Name of Candidate: **Sahar Abdel Aziz Youssef.** Degree: **Ph.D**
Title of Thesis: **Molecular Biology Studies On Some Viruses Affecting Stone Fruit Trees In Egypt**
Supervisors: **Dr. EL Said Ahmed Salama (Late), Dr. Maurice Sabry Mikhail,. Dr.Gamal Amin Ghanem, and Dr. Hamed Mahmoud Mazyad.**
Department: **Plant Pathology**
Branch: **Molecular Biology** Approval:

ABSTRACT

Plum pox virus (PPV) and *Prune dwarf virus* (PDV) are two of the most common viruses infecting stone fruit trees in Egypt and dramatically affecting the yield. A general survey for both viruses was conducted during three successive seasons (2001, 2002, and 2003). Data obtained from the survey depending on ELISA test for the PPV and PDV diseases in the main stone fruit tree production areas showed that, the incidence of the PPV and PDV were 16.94% and 9.0% respectively. The general outlook at the results indicated that, PPV and PDV had a wide host range among members of the family *Solanaceae*. In addition, a few species of *Chenopodiaceae*, *Cucurbitaceae* and *Amrantheaceae* were also susceptible. Grafting transmission gave the best results for both viruses compared with mechanical and seed transmission.

Molecular characterization of the viral genome for the two viruses PPV and PDV were studied. Gene Releaser polymeric matrix proved an effective, reliable method for preparing plant tissue extracts for RT-PCR application to detect PPV and PDV. Fragments of the expected size, 220 bp PPV cDNA and 172 bp PDV cDNA amplified products were directly cloned into pCR™ TOPO plasmid. The recombinant plasmid was successfully transformed into *E. coli* (BL21 strain). White Ampcillin colonies were selected for plasmid minipreparation. Recombinant plasmids which had correct inserts of PPV (220 bp) and PDV (172 bp) were validated using PCR colony and restriction analysis. Nucleotide sequence analysis for 220 bp amplified fragment from the 3'-non coding region (NCR) of the PPV genome showed homology ranged from 93% to 95% when it compared with the other PPV sequenced isolates available in the GenBank such as PPV-D, PPV-M and PPV-C isolates. While it gave a 100% similarity with the published sequences for El Amar strain. This previous evidence strongly supported that the PPV isolate examined corresponded to the PPV-El Amar strain. Multiple sequence alignment of the 172 bp from the PDV coat protein isolated from Egypt with some PDV isolates available in the GenBank indicated that the coat protein gene sequences of PDV isolated from Egypt shared 97 % to 98 % identity with other isolates.

Specific PPV and/or PDV labeled probes in addition to mixed labeled probe were used successfully in non-radioactive hybridization method under high stringency condition to detect the presence of PPV and/or PDV in different plant tissues collected from different locations. Results showed that the apricot and peach

samples which had different levels of infection gave different values of reaction starting with the weak reaction to strong reaction.

Plum pox potyvirus (PPV) isolates divided into four groups according to their serological and molecular differences. Characteristics of the divergent El-Amar strain were investigated using Immuno-capture RT PCR, Silica-capture RT-PCR and restriction fragment length polymorphism (RFLP). IC/RT-PCR proved to be more sensitive and reliable diagnosis for virus detection in the presence of specific antibody. Our results observed that primers designed from conserved sequences in the carboxyl-terminal portion of the coat protein gene of characterized PPV isolates were shown to yield an amplified product of the expected size from all tested field infected trees. On the other hand the primers specific for PPV isolates (D, M and C) did not react with any tested samples which indicated that El Amar was the only unique strain in Egypt. Another interesting result was that the restriction analysis of the amplified fragments by the endoneuclease *Rsa*I revealed the presence of restriction sites at the expected position only in PPV-D isolates and absent in all amplified products from the collected samples, since, they did not contain the *Rsa*I site, and the obtained data indicated that all collected samples were related to El-Amar strain.

For production of polyclonal antibody for PPV El-Amar strain, specific oligonucleotide primers were used to amplify the coat protein gene of PPV El-Amar starin. The amplified products were cloned into pBAD-TOPO TA expression vector and sequenced. Upon induction of *E.coli* cells harboring the recombinant plasmid, a protein with an SDS-PAGE migration commensurate with the calculated molecular weight of the fusion protein 41.5 kDa including the 6xHis-tagged (3.5 kDa) was observed. This fused protein reacted strongly with polyclonal antisera directed against PPV in the western blotting experiments. The appropriate protein was injected subcutaneously into New Zealand White rabbit. By 14 days post the last injection a specific immune response was detected against PPV-CP. Indirect ELISA was carried out to determine the antibody titer. TBIA and DBIA were used to determine the specificity and sensitivity of the produced antisera. The coat protein gene expression in *E.coli* was a promising new technique for generating antibodies in laboratory animals for diagnostic purposes in biological science. The main advantages were the elimination of time and labor and the technically demanding steps of antigen purification.

Key Words: Plum pox virus (PPV), El Amar strain, Prune dwarf virus (PDV),ELISA, RT-PCR, cloning, sequencing, non-radioactive hybridization, RFLP, Coat protein expression, Antiserum, Polyclonal antibody.

CONTENTS

LIST OF ABBREVIATIONS

A	
Agri	Agriculture
Amp	Ampecilin
APS	ammonium persulphate
ATP	Adenosine triphosphate
B	
bp	Bais pair
BCIB	5-bromo-4-chloro-3- indolyl phosphate
C	
cv.	Cultivar
cvs.	Cultivars
cp	Coat protein
cDNA	Complementary DNA
D	
DAS	Double antibody sandwich
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
DTT	Dithiothreitol
DIG	Digoxigenin
dNTP	Dideoxynucleotidetriphosphate
Dept.	Department
E	
E. coli	***Escherichia coli***
ELISA	Enzyme inked immunosorbent assay
EDTA	Ethylene diamine tetraacetic acid
F	
Fac.	Faculty
Fg	Femto gram

Fig.	Figure
G	
g	Grame (s)
H	
h	Hour (s)
HCl	Hydrochloric acid
I	
IC	Immunocapture
IgG	Immunoglobin G
IPTG	Isopropyl B-D-thiogalacto Pyranoside
K	
Kb	Kilobase
KCl	Potassium chloride
KDa	Kilo daltons
L	
LB	Luria-Bertani Medium
M	
min	Minute
mM	Milimolar
mg	Miligrame
ml	Milliliter
μl	Microliter
μg	Microgram
Mw	Molecular wieght
M	Molar
MgCl	Magnesium chloride
N	
NaCl	Sodium chloride
NBT	Nitro blue tetrazolium salt
ng	Nanogram
nM	Nanomolar

nt	Nucleotides
O	
ORF	Open reading frame
P	
PAGE	polyacrylamide gel electrophoresis
Path.	Pathology
PCR	Polymerase chain reaction
PCI	Phenol: chloroform: isoamyl alcohol
PDV	Prune Dwarf Virus
pg	Picogram
pmol	Picomole
PPV	Plum pox virus
R	
Res.	Research
rpm	Revolution per minute
RNA	Ribonucleic acid
RNase	Ribonuclease
RT	Reverse transcriptase
S	
SDS	Sodium dodecyl sulfate
Sec.	Second
T	
TBE	Tris-boric acid -EDTA
TEMED	N,N,N,N,tetramethylethyle diamine
TE	Tris-EDTA buffer
U	
U	Unit
Univ.	University
UV	Ultraviolet
V	
V	Volt

v/v	Volume : volume ratio
w/v	Weight :volume ratio
X	
X-gal	5-bromo-4-chloro-3-idolyl-D-galactoside