

## MOLECULAR IDENTIFICATION AND PHYLOGENETIC ANALYSIS OF POTATO LEAF ROLL VIRUS IN EGYPT

ABDELMAKSOU, H. M., A. M. MANDOUR and A. SH. GAMAL ELDIN

*Plant Virology Research Department, Plant Pathology Research Institute, ARC, Giza – Egypt.*

(Manuscript received 19 March 2013)

---

### **Abstract**

Potato leaf roll virus (PLRV) was isolated from Egyptian grown potatoes. The virus was identified on the basis of host range, symptomatology, insect transmission, electron microscopy, RT-PCR, and PCR-ELISA. The complete nucleotide sequence of genomic PLRV-RNA was obtained from cloned cDNA and submitted in GenBank under Accession No. AY138970. This sequence is 5884 nucleotide long and encodes 5 ORF with an unique read-through protein suggested a conflict with the conceptual translation at amino acid 209. Comparison of PLRV sequence with that of other PLRV strains shows overall similarities of 97.02% and high genetic identity of 100% (Polish Strain), 98.5% (French Strain), 98.4% (Wageningen Strain), 98.3% (UK Strain), 98% (Canadian Strain), and 93.5% (Australian Strain).

Keywords: Phylogenetic analysis, PLRV similarities, Egypt PLRV

### **INTRODUCTION**

PLRV is the most important aphid transmitted potato virus belonged to Luteovirus. The family *Luteoviridae* have long been recognized as a natural group they sharing biological characters and particle features, they also differ in molecular characteristics. The recent taxonomy creates a family *Luteoviridae* that contain 3 genera to accommodate this diversity Genus *Luteovirus* (Type species *Barely yellow dwarf virus* –PAV), Genus *Pelervirus* (Type species *Potato leaf roll virus*), Genus *Enamovirus* (Type species *Pea enation mosaic virus*). PLRV particles is 25 nm in diameter, isometric and contain 5.3 to 9.5 kb ssRNA and a major 23 k coat protein with a “read through” proteins of 60k to 90k. The genomic comprise 5 or 6 large ORFs (Waterhouse *et al* 1988, and Gamal Eldin, A. S. *et al* 2004).

The aim of the present study was to compare the full genomic sequence of an Egyptian strain of PLRV with other PLRV strains from different countries and with other viruses belong to *Luteoviridae* using most up to date genetic software analysis DNASTAR Lasergene version 10 (DNASTAR Inc, MD).

## MATERIALS AND METHODS

### Virus isolation:-

Leaf samples of potato (*Solanum tuberosum*) showing leaf roll symptoms were collected from the potato fields at the Kaluboiua Governorate and were for virus isolation. The samples were serologically tested using PLRV- PAbs ELISA Kit (LOEWE Biochemical GmbH, Germany). The virus was transmitted by *Myzus persicae* Sulz using *Physalis foloridana* seedlings as a test plants and as a virus source for further studies.

### RNA purification and RT-PCR

RNA was extracted by RNeasy Plant Mini Kit (Qiagene, Germany) as recommended by the manufacturer. After total RNA extraction, the PCR detection was carried out using QIAGEN OneStep RT-PCR Kit (Qiagene, Germany) utilized two PLRV-Specific pairs of primers. The foreword primer 5'-AGCGCATAAACTCTACTCATTG and the reverse primer 5'-GTATCCTTCCACAGCCCTCTCATT (metabion GmbH, Germany) corresponding to positions 31-54 and 832-809 of PLRV genome. The PCR products were analyzed by gel electrophoresis on 1% agarose gel prepared in 1X TBE buffer (Sambrook *et al* 1989). The gel was stained with ethidium bromide and examined using UV transilluminater and the PCR fragments of PLRVwas confirmed as 802 bp.

### cDNA Library and PCR Cloning

The entire PLRV-RNA cDNA libraries was constructed using First Strand cDNA Synthesis Kit (Promega, USA) and each represented library was ligated directly into TA cloning vector (Plasmid PCR™II). The ligation products were transformed into competent of *E. coli* (INV& F' cells). White, ampicillin-resistant, colonies were selected and screened for correct inserts.

