

# CONTENTS

	<i>Page</i>
<b>INTRODUCTION .....</b>	1
<b>REVIEW OF LITERATURE.....</b>	2
<b>MATERIALS AND METHODS.....</b>	17
1. Plant Material.....	17
2. Isolation of the Pathogen.....	17
2.1. Isolation from diseased leaves.....	17
2.2. Isolation from diseased stems.....	17
2.3. Isolation from diseased tuber.....	17
3. Identification of the Pathogen.....	17
3.1. Cultural characteristics.....	17
3.2. Mating-type tests.....	18
4. Evaluation of Late Blight Resistance in Potato Cultivars.....	18
4.1. Preparation of inoculum.....	18
4.2. Evaluation of late blight resistance in potato leaves.....	18
4.3. Evaluation of late blight resistance in potato tubers.....	18
5. Genetic Diversity Among <i>P. infestans</i> Isolates and Potato Genotypes.....	19
5.1. Among <i>P. infestans</i> isolates.....	19
5.1.1. Fungal genomic DNA extraction.....	19
5.1.2. RAPD-PCR conditions.....	20
5.1.3. Visualization and analysis of RAPD-PCR products.....	20
5.1.4. Data handling and cluster analysis.....	21
5.2. Among potato genotypes.....	21
6. Detection of <i>P. infestans</i> and Disease Development.....	21
6.1. Symptomatology studies.....	21
6.1.1. In potato leaves.....	21
6.1.2. In potato tubers.....	22
6.2. Serological studies.....	22
6.2.1. Preparation of antigen and antiserum.....	22
6.2.1.1. Preparation of antigen.....	22
6.2.1.2. Preparation of antiserum.....	22
6.2.2. Indirect ELISA.....	22
6.2.3. Determination of antiserum titer.....	23
6.2.4. Dot blot immunoassay (DIA).....	24
6.2.5. Specificity of the <i>P. infestans</i> antiserum.....	25
6.2.6. Reactivity of <i>P. infestans</i> antiserum to the purified antigen of <i>P. infestans</i> .....	25
6.2.7. Serological detection of <i>P. infestans</i> in inoculated potato tissues.....	25
6.2.7.1. In potato tissues inoculated with <i>P. infestans</i> alone.....	25
6.2.7.2. In potato tissues with combined infection.....	25
6.3. Molecular studies.....	26
6.3.1. Genomic DNA extraction.....	26
6.3.2. Determination of DNA concentration .....	26
6.3.3. PCR amplification.....	26

2. Identification of the Pathogen.....	37
2.1. Cultural characteristics.....	37
2.2. Mating type.....	37
3. Evaluation of Late Blight Resistance in Potato Cultivars.....	37
4. Genetic Diversity Among <i>P. infestans</i> Isolates and Potato Genotypes.....	42
4.1. Among <i>P. infestans</i> isolates.....	42
4.2. Among potato genotypes.....	42
5. Detection of <i>P. infestans</i> and Disease Development.....	52
5.1. Symptomatology studies.....	52
5.1.1. In potato leaves.....	52
5.1.2. In potato tuber-discs.....	64
5.2. Serological studies.....	64
5.2.1. Determination of antiserum titer.....	64
5.2.2. Reactivity of <i>P. infestans</i> antiserum to the purified antigen of <i>P. infestans</i> .....	64
5.2.3. Specificity of the <i>P. infestans</i> antiserum.....	64
5.2.4. Serological detection of <i>P. infestans</i> in inoculated potato tissues.....	71
5.2.4.1. In potato tissues inoculated with <i>P. infestans</i> alone.....	71
5.2.4.2. In potato tissues with combined infection.....	71
5.3. Molecular studies.....	80
5.3.1. Specificity of the primer.....	80
5.3.2. Sensitivity of the primer.....	80
5.3.3. Molecular detection of <i>P. infestans</i> in inoculated potato tissues.....	80
5.3.3.1. In potato tissues inoculated with <i>P. infestans</i> alone.....	80
5.3.3.2. In potato tissues with combined infection.....	86
6. Detection of Potato Defense Mechanisms.....	86
6.1. Pathogenesis-related proteins (PR-proteins).....	86
6.1.1. Determination of the changes in total protein content.....	86
6.1.2. Antifungal activity of total protein extracted from potato leaves.....	86
6.1.3. Determination of the changes in polypeptides profiles.....	90
6.1.4. Expression of osmotin like-protein (PR-5).....	90
6.1.4.1. Molecular analysis at the structural level (DNA level).....	97
6.1.4.2. Molecular analysis at the functional level (RNA level).....	97
6.2. Oxidizing enzymes.....	97
6.2.1. Changes in peroxidase (PO) activity.....	97
6.2.1.1. Changes in peroxidase activity in potato leaves.....	100
6.2.1.2. Changes in peroxidase activity in potato tubers.....	100
6.2.2. Changes in peroxidase isozymes activity in potato leaves.....	100
6.2.3. Changes in polyphenoloxidase (PPO) activity.....	100
6.2.3.1. Changes in polyphenoloxidase activity in potato leaves.....	100
6.2.3.2. Changes in polyphenoloxidase activity in potato tubers.....	107
6.3. Reactive oxygen species (ROS).....	107
6.3.1. Changes in (ROS) activity in potato leaves.....	107
6.3.2. Changes in (ROS) activity in potato tubers.....	114
6.4. Lipid peroxidation.....	114
6.4.1. Changes in lipid peroxidation in potato leaves.....	114

## SUMMARY

This study has focused on the role of certain resistance factors affecting the development of late blight disease of potato. The main findings of the present study could be summarized as follows:

