COMPARATIVE STUDIES ON VIRAL INDUCIBLE PROTEIN IN SUSPENSION CULTURE OF SOME *SOLANACEAE* PLANTS

By

ALAA MOHAMED EL-SAEED EL-MINISY

B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2008

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APPROVAL SHEET

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Title of Thesis: Comparative Studies on Viral Inducible Protein in Suspension Culture of *Solanaceae* Plants

Supervisors: Dr. Mohamed Hassanein Soliman

Dr. Salah El-Din Sayed Mohamed El-Assal

Department: Genetics **Approval: 4 /7/ 2017**

ABSTRACT

Potato virus Y (PVY), a member of Potyvirus genus, is one of the most important potato viruses and is the causal agent of several plant diseases in a wide range of host species, causing important economic losses in agriculture. These studies were conducted during the period from 2013 to 2016 at the Department of Plant Biotechnology, National Research Center, Cairo, Egypt, to identify and detect the proteins involved in response to PVY infections in *N.tabacum* Samsun CV. In the first experiment, Total soluble proteins were extracted from *N.tabacum* leaf tissue inoculated with PVY, Protein changes in both healthy and inoculated tobacco leaves were revealed using one dimensional gel electrophoresis (1D-SDS). Proteins that were highly and significantly changed are selected for identification by liquid chromatography mass spectrometry (LC-MS/MS) combined with bioinformatics. These data provide a valuable resource for discovering novel proteins involved in the pathogen response. Such proteins could be introduced into agronomical important species to develop a viral resistant crop plants. A total of 470 proteins were confidently identified, with a predominance of proteins associated with photosynthesis, energy and metabolism and response to stimulus. The second experiment was conducted to study the role of PAP in plant response against PVY infection using *N.tabacum* cv. Samsun. To achieve this goal, pokeweed micropropagation protocol was achieved. After 12 days of inoculation, *N. tabacum* plants pre-treated with 0.1 mg/mL pokeweed crude leaf extract before inoculation with PVY showed less symptoms compared to that inoculated with PVY only. Furthermore, the leaf total soluble proteins of PAP treated and untreated PVY infected tobacco plants were analyzed by 1D-SDS. The electrophoresis profile showed variant accumulation levels at two different molecular weight protein bands. Moreover, the spectrometric analysis of those two bands using LC-MS/MS, has identified about 50 different proteins. According to their functions prediction, it has been classified into seven groups including (Energy& metabolism, stress defense response, carbon fixation, photosynthesis, plant cytoskeleton, structural proteins and signaling). We conclude that the PAP may has a direct role in suppression of the PVY infection mechanism. Moreover, PAP protein could be used as a foliar protective agent against PVY infection in tobacco system.

Key words: *Solanaceae*, PVY, Viral inducible protein, 1 D-SDS, LC-MS/MS.

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INTRODUCTION

Potato plants are subjected to attack by several viral diseases, causing harmful effects and reduce crop yield and tuber quality. Viral diseases, particularly potato leaf roll virus (PLRV) and potato virus Y (PVY), are severe problems for Egyptian potato production.

PVY is one of the most important potato viruses and is spread worldwide and recognized as the fifth most important plant virus regarding its scientific and economic importance (Scholthof *et al.*, 2012). It is mainly infect some plants of family *Solanaceae* and causes great yield losses in major crops.

Agricultural crops worldwide suffer from a vast array of pathogens including bacteria, fungi, and viruses which cause tremendous losses in yield and quality of production (Rodoni, 2009). For a long time, these pathogens have been controlled through conventional measures like crop rotation and other cultivation techniques, early detection, destruction of infected source plants, cross-protection, breeding for resistance, and chemical control (Goldbach *et al.* 2003). It has been known that some plants possess special metabolic pathways to synthesize a number of valuable proteins which can be used for prevention and treatment of diseases (Calixto, 2000). For example, plant genes encoding ribosome-inactivating proteins (RIPs) have been shown to confer disease resistance in recent years. RIPs are found not only in a few higher plants but also in fungi, bacteria, and at least one alga (Stirpe and Battelli, 2006).

