DIVERSITY OF SCLEROTIUM ROLFSII ISOLATES INFECTING SOME OIL CROPS

By HUDA ZAKARIA AHMED ZOHER

B.Sc. Agric. Sc. (Plant Pathology), Ain Shams University, 2003

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in Agricultural Science (Plant Pathology)

Department of Plant Pathology Faculty of Agriculture Ain Shams University

Approval Sheet

DIVERSITY OF SCLEROTIUM ROLFSII ISOLATES INFECTING SOME OIL CROPS

By HUDA ZAKARIA AHMED ZOHER

B.Sc. Agric. Sc. (Plant Pathology), Ain Shams University, 2003

This thesis for M.Sc. degree has been approved by:		
Prof. Dr. Mohamed Anwar Abdel-Sattar	••••	
Prof. Emeritus of Plant Pathology, Faculty of	of Agriculture,	
Suez Canal University		
Prof. Dr. Soad Mohamed Abd-Alla	••••	
Prof. Emeritus of Plant Pathology, Faculty of	of Agriculture,	
Ain Shams University		
Prof. Dr. Dorya Ibrahim Harfoush		
Prof. Emeritus of Plant Pathology, Faculty of	of Agriculture,	
Ain Shams University		

Date of Examination: 7 / 9 / 2010

DIVERSITY OF SCLEROTIUM ROLFSII ISOLATES INFECTING SOME OIL CROPS

By HUDA ZAKARIA AHMED ZOHER

B.Sc. Agric. Sc. (Plant Pathology), Ain Shams University, 2003

Under the supervision of:

Prof. Dr. Dorya Ibrahim Harfoush

Prof. Emeritus of Plant Pathology, Department of Plant Pathology, Faculty of Agriculture, Ain Shams University (Principal Supervisor)

Dr. Medhat Kamel Ali El-Sayed

Associate Prof. of Plant Pathology, Department of Plant Pathology, Faculty of Agriculture, Ain Shams University

Dr. Abeer Mohamed Ahmed Shaltout

Senior Researcher of Plant Pathology, Plant Pathology Research Institute, Agricultural Research Center

ACKNOWLEDGMENT

First I would like to express my deepest gratitude to **Prof. Dr. Dorya I. Harfoush,** Professor Emeritus of Plant Pathology, Faculty of Agriculture, Ain Shams University for her continuous and generous support, and for inspiration all the time.

I also wish to thank Dr. M. K. Ali, Associate Professor of Plant Pathology, Faculty of Agriculture, Ain Shams University for kind help, valuable suggestions and support.

I would like also to thank **Dr. Abeer M. Shaltout**, Senior Researcher, Mycology Res. & Disease Survey Dept., ARC, for her effort and help that made this work achieved.

I am sincerely grateful to **Dr. Ebtisam El-Sherif**, Chief Researcher, Mycology Res. & Disease Survey Dept., ARC, for her help and support.

Special thanks are also due to **Dr. Saad El-Gantiry**, Chief Researcher, Mycology Res. & Disease Survey Dept., ARC for his help and support, and all department members of Mycology Res. & Disease Survey for their cooperation that I always appreciate.

My grateful to **Dr. Aly Abd El-Hady Aly** Chief Researcher, Cotton Diseases Survey Dept., ARC. for his kind help and valuable suggestions.

I would like also due to thank **Dr. Noha F. El-badawy**, Researcher, Central Laboratory of Biotechnology, ARC. Also, **Dr. Hayam S. Abd El-Kader**, Senior Researcher of Virus and Mycoplasma Res. Dept., ARC. for their valuable contributions to this work.

My gratitude is also due to all the professors and lecturers at Plant Pathology Dept. Faculty of Agric., Ain Shams University, for sharing their knowledge and experience with us.

Finally, my deepest and sincere appreciations are due to my parents, my sisters Hadel and Heba and my brother Ahmed for their patience, support and encouragements.

