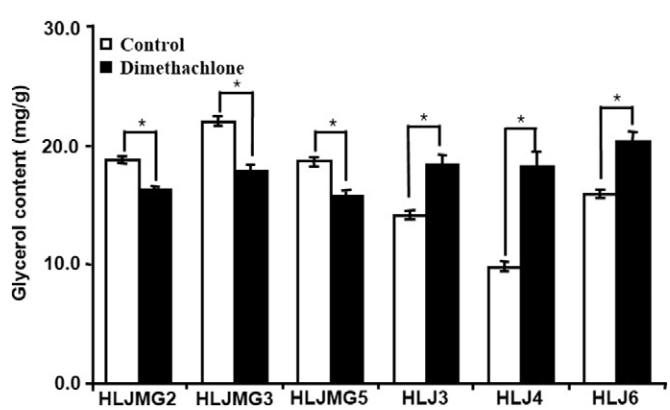

Fig. 1. G *Sclerotinia sclerotiorum*. "*" fi ($\alpha = 0.05$). V (E) . E *t*- .

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

3.3. Peroxidase and polyphenol oxidase activity

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

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

4 *Sshk*: A AC , 232 fi 90- - - (F . 4). 6 GG GA 1087 *Sshk* .

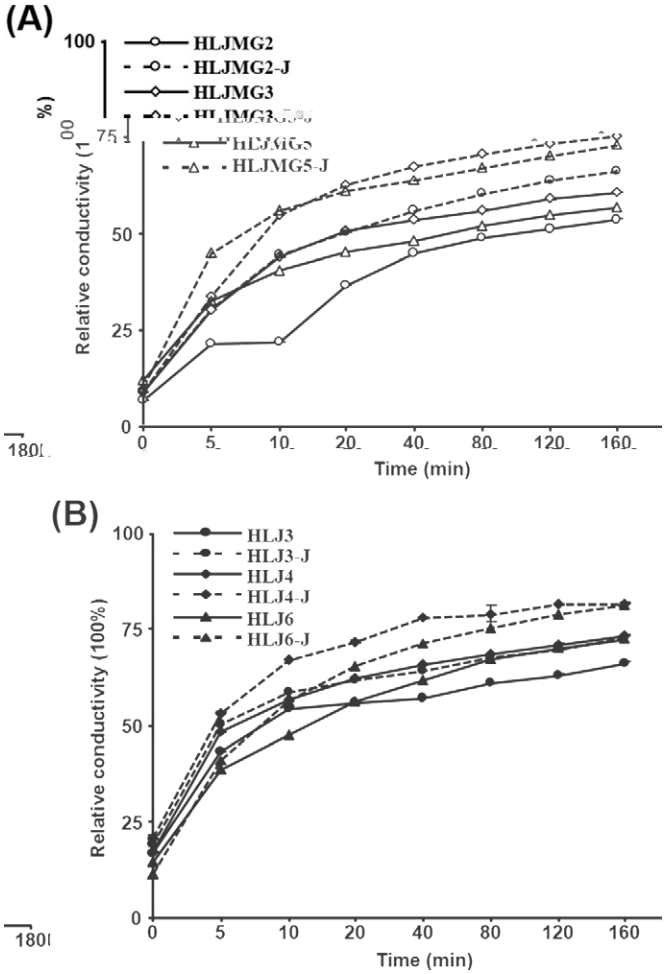

Fig. 2. *Sclerotinia sclerotiorum* . (A) . (B) . , (E) . E *t*- .

