



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكرو فيلم

بسم الله الرحمن الرحيم



MONA MAGHRABY



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكروفيلم



شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلم



MONA MAGHRABY



شبكة المعلومات الجامعية
التوثيق الإلكتروني 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



MONA MAGHRABY

**EFFECT OF SEED TREATMENT WITH UV RADIATION
AND SOME COMPATIBLE COMPOUNDS ON
PLANT RESISTANCE TO BIOTIC STRESS**

By

MOHAMED MAHMOUD ABOUL FOTOUH MESALHI

B.Sc. Agric. Sc. (Agric. Biochemistry), Ain Shams University, 2008

M.Sc. Agric. Sc. (Agric. Biochemistry), Ain Shams University, 2014

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

DOCTOR OF PHILOSOPHY

in

**Agricultural Sciences
(Agricultural Biochemistry)**

**Department of Agricultural Biochemistry
Faculty of Agriculture
Ain Shams University**

2020

Approval Sheet

EFFECT OF SEED TREATMENT WITH UV RADIATION AND SOME COMPATIBLE COMPOUNDS ON PLANT RESISTANCE TO BIOTIC STRESS

By

MOHAMED MAHMOUD ABOUL FOTOUH MESALHI

B.Sc. Agric. Sc. (Agric. Biochemistry), Ain Shams University, 2008

M.Sc. Agric. Sc. (Agric. Biochemistry), Ain Shams University, 2014

This thesis for Ph.D. degree has been approved by:

Dr. Louis Kamel Tadros

Prof. Emeritus of Agricultural Biochemistry, Faculty of Agriculture,
Mansoura University

Dr. Ahmed I. Abo Shadi

Prof. Emeritus of Agricultural Biochemistry, Faculty of Agriculture,
Ain Shams University

Dr. Hany Abd-Allah Mohamed Srouf

Prof. of Agricultural Biochemistry, Faculty of Agriculture, Ain
Shams University

Dr. Farok Guindi Moawad

Prof. Emeritus of Agricultural Biochemistry, Faculty of Agriculture,
Ain Shams University

Date of Examination: 16 / 2 / 2020

EFFECT OF SEED TREATMENT WITH UV RADIATION AND SOME COMPATIBLE COMPOUNDS ON PLANT RESISTANCE TO BIOTIC STRESS

By

MOHAMED MAHMOUD ABOUL FOTOUH MESALHI

B.Sc. Agric. Sc. (Agric. Biochemistry), Ain Shams University, 2008

M.Sc. Agric. Sc. (Agric. Biochemistry), Ain Shams University, 2014

Under the supervision of:

Dr. Farok Guindi Moawad

Prof. Emeritus of Agricultural Biochemistry, Department of
Agricultural Biochemistry, Faculty of Agriculture, Ain Shams
University (Principal Supervisor)

Dr. Mamdouh Abo Mosallam Tag El-Din

Prof. Emeritus of Agricultural Biochemistry, Department of
Agricultural Biochemistry, Faculty of Agriculture, Ain Shams
University

Dr. Hany Abd-Allah Mohamed Srour

Prof. of Agricultural Biochemistry, Department of Agricultural
Biochemistry, Faculty of Agriculture, Ain Shams University

ABSTRACT

Mohamed Mahmoud Aboul Fotouh Mesalhi: Effect of seed treatment with UV radiation and some compatible compounds on plant resistance to biotic stress. Unpublished Ph.D. Thesis, Department of Agricultural Biochemistry, Faculty of Agriculture, University of Ain Shams, 2020.

Rhizoctonia solani is considered as one of the most harmful pathogens affecting green bean productivity. *Phaseolus vulgaris*, L. cv. paulista seeds were subjected to UV-C for 60 min. and/or immersed in one of water, glycine betaine (GB, 5 mM), mannitol (5 mM), salicylic acid (SA, 5 mM) and mix solution of the three compounds with a concentration of 5 mM for each one of them. The immersion lasted for 24 hours to stimulate plant biochemical defenses against *R. solani*. After treatments, seeds were divided into two groups. The first group was used in an *in vitro* experiment to evaluate the effect of treatments on the accumulation of antifungal compounds and cell wall degrading enzymes (CWDE) inhibitory compounds in the extracts of germinated seeds. The second group of treated seeds were sown in either sterilized soil or soil infested with *R. solani*, then root samples were collected after two weeks to evaluate disease index (DI) and plant resistance machinery through determination of total phenols, antiradical activity of roots extracts, lipid peroxidation, proline concentration and some plant defensive enzymes activities. The results clearly demonstrated that DI was significantly ($P \leq 0.05$) reduced by all treatments and the lowest DI was observed in the case of SA, GB and mannitol with decrements of 58, 54.7 and 52.7%, respectively. Seed treatment with UV-C for 60 min, GB (5 mM), mannitol (5 mM) and SA (5 mM) for 24 h alone or together enhanced plant rooting in infected seedlings comparing with control. Also, there was a high correlation between the levels of lipid peroxidation and *Rhizoctonia* root rot disease index. Seed treatments mitigated the effect of infection on the accumulation of malondialdehyde (MDA) resulting from lipid peroxidation in comparison with the infected control. Also, seed treatment with UV, GB + UV and mix + UV significantly ($P \leq 0.05$)

