

بسم الله الرحمن الرحيم





شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



INDUCTION OF TOMATO PLANTS FOR MOSAIC DISEASE RESISTANCE

By

FARIDA MOHAMED MAHMOUD

B.Sc. Agric. Sc. (Plant Pathology), Fac. of Agric., Ain Shams Univ., 2010

**A Thesis Submitted in Partial Fulfillment
Of
The Requirement for the Degree of**

**MASTER OF SCIENCE
in
Agricultural Sciences
(Plant Pathology)**

**Department of Plant Pathology
Faculty of Agriculture
Ain Shams University**

2021

Approval Sheet

INDUCTION OF TOMATO PLANTS FOR MOSAIC DISEASE RESISTANCE

By

FARIDA MOHAMED MAHMOUD

B.Sc. Agric. Sc. (Plant Pathology), Fac. of Agric., Ain Shams Univ., 2010

This thesis for M.Sc. degree has been approved by:

Dr. Amal Abou El-Ela Ahmed Frghaly

Head of Research, Virus and Phytoplasma, Plant Pathology Research
Institute, Agricultural Research Center.

Dr. Tarek Abd El-Kareem Mostafa

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

Dr. Walaa Said Mohamed Shabaan Khalifa

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

Dr. Fawzy Morsi Abo El-Abbas

Prof. Emeritus of Plant Pathology, Faculty of Agriculture, Ain Shams
University.

Date of Examination: / /2021

INDUCTION OF TOMATO PLANTS FOR MOSAIC DISEASE RESISTANCE

By

FARIDA MOHAMED MAHMOUD

B.Sc. Agric. Sc. (Plant Pathology), Fac. of Agric., Ain Shams Univ., 2010

Under Supervision of:

Dr. Fawzy Morsi Abo El-Abbas

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

Dr. Walaa Said Mohamed Shabaan Khalifa

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

Dr. Ahmed Mohsen Bondok

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

ABSTRACT

Farida Mohamed Mahmoud. Induction of Tomato Plants for Moasic Disease Resistance, Unpublished M.Sc. thesis., Department of plant pathology, Faculty of Agriculture, Ain Shams University, 2021.

Tomato plants are most important economic vegetable crop in Egypt and the world. Tomato is subjected to several diseases, which cause considerable yield losses. Virus diseases are considered to be one of the most important problems affecting tomato production. *Tobacco mosaic virus* (TMV) is one of the most economically important virus infecting tomato plants and other different types of plants leading to yield losses and quality degradation. The present study aimed to investigate ability of riboflavin as inducer to induce resistance in different cultivars of tomato plants against TMV infection

Treatment with riboflavin was demonstrated to induce defence responses and systemic acquired resistance against TMV in indicator hosts and tomato plants. Three concentration of riboflavin (0.5, 2.5 and 5.0mM) was studied. Treatments with riboflavin reduced TMV incidence and disease severity in tomato plants. In addition, treatments reduce number of local lesions in indicator hosts.

The present study demonstrated that, exogenous application of 2.5mM riboflavin, 5 days before virus challenge was the most effective concentration. The higher concentration did not increase the effect. The induction disease resistance and reduction of TMV infectivity in tomato plants were determined by indirect ELISA and local lesion host plant assay.

The effect of 2.5mM riboflavin in TMV incidence and disease severity was studied from 0 to 20 days after treatment. In time course investigation, 2.5mM riboflavin treatment reduces the virus symptoms particularly at 9 days, where after the symptoms become evident.

The expression of defence genes *PR10* and *PAL* were studied by reverse transcription polymerase chain reaction (RT-PCR). *PR10* exhibited ribonucleolytic activity against *tobacco mosaic virus* RNA and *PAL* involved in phytoalexine or phenolic compound biosynthesis. Treatment with 2.5 mM riboflavin induced the accumulation of *PAL*-mRNA, particularly at the beginning 3 hours, followed by obviously decrease at 6 h, and raised again at 12 h after treatment.

While, pathogenesis related protein *PR10* gene was efficiently transcribed into an mRNA as detected by the presence of specific amplicons of expected molecular weight (217 bp). *PR10* accumulation was notable increased at 3h, and followed by markedly increase at 6, and 12h after treatment with 2.5 mM riboflavin .

Treatment with riboflavin enhanced activities of peroxidase (PO) and Polyphenol Oxidase (PPO) in treated inoculated plants which catalyze the formation of lignin, and other oxidative phenols that contribute to the formation of defense barriers for reinforcing the cell structure The activity of PO and PPO was examined by specific enzyme assay for each one. The activity of enzyme was higher in treated inoculated plants than those of healthy ones were and reached the highest level at 8 days after treatment.

Finally, histochemical changes in cell death were detected by using trypan blue staining. Formation cell death increased with time at treatment and its reached maximum in 24 hours after treatment.

