Ain Shams University
Faculty of Science
Biochemistry Department

Molecular and biochemical studies on some sclerotia-forming fungi

A Thesis Submitted to
AIN SHAMS UNIVERSITY, FACULTY OF SCIENCE,
BIOCHEMISTRY DEPARTMENT

in partial fulfilment for the requirements of the Degree of Master of Science in Biochemistry

By

HEBA YOUSEF RIZK MOHAMED

B. Sc. in Biochemistry, Ain Shams University

BIOCHEMISTRY DEPARTMENT
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Approval Sheet

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Note

The present thesis is submitted to the Faculty of Science, Ain Shams University in patial fulfilment for the requirements of the Degree of Master of Science in Biochemistry. Besides the research work materialized in this thesis, the candidate did attend eleven post-graduate courses for one academic year in the following topics;

1. Cancer biology
2. Immunology
3. Molecular biology and Genetics
4. Radiobiology
5. Hormones
6. Enzymes and proteins
7. Carbohydrates
8. Instrumental analysis
9. Quality control
10. Biostatistics
11. English Language

She successfully passed the final examinations in these courses held in September 2003.

بِسْمِ اللّهِ الرَّحْمـَنِ الرَّحِيمِ

وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيماً

صدق الله العظيم

﴿سورة النساء – آية 113﴾

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ABSTRACT

Rhizctonia solani and *Sclerotium cepivorum* are two serious plant pathogenic sclerotia-forming fungi. These two fungi are not sporulating under the normal environmental conditions. The isolation of *R. solani* from cotton or tomato and *S. cepivorum* from onion samples from several Egyptian governorates is an indication for the wide spread of those two fungi in Egypt.

Two types of techniques were applied for more distinction among those isolates. Anastomosis grouping (AG) among isolates of *R. solani* and the mycelial comptability grouping (MCG) among *S. cepivorum* isolates. Several biochmical and molecular tools that reveal the genetic variation of similar organisms have arisen. Biochemical studies included electrophoretic profiles of soluble proteins and isozymes. A molecular technique widely used is random amplified polymorphic DNA (RAPD).

The results obtained showed that *R. solani* and *S. cepivorum* classified into Ag4, Ag5 using anstomosis groups (AG) and into MCG1, MCG2 using mycelial compitability groups (MCG). The activities of cellulase, polygalactournase and peroxidase quantitatively showed physiological differences among those isolates regardless to the geographic location. Isozyme patterns of (esterase) for various isolates showed some variability among isolates. Since esterase patterns of S. *cepivorum* from onion showed a specific band in Suhag isolates differed from other isolates with similarity percentage 30.9%. Protein profile of *R. solani* isolated from cotton able to distinguish between the isolates from Upper and Lower Egypt governorates. Analysis of DNA fingerprinting showed that similarity ranged from 38% to 100% depending on the primer used within the *R. solani* isolates isolated from cotton and tomato but among isolates of *S. cepivorum* there is a high genetic resemblance. In conclusion, RAPD technique revealed considerable molecular variation among and within intraspecific groups that have been recognized previously.

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In conclusion, protein profile of *R. solani* isolated from cotton were able to distinguish between the isolates from Upper and Lower Egypt governorates. Since the Upper Egypt isolates fell in one group with similarity percentage ranged from 46.9 % to 85.7% while isolates of the Lower Egypt governorates fell in another similarity group with ratio 50.4% to 87.5%.

Random amplified polymorphic DNA (RAPD) of the fungal pathogen *R. solani* revealed considerable molecular variation among and within intraspecific groups that have been recognized on the basis of anastomosis, morphology and pathogenicity. The differentiation of isolates of root rot pathogens based on pathogenicity and molecular markers can ultimately help in our endeavor of managing this important disease.

The genetic variability was low among isolates of *S. cepivorum* and no clear difference was observed between Upper and Lower Egypt isolates.

INTRODUCTION

Sclerotia-forming fungi are serious plant pathogens in most world countries including Egypt. These pathogens were reported in many economical crops. *Rhizoctonia solani* and *Sclerotium cepivorum* considered as the most serious Sclerotia-forming fungi in Egypt **(Aly *et al.*, 1998 and Haq *et al.*, 2003).**

Sclerotia are compact hyphal structures usually produced asexually by some fungi, including certain soilborne plant pathogenic species. They play a vital role in the survival of these organisms for long period under unfavorable conditions because of their high resistance to chemical and biological degradations **(Kuo and Alexander, 1967; Coley-Smith and Cooke, 1971; Willetts, 1971 and Agrios, 1988)**. Elucidating the mechanism of formation of sclerotia may lead to novel approaches of controlling sclerotia-forming fungi **(Georgiou *et al.*, 2000a and Georgiou *et al.*, 2000b)**.

The survival of sclerotia is greatly influenced by many factors such as water status, depth of burial, soil temperature, soil gases and antagonistic and mycoparasitic organisms. Longevity of sclerotia in soil has been reported to be as short as a few weeks to as long as 8 years or more **(Adams and Ayers 1979 and Huang, 1983)**.

Rhizoctonia and *Sclerotium* are soil inhabitants that cause serious diseases for many plants by affecting the roots, stems, tubers, corms and other plant parts that develop in or on the ground. These two fungi are known as sterile fungi because for many years they were though to be incapable of producing spores of any kind, either sexually or