INSERTION OF CHITINASE GENE TO ATTENUATE EARLY BLIGHT DISEASE IN SOME POTATO VIRUS RESISTANT LINES

By

SALLY MOHAMED HASSAN
B.Sc. Agric. Sci. (Genetic), Fac. Agric., Ain Shams Univ. 2008

A Thesis Submitted in Partial Fulfillment

Of

The Requirements for the Degree of

MASTER OFSCIENCES

in

Agricultural Sciences
(Genetics)

Department of Genetics
Faculty of Agriculture
Ain Shams University

2017

Approval Sheet

INSERTION OF CHITINASE GENE TO ATTENUATE EARLY BLIGHT DISEASE IN SOME POTATO VIRUS RESISTANT LINES

By

SALLY MOHAMED HASSAN
B.Sc. Agric. Sci. (Genetic), Fac. Agric., Ain Shams Univ. 2008

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

Dr. Abdel-Hady Ibrahim Hasan **……..……...**
Prof. Emeritus of Genetics, Faculty of Agriculture, Al Azhar University.

Dr. Ahmed Fahmy Hussein **…….……**
Prof. of Genetics, Faculty of Agriculture, Ain Shams University.

Dr. AimanHanafy Abdel-Azeem **.....**
Prof. of Genetics, Faculty of Agriculture, Ain Shams University.

Dr. Mohamed Abdel-SalamRashed **……..……...……**
Prof. Emeritus of Genetics, Faculty of Agriculture, Ain Shams University.

Date of Examination: / /2017

INSERTION OF CHITINASE GENE TO ATTENUATE EARLY BLIGHT DISEASE IN SOME POTATO VIRUS RESISTANT LINES

By

SALLY MOHAMED HASSAN

B.Sc. Agric. Sci. (Genetic), Fac. Agric., Ain Shams Univ. 2008

Under the Supervision of:

Dr. Mohamed Abdel - SalamRashed

Professor Emeritus of Genetic, Department of Genetic, Faculty of Agriculture, Ain Shams University (Principal Supervisor).

Dr. AimanHanafy Abdel-Azeem

Professor of Genetic, Department of Genetic, Faculty of Agriculture, Ain Shams University.

Dr. EmadAnisMetry

Head research of Genetic, Agriculture Genetic Engineering Research Institute, Agriculture Research Center.

ABSTRACT

Sally Mohamed Hassan. Insertion of Chitinase Gene to Attenuate Early Blight Disease in some Potato Virus Resistant Lines. Unpublished M.Sc. Thesis, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2017.

Potato (*Solanum tuberosum* L.) an agro-economically important food crop in the world, is sensitive to many fungal pathogens including *Alternaria solani*, the causal agent of early blight disease. Chitinase is cell wall degrading enzyme which has been shown to have high antifungal activity against a wide range of phytopathogenic fungi. In the present study, plasmid pRI 201-AN binary vector, containing the kanamycine selectable marker in plant and the chitinase gene was used in potato transformation. Leaves of Desriee cultivar, PVY5 and PVY15 lines were transformed with the pRI 201-AN construct *via* the *Agrobacterium tumefaciens* delivery system. Transformed leaves were incubated for 5 days in dark on callus induction media which contained MS with 5 mg/l 2-4, D, 1 mg/l cefatoxine (200mg/ml) and 1 mg/l kanamycine (25mg/ml). After that callus was transferred to regeneration media which contained MS with 1 mg/l IAA, 1 mg/l BA, 10 mg/l GA3, 1 mg/l cefatoxine (200mg/ml) and 250 µg/l kanamycine (25mg/ml) and their expression at the transcriptional level was confirmed by polymerase chain reaction (PCR) by using chitinase and vector primers. After the transformed plants were evaluated, the positive transgenic plants were detected by using forward and reverse primer of kanamycine.

Keywords: Chitinase; Potato; *Alternaria solani*; early blight disease; *Agrobacterium tumefaciens*.

ACKNOWLEDGEMENT

In the beginning of this thesis, all praise and thanks to Allah as I complete my studies of this thesis.

I would like to extend thanks to everyone, in many ways, who so generously contributed to the work presented in my present thesis.

As a coronation for my researches in the context of my thesis that is accomplished in the Department of Genetics, Faculty of Agriculture, Ain Shams University, which I am proud to belong to. It is a great honor to be a person in this department.

The accomplishment of this thesis is a result of the help and direction from my supervisor **Prof. Mohamed Abdel Salam** who is always working hardly to give me the willing to solve the confusions and direct me approach to the final result of the thesis. On top of that, without his encouragement, I would not finish this final work in my master study. Thank you very much.

Special mention goes to my enthusiastic supervisor, **Prof. Aiman Hanafy**. Really my master has been an amazing experience by working with him and I thank him wholeheartedly, not only for his tremendous academic support, but also for his always encouragement to me.

It is also a matter of pride and honor to have AGERI is the operations lab and research headquarters related of this thesis. I am very proud of my affiliation to the institute.