ABSTRACT

Huda Zakaria Ahmed: Diversity of *Sclerotium rolfsii* isolates infecting some oil crops. Unpublished M.Sc. Thesis, Department of Plant Pathology, Faculty of Agriculture, Ain Shams University, 2010.

The fungus Sclerotium rolfsii Sacc. causes severe root rot to peanut and sunflower plants, pathogenicity tests of *S. rolfsii* 21 isolates on peanut were realized. All isolates from different hosts affect peanut plants, with different degrees. Pathogenicity tests of 21 isolates of S. rolfsii on peanut (Giza 6) and sunflower (hybrid 109) were carried out. All isolates from different hosts and locations were pathogenic to peanut and sunflower. Mycelial growth on natural media was higher than that on synthetic media. The best mycelial growth was obtained on PDA and Br media. For sclerotial formation, isolates varied in the number production on different media according to the type of media and isolate. The highest mycelial growth was recorded at 30°C and the least was recorded at 15°C. While, there was no clear differences found between isolates grew on different carbon sources, a great variation was obtained on sclerotia formation on media amended with different carbon sources. Peptone as a nitrogen source was the best for growing S.rolfsii isolates while medium consisted of sodium nitrate gave the lowest growth. Total of 441 combinations only 76 isolates (17.2%) showed compatible reactions. Based on mycelial compatibility seven groups were identified among the different isolates. MCG 7 was a unique group consist only one isolate isolated from guar plant. There was no correlation between host, geographic locations and members in certain MCG. The genetic relationships among 12 isolates of *S. rolfsii* showed various reactions by using 4 primers. High degree of genetic similarity was obtained between isolates P2 and P6 isolated from peanut from Giza and Behira when using the three primers 1, 2 and 3. These two isolates were also compatible and gave the same trend of disease incidence. Isolates from the same host showed variability in their genetic characters. The isolates were varied in their ability to produce oxalic acid. Isolate P3 produced the highest

amount of oxalic acid, (150µg/mg), also, this isolate was the high virulence one. *Trichoderma harzianum* was the best bioagent used inhibiting mycelial growth and sclerotia formation *in vitro*. Comparing with two bioagents and two fungicides (Moncut and Rizolex T) in controling the disease, fungicides were more effective in suppressing the disease incidence (pre-emergence damping off). All treatments completely suppressed the disease in post-emergence damping off.

Key words: *Sclerotium rolfsii*, rot root, mycelial compatible group, random amplified polymorphic DNA markers, biological control, and chemical control

CONTENT

LI	ST OF TABLES	11
LI	ST OF FIGURES	III
LI	ST OF ABBREVIATIONS	V
I.	INTRODUCTION	1
II.	REVIEW OF LITERATURE	3
III.	MATERIALS AND METHODS	25
IV.	RESULTS	36
	1. Symptoms on oil crops	36
	2. Isolation and identification of <i>Sclerotium rolfsii</i>	36
	3. Variation in virulence among isolates of <i>Sclerotium rolfsii</i>	38
	3.1.Pathogenicity of <i>Sclerotium rolfsii</i> isolates on peanut plant	38
	3.2. Pathogenicity of <i>S. rolfsii</i> isolates on sunflower plant	40
	4. Characterization of <i>S. rolfsii</i> isolates	42
	4.1. Morphological characterization of <i>S. rolfsii</i> isolates among	
	different media	42
	4.1.1. Variation among <i>S. rolfsii</i> isolates on natural media	42
	4.1.2. Variation among <i>S. rolfsii</i> isolates on synthetic media	48
	4.2. Variation of S.rolfsii isolates among different temperature	
	degrees	53
	4.3. Variation of <i>S. rolfsii</i> isolates among different carbon sources	57
	4.4. Variation of <i>S. rolfsii</i> isolates among different nitrogen sources	60
	5. Mycelial compatibility grouping	63
	6. Random Amplified Polymorphic DNA markers (RAPD)	68
	7. Variation of <i>S. rolfsii</i> isolates among oxalic acid production	82
	8. Variation of <i>S. rolfsii</i> isolates among different bioagents	83
	8.1. <i>In vitro</i>	83
	8.2. In vivo	86
V.	DISCUSSION	87
VI.	SUMMARY	103
VII	. REFERENCES	108
BI	C SUMMRY	

