Abstract

Hoda Mahmoud Waziri , Physicochemical Studies on Barley Yellow Dwarf Virus., Unpublished Ph.D. Thesis, Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, 2003.

Barley Yellow Dwarf luteovirus–PAV (BYDV–PAV) is considered to be the most common virus for small grains, especially barley and wheat in Egypt.

In this study, An Egyptian isolate of BYDV-PAV (EGY-PAV) was purified from infected oat leaf tissue by modified procedure which included clarification of sap extracts with chloroform-amylalcohol, followed by concentration with polyethelene glycol (PEG), then pelleted through a 30% sucrose cushion and further purified by sucrose density gradients. Ultraviolet spectrum showed a typical curve of nucleoprotein with an $A_{260/280}$ ratio ranged from 1.75 to 1.80. The yield of purified virus was ranged from 1.5 to 1.6 mg/kg of infected tissue. Isometric virus particles of 25 nm in diameter were detected in the purified preparation when examined by the transmission electron microscope. The polyclonal antibodies against EGY-PAV was produced and the dilution end point of IgG and IgG conjugate was determined using DAS-ELISA.The genomic ssRNA was extracted from EGY-PAV purified particles and its size length was about 5.6 kb when determined by agarose gel electrophoresis. The IC/RT-PCR was carried out using EGY-PAV-IgG and specific primers to amplify the entire coat protein (CP) gene of *c.*600 bp from EGY-PAV genomic RNA. The CP gene was cloned into plasmid vector, and then the recombinant plasmid was transformed into *E.coli* competent cells. Our results using endonuclease *Eco*R1 restriction enzyme identified the inserts of CP gene with the expected size (*c.*600 bp).

Data of nucleotide sequencing identified exactly 600 bp for EGY-PAV CP gene and be translated into 200 amino acids with molecular weight of 22 kDa. Our results proved the presence of 17 kDa virion protein genomic linked (VPg) embedded in the open reading frame (ORF) of the CP gene. The computer data of multiple alignment of nucleotide and deduced amino acid sequences between the EGY-PAV and other PAV isolates from the GenBank indicated that the highest (99.0 and 98.5%)and the lowest (76.0 and 73.0%) percent similarity were observed with the French and Chinese isolates, respectively.

Key words: Barley yellow dwarf virus (BYDV), IC/RT-PCR, CP gene, Cloning, Nucleotide sequence, Similarity.

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LIST OF ABBREVIATIONS

(A)

AMV	Avian Myeloblastosis Virus
App	Appendix

(B)

bp	base pair
BSA	Bovine serum albumin

(C)

cDNA	Complementary deoxyribonucleic acid

(D)

DEPC	Diethypyrocarbonate
DIECA	Diethyl dithiocarbanate
DMF	Dimethylformamid
DNA	deoxyribonucleic acid
DTT	Dithiothreotol

(E)

EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme linked Immuno Sorbant Assay
E.coli	*Escherichia coli*

(G)

GTC	Guanidine thiocyanate

(H)

h	hour (s)

(I)

IPTG	isopropyle – β -D-thiogalactoside

(L)

LB	Luria –Bertani medium

(M)

min	minute (s)
MMLV	Moloney murine leukemia virus

(O)

O.D.	Optical density

(P)

PAGE	Polyacrylamide gel electrophoresis
PB	Phosphate buffer
PBS	Phosphate buffer saline
PBST	Phosphate buffer saline with tween 20
PCR	Polymerase chain reaction
pNPP	Para-Nitrophenyl phosphate
PVP	Polyvinylpyrolodin

(R)

RNA	Ribonucleic acid
RNase	Ribonuclease enzyme
RNase Inh	Ribonuclease Inhibitor
rpm	round per minute
RT	Reverse Transcription
RT-PCR	Reverse Transcription-Polymerase Chain Reaction

(S)

s	Second(s)
SDS	Sodium Dodecyl Sulphate

(T)

TAE	Tris-Acetate –EDTA (buffer)
Taq	Thermus aquaticus
TE	Tris/ EDTA (buffer)
Tfl	*Thermus flavus*

(U)

UV	ultraviolet

(X)

X-gal	5-bromo-4-chloro-3-indolyle – β -D-galactoside