Furthermore, I would like to show my grateful feeling to **Prof. Emad Anis** who taught me and was the most near for me of my project supervisors in my long way. He is a discipline-keeping person, with whose supervision I accomplish my master study in time. Although He is always nervous and tough in dealing with me but also for giving me so many wonderful opportunities motivating me in terms of administration and rules. Thank you very much.

I am also hugely appreciative to **Dr. Ahmed Mohamed** professor of genetics in AGERI especially for sharing their laboratory expertise.

Great thanks for my colleagues **Mervat Ragab** and **Heba Sayed,** they supported me as a real sisters and good teachers. My warm greetings for those team mates who were my main supporter in my lab.

Finally, but by no means least, thanks go to my great mom, marvelous husband, lovely sisters, my son Yassin and also my dad.

Thanks for almost unbelievable support. They are the most important people in my world and I dedicate this thesis to them.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Potato (*Solanum tuberosum*) is the fourth most important food crop in the world and is a critical crop in terms of food security. During the growing season and under storage conditions potato usually suffers from several fungal and viral diseases that cause serious damage and losses in yield. Such as leaf diseases, wilts, diseases of young plants, tuber diseases, virus and fungal diseases **(Jalli *et al.*, 2011)**.

Some of the fungal diseases are early blight caused by *Alternaria solani* and late blight caused by *Phytophthora infestant*, some of the virus diseases are Potato leafroll virus (PLRV), Potato virus X (PVX) and Potato virus Y (PVY).

Alternaria solani is a fungal pathogen, that produces a disease in tomato and potato plants called early blight; it is also one of the most important foliar pathogens of potato. Yield loss estimates attributed to foliar damage, which results in decreased tuber quality and yield reduction, can reach 20-30% **(Olanya *et al.*, 2009)**.

In storage, *Alternaria solani* can cause dry rot of tubers and may also reduce storage periode, which diminish the quantity and quality of marketable tubers.

The cell walls of bacteria, fungi, mushrooms, the exoskeleton of crustaceans (crabs, shrimp, etc.) and insects, the microfilarial sheath of parasitic nematodes, and the lining of the digestive tracts of many insects have chitin compound **(Araujo *et al.*, 1993)**.

Chitin is used for protection of chitin-containing organisms against the harsh conditions in their environment and host antiparasite/pathogen immune responses. Thus, the absence of chitin can lead to the death of the pathogen **(Lee, 2009).**

INTRODUCTION

Chitinases contribute to the life cycle of chitin-containing fungi and parasites, in which they control growth and molting. Chitinases are also used by pathogens to invade or exploit chitin-containing structures in the host and thus play a critical role(s) in the transmission of infection from one vertebrate host to another by insect vectors **(Shahabuddin and Kaslow, 1994)**.

The chitinases are the key degrading enzymes; they are produced in significant quantities by hosts as a defense against infection with chitin-containing organisms. This attempt to damage the chitin coat of the infecting organism is part of the innate immune response.

The major goal of this thesis is producing resistant shoots to early blight disease by transforming the chitinase gene into two different lines of potato resistant to virus Y (PVY5 and PVY15) and potato cultivar (Desiree). The object of this study has been carried out through the following steps:

1- Genetic transformation using *Agrobacterium tumefaciens*.

2- Regeneration of Desiree cultivar, PVY5 and PVY15 lines.

3- Determination of potative transformed shoots using PCR.

REVIEW OF LITERATURE

1- Potato

Potato (*Solanum tuberosum*) is the fourth most important food crop in the world, it contains vitamins and minerals, as well as an assortment of phytochemicals, such as carotenoids and natural phenols **(Ferretti, 2011)** and the predominant form of this carbohydrate is starch.

The total world potato production is estimated at about 365.000.000 tones **(Tubiello *et al.*, 2015)**. Potato is a critical crop in terms of food security and more than one billion populations around the globe consume potato. Potato is vegetatively propagated, meaning that a whole plant can be grown from a potato tuber or a piece of it.

One plant can produce 5-20 new tubers, which will be genetic clone of the mother plant. Potato has a long history of improvement through traditional breeding. Breeders target multiple traits, including resistance to biotic and abiotic stresses, and tuber quality **(Singh *et al.*, 2000)** . Adaptation and response to these stresses is highly complex and involve changes at the molecular, cellular, and physiological levels.

2- Abiotic and biotic stresses

Abiotic stress factors such as heat, drought, and salinity have a significant impact on cultivated potato, affecting yield, tuber quality, and market value **(Ishitani *et al.*, 2011)**.

Biotic stress is stress that occurs as a result of damage plants by other living organisms, such as bacteria, viruses, fungi, parasites, beneficial and harmful insects, weeds, and cultivated or native plants, among them late blight and early blight are fungal diseases , viruses as potato virus X (PVX), potato virus S (PVS), Potato virus Y (PVY) and potato leaf-roll virus (PLRV), bacterial wilt, wart and cyst nematodes **(Flynn, 2003).**