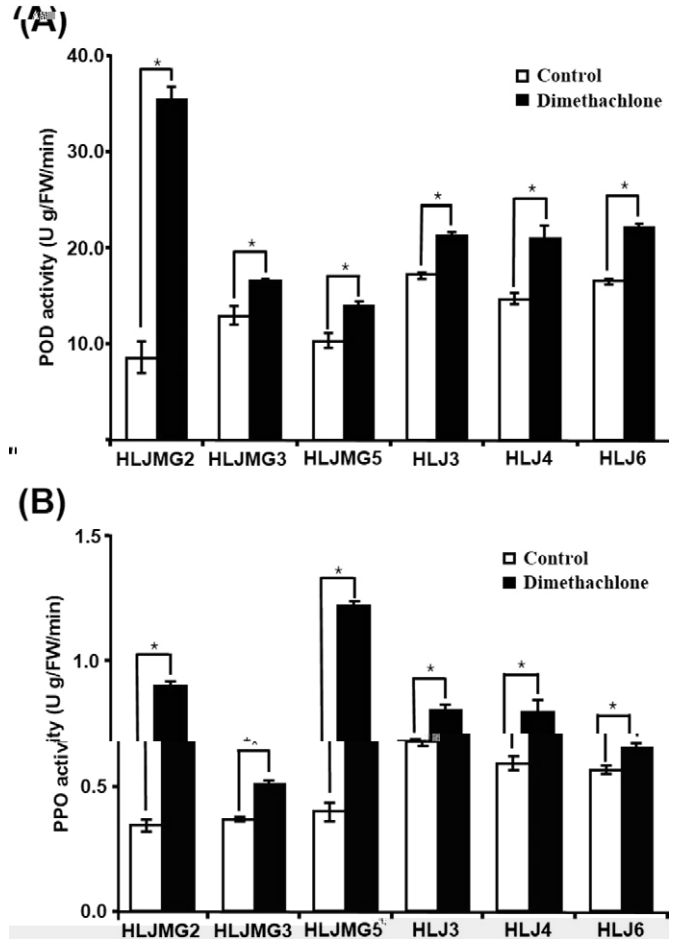

Fig. 3. (D) (A) ( ) (B) *Sclerotinia sclerotiorum*. "*" fi (α = 0.05). V (E) . E *t*- .

, 90- - . A *SsPbs* - CCA C A 96 4. - *SsYpd* (324C), *Sssk*1 (A759G), *Sssk*2 (C48 , A3447 1736G), . *SsHog* , .

3.5. Transcription levels of the Sshk gene

Sshk fi ($P < 0.05$) 4 6 - 3 (F . 5). F , *Sshk* - fi ($P < 0.05$) - - . 4 6 .