increased total phenols concentration in infected roots as compared with control and there was a correlation between the accumulation of phenolic compounds and their antiradical activity in plant roots grown from UV-treated seeds. Among all treatments, UV radiation was the only treatment that significantly ($P \leq 0.05$) increased proline concentration in infected roots as compared to control. Regarding to the plant defensive enzymes, chitinase activity was enhanced by UV, mannitol, mix and mix + UV treatments in infected roots. While, the levels of phenylalanine ammonia lyase was increased by UV and SA + UV treatments. Also, SA + UV and GB treatments were the only treatments that triggered significant ($P \leq 0.05$) increments in the superoxide dismutase (SOD) activity in infected roots as compared to the control. On the other hand, guaiacol peroxidase (G-POD) and polyphenol oxidase (PPO) activities were elevated by all treatments in infected plant roots in comparison with control. Furthermore, the *in vitro* experiment revealed that each of UV, SA, SA+UV, GB+UV, mix and mix + UV treatments led to significant increases in antifungal compounds in germinated seeds extracts. Such effect was highly observed with mix + UV as the inhibition percentage of hyphal radial growth of *R. solani* reached to 59.4% as a result of treatment with the extract of treated germinated seeds. The inhibitory effects of treated germinated seeds extracts against CWDE of *R. solani* were tested. Results indicated that seed treatments led to accumulation of inhibitors of pectin methyl esterase, polygalacturonase, pectate lyase and cellulase. Finally, it can be concluded that treatment of green bean seeds with UV-C, GB, mannitol and SA enhanced green bean seedlings resistance against *R. solani* by accumulation of phytoalexins, CWDE inhibitors, activation of the antioxidant system and induction of pathogenesis-related proteins (PR proteins) such as chitinase.

Keywords: *Rhizoctonia solani* – *Phaseolus vulgaris* – glycine betaine – Mannitol - Salicylic acid - UV – Seed treatment – Induced resistance.

ACKNOWLEDGMENT

First of all, great thanks and praises to **ALLAH** who gave me strength and patience to accomplish this work. Really, no word can express how I am grateful to **ALLAH**.

Foremost, I would like to express my deepest and sincere gratitude to **Prof. Dr. Farok Guindi Moawad** Prof. of biochemistry, Faculty of Agriculture, Ain Shams University for his guidance, patience, motivation, assistance in preparing the manuscript and for allowing me to grow as a scientist. Also, I would like to thank **Prof. Dr. Mamdouh Abo Mosallam Tag El-Din** Prof. of biochemistry, Faculty of Agriculture, Ain Shams University for his supervision, encouragement, valuable help and priceless advices in preparing the manuscript. Moreover, I would like to express my appreciation to **Prof. Dr. Hany Abd-Allah Mohamed Srour** Prof. of biochemistry and the head of biochemistry department, Faculty of Agriculture, Ain Shams University for his efforts in providing all facilities required to successfully finish this work, for his valuable contribution in designing the plan of this work and for his precious advices in the preparation of the manuscript.

Thanks are also extended to all biochemistry department staff and my colleagues for their support and encouragement.

Moreover, I would like to offer my sincere gratitude to **Dr. Maha Helmy Mohamed** the lecturer of plant pathology for her great effort in the preparation of the inoculum for the green house experiment and for her contribution in the antifungal assay and preparation of cell wall degrading enzymes.

Finally, words are not enough to express how I am grateful to my parents who pushed me to accomplish success in my life. The last but not the least, I would like to express my appreciation to my beloved wife for her unconditional limitless support to me.

CONTENTS

	Page
LIST OF TABLES.....	IV
LIST OF FIGURES.....	VI
LIST OF ABBREVIATIONS.....	VIII
1. INTRODUCTION.....	1
2. REVIEW OF LITERATURE.....	4
2.1. Adverse effects of <i>Rhizoctonia solani</i> on plant.....	4
2.2 Biochemical responses to infection in green bean	6
2.3. The effect of seed priming on plant resistance.....	7
2.3.1. Effect of seed priming with glycine betaine on plant resistance.....	8
2.3.2. Effect of seed priming with mannitol on plant resistance.....	9
2.3.3. Effect of seed priming with salicylic acid on plant resistance	10
2.3.4. Effect of seed priming with UV-C on plant resistance.....	11
3. MATERIALS AND METHODS.....	14
3.1. Plant material	14
3.2. Pathogen isolation and preparation of inoculum.....	14
3.3. Seed Treatments.....	14
3.4. The <i>in vitro</i> experiment.....	15
3.4.1. Preparation of ethanolic extract.....	15
3.4.2. Antifungal assay.....	15
3.4.3. Inhibition studies on hydrolytic enzymes	16
3.4.3.1. Production of hydrolytic enzymes.....	16
3.4.3.2. Percentage of inhibition for Pectin methyl esterase (PME)	16
3.4.3.3. Percentage of inhibition for polygalacturonase.....	17
3.4.3.4 Percentage of inhibition for pectate lyase.....	17
3.4.3.5. Percentage of inhibition for cellulases.....	18
3.5. Green house experiment.....	19
3.5.1. Preparation of fungal inoculum and soil infestation	19
3.5.2. Experimental design.....	19