### DNA Sequencing

The nucleotide sequence of PLRV clone liberary was determined by the method of Beck (1993). The sequence was performed with ALF DNA Sequencer based on an adaptation of Sanger dideoxy methodology (Sanger *et al*, 1977). The nucleotide sequence of PLRV was carried out at Molecular Virology Group-Biotechnology Group, Department of Plant Biology-The Royal Veterinary and Agricultural University (KVL), Copenhagen-Denmark. The PLRV sequence was computer translated with the program Fragment Manger Software (Amersham Inc) and further analyzed by DNASTAR Lasergene (DNASTAR Inc, MD).

**RESULTS**

The complete genomic nucleotide sequence is shown in Fig (1). It contains 5884 bp. The base comparison of PLRV RNA (22.37% U, 25.24% C, 27.88% A and 24.31 G), while (A+T) = 50.45 and (C+G)= 49.55% and the base count was 1640 A, 14850 C, 1430 G, and 1328 U and the predicated translation of the amino acids is shown in Fig (1), below each triplet.

**Fig. 1. The complete nucleotide sequence of PLRV-Egypt and its predicted encoded amino acid sequence**

```

1  caaaaagaataccaggggaattgtagcgttttagcgcataaactctacactcattgcaaacggttatagcat atg att
    M I
76  gta ttg acc cag tct gga acc ttg ctt ttt gac caa aga ttt aaa ctc tca aag ttt ctc
    V L T Q S G T L L F D Q R F K L S K F L
136  ttc gtt gtc att gca aca ggc ttt cct ctt ctc ctg cag caa gcg agc tta att tac ggc
    F V V I A T G F P L L L Q Q A S L I Y G
196  tat aat cat gaa cag att tac cgc ata tgc cgc tct ttt ctt tat gtt ctc cct ttg ctc
    Y N H E Q I Y R I C R S F L Y V L P L L
    M N R F T A Y A A L F F M F S L C S
256  aac tgc aaa aga ggc agg att tca aca tcc ggc ctt caa ctt ccg agg cac ctc cac tat
    N C K R G R I S T S G L Q L P R H L H Y
    T A K E A G F Q H P A F N F R G T S T M
316  gag tgc ctt gag tgg gga tta ctc tgc ggc acc cac ccc gct ata caa atc gtg ggc cct
    E C L E W G L L C G G T H P A I Q I V G P
    S A L S G D Y S A A P T P L Y K S W A L
376  acc atc gtc att aaa ctt gac gac cca acc act gcc gcc gct tac aga tgc gag cta cta
    T I V I K L D D P T T A A A Y R S E L L
    P S S L N L T T Q P L P P L T D R S Y Y
436  cga gtt agt tca agc tct tat atc caa aat gcg gct gga ttg tca aac ggt tgg gga cat
    R V S S S S Y I Q N A A G L S N G W G H
    E L V Q A L I S K M R L D C Q T V G D M
496  gac atg gag gca ttt gtc aga aat gct att tgc ctc ctg gaa ctc cgt gaa aga agt atc
    D M E A F V R N A I C L L E L R E R S I
    T W R H L S E M L F A S W N S V K E V S
556  cct caa agc ggc ctc cgt gac ctt atg ggc aat tat caa cat ttg gtt cgt tct cta ttg
    P Q S A G L R D L M G N Y Q H L V R S L L
    L K A A S V T L W A I I N I W F G L Y W
616  gac gct tgc aag gtt gat cac ttt gtt cct ctg gac ttt cag cat aga agc ctt atg ctt
    D A C K V D H F V P L D F Q H R S L M L
    T L A R L I T L F L W T F S I E A L C L
676  aat ttg gct cgg ttg tat aac cag ctt gat cta caa ggg cgc gct aag tct ttc aga gca
    N F A R L Y N Q L D L Q G R A K S F R A
    I L L G C I T S L I Y K G A L S L S E H
736  ctt acc ggt ttt cct gtt tat gtc ccc tct gaa gat tat ttg gag ggc agc ttt ctc caa
    L T G F P V Y V P S E D Y L E G S F L Q
    L P V F L F M S P L K I I W R A A F S K
796  aag gaa tta caa gaa tga gag ggc tgt gga agg ata caa agg gtt ttc ggt ccc aca gaa
    K E L Q E *
    R N Y K N E R A V E G Y K G F S V P Q K
856  acc gcc aaa gtc tgc cgt aat tga act aca aca tga aaa cgg cag cca tct cgg gta cgc
    P P K S A V I E L Q H E N G S H L G Y A
916  gaa ctg cat tgc ctt gta cag tgg aga gaa cgc ctt ggt gac agc tga aca ctg tct aga
    N C I R L Y S G E N A L V T A E H C L E
976  agg cgc ctt cgc aac gtc gtt gaa aac tgg aaa cag gat tcc gat gtc gac ttt ctt tcc
    G A F A T S L K T G N R I P M S T F F P
1036  cat ttt caa aag tgc ccg taa tga tat ctc cat act agt agg tcc acc caa ctg gga agg
    I F K S A R N D I S I L V G P P N W E G
1096  tct act atc agt caa agg agc tca ttt cat tac agc tga caa aat cgg caa agg tcc tgc
    L L S V K G A H F I T A D K I G K G P A
1156  ctc ttt cta cac tct tga gaa agg gga gtg gat gtg cca tag tgc cac cat aga tgg agc
    S F Y T L E K G E W M C H S A T I D G A
1216  cca tca cca gtt cgt gtc tgt ttt atg caa cac tgg acc cgg ata ttc cgg aac agg gtt
    H H Q F V S V L C N T G P G Y S G T G F
1276  ttg gtc ttc aaa gaa tct gct tgg tgt gct taa agg ctt ccc act gga aga gga gtg taa
    W S S K A N L L G V L K G F P L E E E C N
1336  cta caa tgt tat gtc tgt tat acc ctc gat ccc agg aat cac ttc ccc aaa tta tgt gtt
    Y N V M S V I P S I P G I T S P N Y V F
1396  tga gtc gac cgc cgt aaa agg ccg cgt ctt ctc gga tga agc tgt gaa aga gct aga ggc
    E S T A V K G R V F S D E A V K E L E R
1456  gga agc atc cga agc cgt caa gaa gct tgc cag att taa atc act tac cgg caa gaa ctg
    E A S E A V K K L A R F K S L T G K N W
1516  ggc tga tga tta tga ctc cga tga gga tta cgg tct gga gag aga ggc tgc aac aat tgc
    A D D Y D S D E D Y G L E R E A A T N A
    G L R S G E R G C N K C
1576  gcc cgc aga gaa aac tgc tca aac aaa ctc agc aga gaa gac tgc tcc atc aac ttc agc
    
```