LIST OF TABLES

No.	
1.	Sources of <i>S. rolfsii</i> isolates and locations
2.	Pathogenicity of different S. rolfsii isolates on peanut (Giza 6) recorded 15 and 30
	days after sowing
3.	Pathogenicity of different S. rolfsii isolates on sunflower plants (hybrid 109)
	recorded 15 and 30 days after sowing
4.	Effect of different natural media on mycelial growth (cm) (mg) after 5 days and sclerotial number (Sc. No./ plate) after two weeks incubation at 30°C of <i>S. rolfsii</i>
	isolates
5.	Effect of different synthetic media on mycelial growth (cm) (mg) after 5 days and
	sclerotial number (Sc. No./ plate) after two weeks incubation at 30°C of S. rolfsii
	isolates
6.	Effect of different temperature on mycelial growth (cm) (mg) after 5 days and
	sclerotial number (Sc. No./ plate) after two weeks incubation at 30°C of S. rolfsii
_	isolates
7.	Effect of different carbon sources on mycelial growth (cm) (mg) after 5 days and
	sclerotia number (Sc. No./ plate) after two weeks incubation at 30°C of S. rolfsii
0	isolates
8.	Effect of different nitrogen sources on mycelial growth (mg) (cm) after 5 days and
	sclerotia number (Sc. No./ plate) after two weeks incubation at 30°C of S. rolfsii
0	isolates
9.	Mycelial compatibility groups of 21 <i>S.rolfsii</i> isolates according to dendrogram
10.	Mycelial compatibility among different <i>S. rolfsii</i> isolates on PDA medium after 7
10.	days
11.	Digitized patterens and dendrogram derived from RAPD profile analysis of 12
11.	isolates of S. rolfsii using primer 1
12.	Banding patterens MW of 12 isolates of <i>S. rolfsii</i> obtained from RAPD analysis
14.	using primer 1
13.	Digitized patterens and dendrogram derived from RAPD profile analysis of 12
13.	
	isolates of <i>S. rolfsii</i> using primer 2

14.	Banding patterens MW of 12 isolates of S. rolfsii obtained from RAPD analysis	74
	using primer 2	
15.	Digitized patterens and dendrogram derived from RAPD profile analysis of 12	
	isolates of S. rolfsii using primer 3	77
16.	Banding patterens MW of 12 isolates of S. rolfsii obtained from RAPD analysis	
	using primer 3	77
17.	Digitized patterens and dendrogram derived from RAPD profile analysis of 12	
	isolates of S. rolfsii using primer 4	80
18.	Banding patterens MW of 12 isolates of S. rolfsii obtained from RAPD analysis	
	using primer 4	81
19.	Amounts of oxalic acid produced by 12 S.rolfsii isolates on PDB	82
20.	Effect of biocontrol agents on reduction of mycelial growth and sclerotial	
	production of S. rolfsii isolates	84
21.	Effect of biocontrol agents and fungicides on disease incidence of peanut root rot	
	caused by S. rolfsii isolate (P3)	86