Keywords: Tomato, *Tobacco moaic virus* (TMV), Peroxidase, Polyphenol oxidase, Phenylalanine ammonia-layse, PR proteins, PR gene expression

ACKNOWLEDGMENT

All praises are due to ALLAH who fulfilled my all dreams and blessed me with kind professors and gave me the support to dispatch this work.

I wish to express my deepest gratitude to Prof. Dr. Fawzy M. Abo El-Abbas, Professor of Plant Pathology, Faculty of Agriculture, Ain Shams University for his kind supervision, kind encouragement, his effective, valuable help and his endless support during the progress of this study and facilities offered to carry out this work. I will not forget what he did for me. All the words of thanks cannot describe my feelings.

I am indebted to Dr. Walaa Said Khalifa, Associate Prof. of Plant Pathology, Faculty of Agriculture, Ain Shams University, for her effective, kind attention, kind advice. She is my dear teacher, beautiful sister and my idol. I would like to express my thanks and appreciation for her great effort. Thanks for everything she have given me.

Thanks are also due to Dr. Ahmed Mohsen Bondok, Associate Prof. of Plant Pathology, Faculty of Agriculture, Ain Shams University, for supervising, his kind attention and kind encouragement. I would like to express my thanks and appreciation for his great effort.

I am very grateful to my late great professorial Dr. Mostafa Helmy El-Hamady, Professor of Plant Pathology, Faculty of Agriculture, Ain Shams University for his encouragement, kind attention and confidence for me. He has all my respect and appreciation. May God have mercy on him and make him live in Al-Fardouss. Words can't describe how sorry I am loss him. He was such an incredible person. I am going to miss him but he will live on our memories for ever.

Thanks are also due to Dr, Tarek A. Mostafa, Associate Prof. of Plant Pathology, Faculty of Agriculture, Ain Shams University, for his kind attention, and make through the course of realization of this study.

I am particularly grateful to my dear professor. Dr. Samir El-Deeb, Professor of Plant Pathology, Faculty of Agriculture, Ain Shams University for his continuous encouragement, valuable help and moral support during the critical times. Really, I will not forget this favor, thanks with all my heart.

Thanks also extended to all staff members and colleges at Plant Pathology Department, Faculty of Agriculture, Ain Shams University, for their valuable help and encouragement.

CONTENTS

	Page
LIST OF TABLE	III
LIST OF FIGURES	V
LIST OF ABBREVIATIONS	III
I.INTRODUCTION	1
II.REVIEW OF LITRATURE	4
1. Tomato plants	4
2. <i>Tobacco mosaic virus</i> (TMV)	5
3. Systemic acquired resistance	6
4. Inducers for induction of plant resistance	7
5. Mechanisms of SAR	13
5.1. Salicylic acid (SA)	13
5.2. Pathogenesis-related proteins (PR proteins)	14
5.3. Defence enzymes	16
5.4. Histochemical changes in induced plants	18
5.4.1. Reaction oxygen species (ROS)	19
6. Induction of SAR in plants against viral diseases	21
III.MATERIALS AND METHODS	23
1.Source and maintaining of <i>Tobacco mosaic virus</i> (TMV)	23
2.The effect of different concentration of riboflavin on some growth characters of tomato cultivars	23
2.1. Stem length	24
2.2.Number of leaves	24
3.The effect of different concentration of riboflavin on TMV infection	24
3.1.TMV-incidence and severity	24
3.1.1.On indicator hosts	24
3.1.2.On tomato plants	25
3.2.Quantification of TMV infectivity	25
3.3.Determination of TMV concentration by using ELISA test	26

	Page
3.4.Detection of TMV infection in riboflavin treated plants using NCM-ELISA	26
4.Time course of SAR induction against TMV on 2.5 mM riboflavin treated plants	27
5.Determination the transcription level of PAL- and pathogenesis related genes in 2.5 mM riboflavin treated tomato cv. Strain B plants	28
5.1.Extraction of nucleic acid RNA	28
5.2.Reverse transcription-polymerase chain reaction (RT-PCR)	29
5.3.Analysis of RT-PCR product	30
6.Biochemical changes associated with 2.5 mM riboflavin treatment	30
6.1.Peroxidase activity assay	31
6.2.Polyphenol Oxidase activity assay	31
7.Histological detection of cell death in 2.5 mM riboflavin treated plant	32
IV.RESULTS	33
1. The effect of different concentration of riboflavin on some growth characters of tomato cultivars	33
1.1. Stem length	33
1.2.Number of leaves	34
2.The effect of different concentration of riboflavin on TMV infection	37
2.1.TMV-incidence and severity	37
2.1.1.On indicator hosts	37
2.1.2.On tomato cultivars	38
2.2.Quantification of TMV infectivity	41
2.3.Determination of TMV concentration by using ELISA test	43
2.3.1.On local lesion hosts	43
2.3.2.On tomato plants	43