ABSTRACT

SOZAN EID MOHAMED EL-ABEID. VARIANCE AMONG ALTERNARIA SPP. IN RELATION TO THEIR PATHOGENIC CAPABILITY TO SOME PLANT HOSTS. Un-published Master of Science Thesis, Department of Plant Pathology, Faculty of Agriculture, Ain Shams University (2005).

The present investigation was planned to study the morphological diversity among *Alternaria* spp. isolated from some economic crops (potato, tomato, eggplant, pepper, cotton and soybean) to determine the pathogenicity of the different morphological types and their potential as causal agents of *Alternaria* spot or blight of these economic plants. To evaluate the phylogenetic relationships among isolated *Alternaria* spp using varieties of molecular technique to further our understanding of the taxonomic status of this *Alternaria* spp.

Alternaria spp were isolated from all crops under study growing in six governorates, i.e. Ismaillia, Sharkia. Giza, Beni-suef, Behairh and Gharbia. The other genera of fungi that isolated were Cladosporium, Stemphylium. Helminthosporium and Ulocladium. Study revealed that seventeen isolates of Alternaria spp. were significantly differed in their pathogenic capability. The most pathogenic isolates were two isolates isolated from potato and eggplant samples collected from Ismallia governorate, followed by two isolates isolated from potato and cotton samples collected from Sharqia and Beni- Suef governorates.

However, the collections of 17 Alternaria isolates

were grouped into 6 species, *i.e.* A. alteranta, A. citri, A. tenusima, A. dianthi, A. raphani and A. solani, based on morphological and characteristics of sporulation apparatus. For the characterization of colony morphology, the incubation of single spored colonies at 23°C in darkness on Potato Carrot Agar medium resulted in diagnostic features that could be used to differentiate representative isolates of the A. alternata, A. tenussima and A. raphani species.

Among seventeen *Alternaria* isolates, fourteen isolates were distinguished and identified according to their biochemical and molecular fingerprinting. Biochemical fingerprints were investigated based on proteins banding patterns using SDS-PAGE and three isozymes system (Estrase, Malic dehydrogenase, acid phosphatase) for fresh mycelium, in addition, molecular genetic finger printing using RAPD-PCR technique. In this respect, three primers out of the tested ten oligomers succeeded to amplify polymeric DNA. Hence, each isolate has a different and distinct molecular fingerprint.

Keywords: Alternaria alteranta, A. citri, A. tenusima, A. dianthi, A. raphani, A. solani, potato, tomato, eggplant. pepper, cotton, soybean, fingerprinting, SDS-PAGE, Isozyme and RAPD-PCR.

CONTENTS

	page
ABSTRACT	I
ACKNOWLEDGEMENTS	Ш
LIST OF TABLES	IV
LIST OF FIGURES	V
I. INTRODUCTION	l
II. REVIEW OF LITERATURE	4
2.1.Plant pathogen Alternaria	4
2.2.1. Losses caused by <i>Alternaria</i> spp.	7
2.2. Identification of the causal pathogen	8
Alternaria	11
2.3. Effect of media	14
2.4. Effect of temperature	16
2.5. Effect of light	18
2.6. Genetic variability	22
III. MATERIALS AND METHODS	22
3.1. Isolation of <i>Alternaria</i> pathogen	23
3.2. Morphological characterization and	24
taxonomy	25
3.2.1. Light microscopy	25
3.2.2. Scanning Electron Microscopey (SEM)	27
3.3. Pathogenicity tests	
3.4. Effect of media on the mycelial growth and	29
sporulation of Alternaria spp.	
3.5 .Effect of temperature on the mycelial	29
growth and sporulation of Alternaria spp.	
3.6. Effect of darkness on the mycelial growth	30
and sporulation of Alternaria spp.	34
3.7. protein electrophoresis	

3.8. Enzyme extraction gel electrophoresis and	37
analysis of isozyme data.	38
3.9. DNA extraction.	39
3.9.1. RAPD markers.	41
3.9.2. RAPD analysis.	41
IV RESULTS	
4.1 .Isolation of plant pathogenic Alternaria	51
spp.	
4.2. Morphological characterization and taxonomy	57
4.3. Pathogenicity test 4.4. Effect of media on the mycelial growth and sporulation of <i>Alternaria</i> spp.	63
4.5. Effect of temperature on the mycelial growth and sporulation of <i>Alternaria</i> spp.	66
4.6. Effect of light on the mycelial growth and sporulation of <i>Alternaria</i> spp.	66
4.7. Biochemical genetic marker for different Alternaria spp.	66
4.7.1. Fungal total protein 4.7.2. Isozym pattern	69
4.7.3. RAPD-PCR	73
V DISCUSSION	81
VI SUMMARY	95
VII REFERENCES	98
ARABIC SUMMARY	120