LIST OF FIGURES

No.		Page
1.	Symptoms caused by S. rolfsii pathogen on peanut (a) and sunflower (b)	
	plants artificially inoculated 45 days after sowing. Note:(a) arrow show	
	mycelium on crown area on peanut and(b) on sunflower	36
2.	Symptoms of peanut root rot caused by S.rolfsii (artificial inoculation) 45	
	days after sowing. Note: (a) arrow show mycelium on crown area, (b)	
	mycelium and sclerotia initially were formed on crown area	40
3.	Symptoms of sunflower root rot artificial inoculation with S.rolfsii 45	
	days after sowing. Note: (a) white mycelium on crown area, (b) mycelium	
	and sclerotia initially were formed on crown area	42
4.	Effect of different natural media on mycelial growth of S. rolfsii isolates	45
5.	Effect of different natural media on sclerotial number of S. rolfsii isolates.	46
6.	Effect of different natural media on mycelium condition of S. rolfsii,(a)	
	PDA, radial fluffy growth(b) Yeast ex., compact radial and (c) V8., poor	
	and light growth lay to the base	47
7.	Effect of different synthetic media on mycelial growth of S. rolfsii isolates	50
8.	Effect of different synthetic media on sclerotial number of S. rolfsii	
	isolates	51
9.	Effect of different synthetic media on mycelium condition of S. rolfsii,(a)	
	Br, heavy, cotton and fluffy(b) Cz., radial light mycelium and (c)	
	Brown's, poor light mycelia lay to the base	52
10.	Effect of different temperature degrees on mycelial growth of S. rolfsii	
	isolates	55
11.	Effect of different temperature degrees on sclerotial number of S. rolfsii	
	isolates	56
12.	Sclerotia production on PDA at different temperature, mycelial condition	
	at 30°C show radial fluffy growth	57
13.	Effect of different carbon sources on the means (a) on mycelial growth	
	and (b)sclerotial number of S. rolfsii isolates	59

14.	Effect of different nitrogen sources on the means (a) on mycelial growth	
	and (b) sclerotial number of <i>S. rolfsii</i> isolates	62
15.	Pairings of three isolates of S. rolfsii from three different MCGs showing	
	the development of A,B,and C incompatible reactions (clearing zones) in	
	the region of mycelial contact. Photograph was taken 2 weeks after	
	growth on potato dextrose ager. D. Pairing between isolates of S.rolfsii	
	showing mycelial compatible. Note. A) arrow show the sclerotia on the	
	line formation between incompatible isolates, B) barrage zone formation	
	on the area between incompatible isolates, C) arrow show lyses on the area	
	between incompatible isolates and D) show intermingled or produced a	
	knitted ridge between mycelium in the zone of mycelial	- 1
1.0	contact	64
16.	A and B) Anastomosis between two compatible isolates show H&A	
	shape.C) Pairings of two isolates of <i>S. rolfsii</i> from different MCGs show	6 5
17	the development of incompatible reactions (lysis between hyphae)	65
17.	Dendrogram for protein profile of 21 isolates of <i>S. rolfsii</i> from different plants and locations	66
18.	RAPD profile analysis of 12 isolates of <i>S. rolfsii</i> using primer1	69
10. 19.	Dendrogram divied from RAPD analysis of 12 isolates of <i>S. rolfsii</i> using	0)
1).	primer 1	69
20.	RAPD profile analysis of 12 <i>S. rolfsii</i> isolates using primer 2	72
21.	Dendrogram divied from RAPD analysis of 12 isolates of <i>S. rolfsii</i> using	. –
21,	primer 2	72
22.	RAPD profile analysis of 12 <i>S. rolfsii</i> isolates using primer 3	76
23.	Dendrogram divied from RAPD analysis of 12 isolates of <i>S. rolfsii</i> using	
	primer 3	76
24.	RAPD profile analysis of 12 isolates of <i>S. rolfsii</i> using primer 4	79
25.	Dendrogram divied from RAPD analysis of 12 isolates of <i>S. rolfsii</i> using	
	primer 4	79
26.	Amounts of oxalic acid produced by 12 S. rolfsii isolates after 6 days	83
27.	Effect of different bioagents on reduction of mycelial growth of S. rolfsii	
	isolaltes. A) T.harzianum B) T. atroviride C) T. viride and D) B. subtilis	85