Attempts to transform potato plants with the pokeweed antiviral protein (PAP), a ribosome-inactivating protein found in the cell walls of *Phytolacca Americana* (pokeweed), thought to give a broad-spectrum protection against several viruses, gave only limited protection against aphid transmitted PVY. When PAP was applied exogenously it protected potato plants from mechanical inoculation with PVY (Lodge *et al.*, 1993).

In recent years, several efforts have been made to study plant-virus interaction at the proteome level in both compatible (susceptible host) and incompatible (resistant host) infections. In both compatible and non-compatible interactions, viruses utilize plant host proteins to complete the infection process. In case of incompatible host–pathogen interactions, damage caused by the pathogen remains restricted because of the plant's defensive response. Plant defensive response is hypersensitive reaction (HR), in which the cells around the infection site rapidly undergo necrosis, integrated with set of metabolic alterations that restricts pathogen ingress (Van Loon and Van Strien, 1999). Metabolic alterations included defense mechanisms like physical strengthening of the cell wall through lignification, suberization, callose deposition and synthesis of the pathogenesis related (PR) proteins, which include β-1,3-glucanases (PR-2), chitinases (PR-3, -4, -8, and -11) and thaumatin-like proteins(PR-5) (Bowles, 1990) . Other HR responses to contain the pathogen is changes in ion fluxes, lipid hyper peroxidation, protein phosphorylation, nitric oxide generation, antimicrobial compounds and burst of reactive oxygen species (ROS).

The ability of plants to defend themselves against pests and disease is associated with a number of proteins that can be up or down-regulated (Afroz, 2011) after challenged. Thus, proteomic analysis to identify host's proteins and their changes in abundance linked to biochemical and cellular processes that control pathogen recognition, defense signal transduction and confer resistance (Zimaro, 2001; Mandelc, 2013) is of paramount importance.

The objective of our study is to detect and identify the proteins involved in the response to PVY infection and exogenous application of crude extract of PAP in tobacco plants. The scope of the study was:

- isolate and identify potato virus Y (PVY).
- mechanically inoculate plants of host range with PVY.
- confirm the inoculation with PVY.
- Pokeweed micropropagation and extract pokeweed antiviral protein (PAP).
- detect the plant protein response due to PVY infection and exogenous application of crude extract of PAP
- in gel digestion of excised selected protein bands for liquid chromatography mass spectrometry (LC MS/MS) analysis.
- LC MS/MS analysis and *in silico* analysis for identification and functional prediction of sequenced proteins (proteomics).

REVIEW OF LITERATURE

1. *Solanaceae* family and plant viruses

Solanaceae is a family of flowering plants that consists of about 100 genera and 2500 species (Olmstead *et al.,* 2008). Many of them are considered from the world's most important agricultural species, including tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum*), egg- plant (*Solanum melongena*), tobacco (*Nicotiana tabacum*), pepper (*Capsicum annuum*) and petunia (*Petunia spp*.). Also, *Solanaceae* has many species that have wide variety of uses (Mueller *et al.,* 2005). Considering the economic importance, some *Solanaceae* plants are important model systems for biology. For example, tomato for fruit ripening and plant defense, tobacco for plant defense, and petunia for the biology of anthocyanin pigments. Wide range of diseases affects solanaceous vegetables. The most important viruses isolated from potato are Potato leaf roll virus (PLRV), Potato virus Y (PVY), Potato virus A (PVA), Potato virus X (PVX) and Potato virus M (PVM) (Valkonen, 1994).

Potato Virus Y (PVY; family Potyviridae, genus Potyvirus), and Potato Leafroll Virus (PLRV; family Luteoviridae, genus Polerovirus), are considered highly important. However, PVY regarded as the most economically significant virus of potato (De Bokx and Van der Want, 1987). Potato leaf roll virus (PLRV) and potato virus Y (PVY), are critical problems for Egyptian potato production. They decrease crop yield and tuber quality.