4. Discussion

fi - fi - *S. sclerotiorum*, - . *B. cinerea* *S. sclerotiorum*, fi

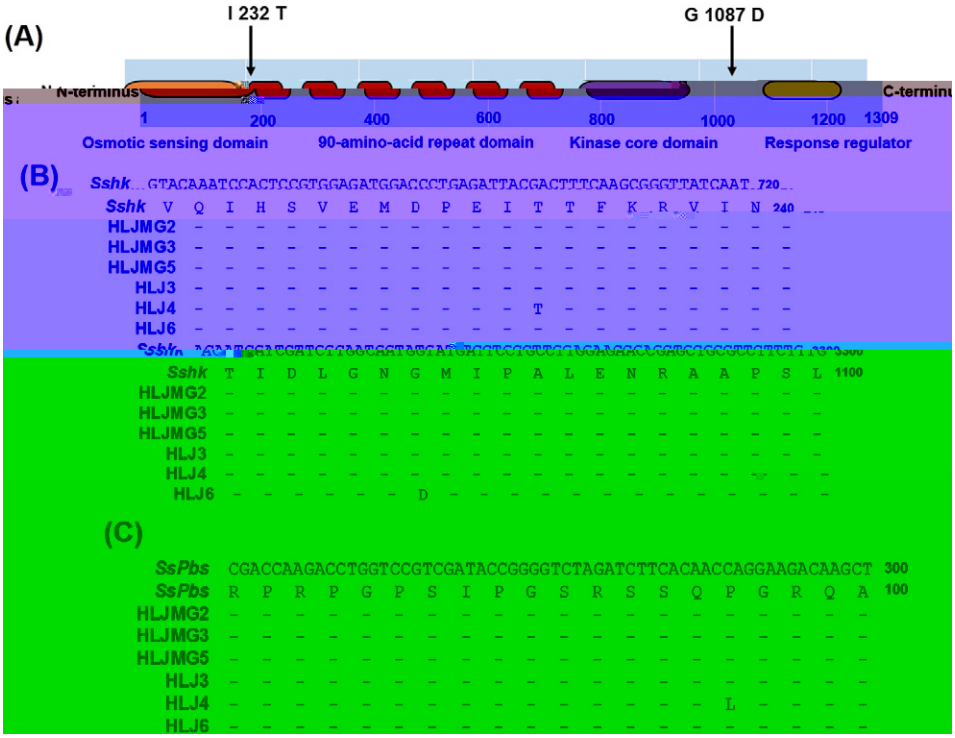

Fig. 4. C *Sshk* (1G 12694.3) . (A) *Sshk* ( ). (B) B *Sshk* B A D A , 4 6 *Sshk* . (C) *SsPbs* 96 4.

-

6,42 , fl . ., .

S. sclerotiorum 14 .

S. sclerotiorum -

fi 14,16 ,

fi -

11,13, 48 . -

15,31,48 . DCF

S. sclerotiorum , . ,

- fi

9,16–17 . . D fi

. B ,

, fi- 16 , -

S. sclerotiorum.

. E , D

16,32 ,

19,32 . , D

fi -

, -

. , D

- *S. sclerotiorum* -

, D 14,16 .

D *Fusarium*

oxysporum . . *fragariae* -

20 . -

D . A

S. sclerotiorum

, .

DCF

, 90- - -

DCF -

, *S. sclerotiorum, B. cinerea, A. alternata, M.*

fructicola 13,16,29–30 . , -

232 fi 90- -

4, G1087D

6. , fi

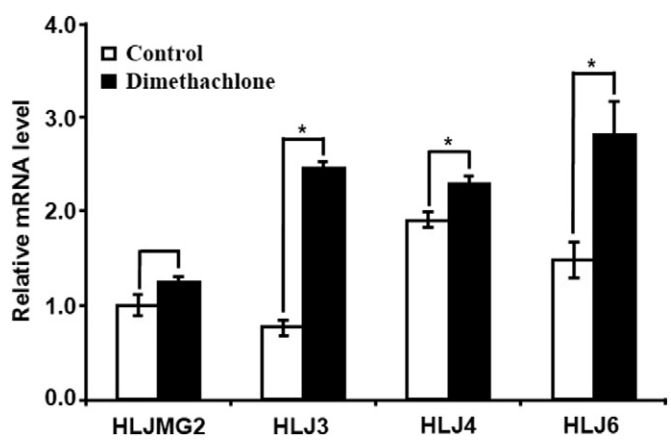

Fig. 5. A *Sshk* *Sclerotinia sclerotiorum.* "*" fi (α = 0.05). V (E) . E *t*- .

S. sclerotiorum. A CGA GA 1040 - (*Bos1*) DCF - *B. cinerea* 31 . - *SsYpd*, *Sssk*1, *Sssk*2 . A ,

. -

, , A . - 1 (D 1) fi 21 . *Sshk* 4 6 G2 - 3. 3 *Sshk* . A -

,

DCF *S. sclerotiorum*, *A. longipes* *B. cinerea* 17,18,26 . A - V238A *Ssos1* fi *S. sclerotiorum* 17 . *Sshk*

,

S. sclerotiorum.

, - (232 G1087D) *Sshk* D *S. sclerotiorum*. - *S. sclerotiorum* . A ,

S. sclerotiorum - fi *Sshk* *Sshk* D, , ( ) 13, 16,20,29–31 .

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Competing interests

.

Acknowledgements

F C (31371964).

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F 29 (2004) 40–43.
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