- 1- Thirty two isolates of *Phytophthora infestans* have been isolated from infected potato samples (leaves, stems and tubers) collected from different locations in Alexandria and El-Behera Governorates, during 2003-2004 and 2004-2005 growing seasons.
- 2 - Mating types trails showed that, both mating types A<sub>1</sub> and A<sub>2</sub> were detected in Alexandria and El-Behera Governorates. The percentage of mating type A<sub>1</sub> (88.5%) was higher than A<sub>2</sub> (11.5%).
- 3- Twenty different isolates of *P. infestans* were chosen for the evaluation of late blight resistance in leaves and tubers of eleven potato cultivars. The tested potato cultivars were Cara, Diamant, Hanna, Hermes, Lady-Rosetta, Lady-Olympia, Nicola, Nieta, Slaney, Spunta and Valor. Obtained data showed the following:
  - (A)- All the tested isolates were found to be pathogenic but differed in their pathogenic capabilities. Isolates P3, P14 and P24 were considered to be the highest virulent isolates. On the other hand, isolates P1, P4 and P7 were the lowest virulent isolates on the tested potato cultivars.
  - (B)- The resistibility of the tested potato cultivars varied in their response to infection by *P. infestans* isolates. Also, in certain cases, the resistibility of the leaves and tubers varied in the same cultivar. Hanna and Cara were the highest significant resistant cultivars, while Lady-Rosetta and Lady-Olympia were the lowest in this respect.
- 4- RAPD polymerase chain reaction analysis was used to study the genetic diversity among 15 isolates of *P. infestans* varied in their virulence to potato cultivars. Obtained data showed the following:
  - (A)- Cluster analysis of 15 isolates showed that, all the tested isolates were separated into two clusters (1 and 2). All the low virulent isolates P1, P4 and P7 were grouped in cluster 1 with an overall similarity coefficient of 0.805. Cluster 2 included highly and moderately virulent isolates.
  - (B)- Less virulent isolates produced identical marker band (885 bp) which was distinctive of other tested isolates using RAPD primer K2. The same primer (K2) yielded a RAPD fragment (798 bp) found only in highly and moderately virulent isolates.
- 5- RAPD polymerase chain reaction analysis was used to study the genetic diversity among a wild potato variety *Solanum demissum* (very resistant to late blight) and six potato cultivars (Hanna, Lady-Olympia, Lady-Rosetta, Spunta, Diamant and Cara) varied in their resistance to *P. infestans*. Obtained data showed the following:

- (c)- In potato tissues inoculated with *P. infestans* alone, the antiserum detected positively the pathogen in the susceptible and resistant potato cultivars, 48hpi in inoculated leaves and tuber-discs. Results of DIA were in line of those obtained by the indirect ELISA test.
- (d)- In potato tissues with combined infection indirect ELISA and DIA tests indicated that, the antiserum detected the late blight pathogen 48hpi in leaves of the susceptible potato cultivar inoculated with *P. infestans* and *A. solani*. Potato tuber-discs of resistant and susceptible cultivars inoculated with *P. infestans* and *E. carotovora* or *R. solanacearum*, showed no positive ELISA values.
- (C)- Results obtained from molecular studies showed that:
- (a)- Using PCR the specificity of the specific primer to *P. infestans* was tested against DNA isolated from 9 isolates of *P. infestans* and DNA of *Fusarium* sp., *Pythium* sp., *R. solani*, *M. phaseolina*, *A. solani*, *E. carotovora* and *R. solanacearum*. All the tested isolates of *P. infestans* amplified a product of approximately 813bp with the primer. No amplification products were obtained with the other tested organisms.
- (b)- Using the specific primer only 1pg of *P. infestans* purified DNA isolated from mycelium or DNA isolated from 500 sporangia was needed to detect the pathogen.
- (c)- In Potato tissues inoculated with *P. infestans* alone, the specific primer detected the pathogen in infected potato cultivars (resistant and susceptible) at 12hpi in inoculated leaves and 48hpi in inoculated tuber-discs.
- (d)- In potato tissues with combined infection, the specific primer detected the late blight pathogen at 12hpi in leaves of susceptible potato cultivar inoculated with *P. infestans* and *A. solani*. The primer detected fungal DNA in tuber-disc, inoculated with both *P. infestans* and *R. solanacearum* only 48hpi.
- 7- Studies were performed in order to investigate the role of pathogenesis-related proteins (PR-proteins), oxidizing enzymes, reactive oxygen species (ROS), lipid peroxidation and transcription activators, in relation to resistance to late blight disease.
- (A)- Data concerning the role of the PR- proteins showed that:
- (a)- In both resistant and susceptible potato cultivars, inoculated leaves showed a significantly higher amount of total protein than the healthy ones, and the total protein content increased gradually with time. The resistant potato cultivars (Hanna and Cara) showed a significant higher increase in the content of total proteins which reached 0.822 and 0.793 mg g<sup>-1</sup> fresh weight respectively. The susceptible cultivars showed less increase.
- (b)- In a bioassay experiment, the effect of crude protein extracted from leaves of resistant potato cultivars showed the lowest percentage of fungal growth. Conversely, crude protein extracted from susceptible potato cultivars showed the highest percentage of fungal growth. It can be noticed that, crude protein extracted from leaves of resistant cultivars resulted on inhibitory effect on the growth of *P. infestans*.
- (c)- SDS-PAGE analysis of acidic soluble proteins extracted from leaves of the resistant and susceptible potato cultivars at different periods of inoculation

- (8)- The role of common antigen in relation to potato resistance was studied using the indirect ELISA and dot blot immunoassay (DIA) to detect the cross-reactivity between antigens of potato cultivars (resistant and susceptible) and *P. infestans* antiserum. Results indicating that, the higher reaction and more specific antigens were detected in the susceptible cultivars than in the resistant ones.