II

3.6. Determination of disease index.....	19
3.7. Biochemical analysis.....	20
3.7.1. Determination of proline concentration.....	20
3.7.2. Determination of lipid peroxidation.....	20
3.7.3. Determination of total phenols concentration.....	21
3.7.4. Determination of free radical scavenging activity.....	21
3.7.5. Enzyme assays.....	22
3.7.5.1. Enzyme extraction.....	22
3.7.5.2. Superoxide dismutase (SOD).....	22
3.7.5.3. Guaiacol peroxidase (G-POD).....	22
3.7.5.4. Polyphenol oxidase (PPO).....	23
3.7.5.5. Phenylalanine ammonia lyase (PAL).....	23
3.7.5.6. Chitinase assay.....	24
3.7.5.7. Preparation of colloidal chitin.....	24
3.8. Statistical analysis.....	25
4. RESULTS AND DISCUSSION	26
4.1. Glycine betaine (GB), Mannitol, Salicylic acid (SA), and UV induce resistance against <i>Rhizoctonia solani</i> in green beans.....	26
4.2. Biochemical changes associated with induction of plant resistance by treatment with UV-C, Salicylic acid (SA), Glycine brtaine (GB) and Mannitol.....	29
4.2.1. Lipid peroxidation.....	29
4.2.2. Proline concentration.....	32
4.2.3. Total phenols concentration.....	35
4.2.4. Free radical scavenging activity of root extracts.....	37
4.2.5. Phenylalanine ammonia lyase (PAL) activity.....	41
4.2.6. Superoxide dismutase (SOD) activity.....	42
4.2.7. Guaiacol peroxidase activity (G-POD).....	45
4.2.8. Polyphenol oxidase (PPO) activity.....	47
4.2.9. Chitinase activity.....	49
4.2.10. Antifungal effect of ethanolic extracts of treated germinated seeds.....	52

III

4.2.11. Effect of seed ethanolic extracts on cell wall degrading enzymes (CWDE) activities.....	56
5. SUMMARY AND CONCLUSION.....	62
6. REFERENCES.....	68
7. ARABIC SUMMARY.....	

LIST OF TABLES

No.		Page
1	Effect of seed treatment with UV-C radiation and/or some compatible compounds on disease index (DI) in green beans infected with <i>R.solani</i> .	27
2	Effect of seed treatment with UV-C radiation and/or some compatible compounds on lipid peroxidation (malondialdehyde concentration, nmol. g ⁻¹ FW) in green bean roots	31
3	Effect of seed treatment with UV-C radiation and/or some compatible compounds on proline concentration (μg.g ⁻¹ FW) in green bean roots	33
4	Effect of seed treatment with UV-C radiation and/or some compatible compounds on total soluble phenols concentration (μg.g ⁻¹ FW) and free radical scavenging activity (% of DPPH inhibition) in ethanolic extracts of green bean roots	39
5	Effect of seed treatment with UV-C radiation and/or some compatible compounds on phenylalanine ammonia lyase (PAL) activity (Unit. mg ⁻¹ protein) in green bean roots	41
6	Effect of seed treatment with UV-C radiation and/or some compatible compounds on superoxide dismutase activity (Unit. mg ⁻¹ protein) in green bean roots	44
7	Effect of seed treatment with UV-C radiation and/or some compatible compounds on guaiacol peroxidase (G-POD) activity (Unit. mg ⁻¹ protein) in green bean roots	46
8	Effect of seed treatment with UV-C radiation and/or some compatible compounds on polyphenol oxidase (PPO) activity (Unit. mg ⁻¹ protein) in green bean roots	48
9	Effect of seed treatment with UV-C radiation and/or some compatible compounds on chitinase activity (Unit. mg ⁻¹ protein) in green bean roots	50

- 10 Antifungal effect of ethanolic extracts of green bean germinated seeds treated with UV-C radiation, mannitol (5 mM), salicylic acid (5 mM), glycine betaine (5 mM), individually or in combination, against *R.solani* (AG4-HGI isolate). 54
- 11 Effect of ethanolic extracts of germinated green bean seeds treated with UV-C radiation, Mannitol, Salicylic acid, Glycine betaine, individually or in a mixture on activities of pectinases and cellulase produced by *R.solani* (AG4-HGI isolate) 58