LIST OF ABBREVIATIONS

BAM Basel agar medium

Cz Czpek's

MCG Mycelial compatibility groups

mg mycelial growth

PCR Polymerase chain reaction

PDA Potato dextrose agar PDB Potato dextrose broth

RAPD Random Amplified Polymorphic DNA markers

Red Reduction

Sn Sclerotial number
BAM Basel agar medium

LIST OF ABBREVIATIONS

BAM Basel agar medium

Cz Czpek's

MCG Mycelial compatibility groups

mg mycelial growth

PCR Polymerase chain reaction

PDA Potato dextrose agar PDB Potato dextrose broth

RAPD Random Amplified Polymorphic DNA markers

Red Reduction

Sn Sclerotial number
BAM Basel agar medium

LIST OF TABLES

No.		Pa
1.	Sources of <i>S. rolfsii</i> isolates and locations	37
2.	Pathogenicity of different S. rolfsii isolates on peanut (Giza 6) recorded 15 and 30	
	days after sowing	39
3.	Pathogenicity of different S. rolfsii isolates on sunflower plants (hybrid 109)	
	recorded 15 and 30 days after sowing.	41
4.	Effect of different natural media on mycelial growth (cm) (mg) after 5 days and	
	sclerotial number (Sc. No./ plate) after two weeks incubation at 30°C of S. rolfsii	
	isolates	44
5.	Effect of different synthetic media on mycelial growth (cm) (mg) after 5 days and	
	sclerotial number (Sc. No./ plate) after two weeks incubation at 30°C of S. rolfsii	
	isolates	49
6.	Effect of different temperature on mycelial growth (cm) (mg) after 5 days and	
	sclerotial number (Sc. No./ plate) after two weeks incubation at 30°C of S. rolfsii	
_	isolates	54
7.	Effect of different carbon sources on mycelial growth (cm) (mg) after 5 days and	
	sclerotia number (Sc. No./ plate) after two weeks incubation at 30°C of S. rolfsii	~ (
0	isolates	58
8.	Effect of different nitrogen sources on mycelial growth (mg) (cm) after 5 days and	
	sclerotia number (Sc. No./ plate) after two weeks incubation at 30°C of <i>S. rolfsii</i> isolates	61
9.	Mycelial compatibility groups of 21 S. rolfsii isolates according to	01
<i>)</i> .	dendrogramdendrogram	65
10.	Mycelial compatibility among different <i>S. rolfsii</i> isolates on PDA medium after 7	0.
10.	days	67
11.	Digitized patterens and dendrogram derived from RAPD profile analysis of 12	0.
	isolates of S. rolfsii using primer 1.	70
12.	Banding patterens MW of 12 isolates of <i>S. rolfsii</i> obtained from RAPD analysis	
	using primer 1	70
13.	Digitized patterens and dendrogram derived from RAPD profile analysis of 12	
	isolates of <i>S. rolfsii</i> using primer 2.	73

14.	Banding patterens MW of 12 isolates of <i>S. rolfsii</i> obtained from RAPD analysis	
	using primer 2	74
15.	Digitized patterens and dendrogram derived from RAPD profile analysis of 12	
	isolates of S. rolfsii using primer 3	77
16.	Banding patterens MW of 12 isolates of S. rolfsii obtained from RAPD analysis	
	using primer 3	77
17.	Digitized patterens and dendrogram derived from RAPD profile analysis of 12	
	isolates of S. rolfsii using primer 4	80
18.	Banding patterens MW of 12 isolates of S. rolfsii obtained from RAPD analysis	
	using primer 4	81
19.	Amounts of oxalic acid produced by 12 S.rolfsii isolates on PDB	82
20.	Effect of biocontrol agents on reduction of mycelial growth and sclerotial	
	production of S. rolfsii isolates	84
21.	Effect of biocontrol agents and fungicides on disease incidence of peanut root rot	
	caused by S. rolfsii isolate (P3).	86