P A E K T A Q T N S A E K T A P S T S A  
 1636 A R R E N C S N K L S R E D C S I N F S A  
 aga gaa aac tgc tcc aac aaa caa gcc ttt aaa tgg gca agc ggc acc gtc cgc caa aac  
 E K T A P T N K P L N G Q A A P S A K T  
 R E N C S N K Q A F K W A S G T V R Q N  
 1696 aaa cgg caa ctc cga cat ccc cga cgt cgc tac aag cgc acc acc aat gga caa aat ggt  
 N G N S D I P D V A T S A P P M D K M V  
 K R Q L R H P R R R Y K R T T N G Q N G  
 1756 cga aca gat cat cac agc tat ggt ggg gag aat caa tct ctc gga gat aga gga gaa gat  
 E Q I I T A M V G R I N L S E I E E K I  
 R T D H H S Y G G E N Q S L G D R G E D  
 1816 agt ggt cag ggt gtc tca gaa agc cct gca gaa gcc caa aca aaa gaa gcg cgg aag gcg  
 V S R V S Q K A L Q K P K Q K K R G R R  
 S E Q G V S E S P A E A Q T K E A R K A  
 1876 tgg agg gaa gaa caa gca aaa cag ttc acc tcc tac ttc aac gca atc tac aag tgg ggc  
 G G K N K Q N S S P P T S T Q S T S G A  
 W R E E Q A K Q F T S Y F N A I Y K W G  
 1936 gcc caa gaa gaa ggc tgt ccc cca gcc ttc agg aag tgc ggg cac atc ccc ggc tac tac  
 P K K K A V P Q A S G S A G T S P A T T  
 A Q E E G C P P G F R K C G H I P G Y Y  
 1996 cac ccc cgc acc aga ggc gaa acc cag tgg ggg caa aaa ctc tgc caa gtt cat ccc gag  
 T P A P E A K P S G G K N S A K F I P S  
 H P R T R G E T Q W G Q K L C Q V H P E  
 2056 ctg gcg gag aaa aca aca gga ttc ggc tgg cca aaa gcc gga tct gaa gct gaa ctc caa  
 W R R K Q Q D S A G Q K P D L K L N S K  
 L A E K T T G F G W P K A G S E A E L Q  
 2116 agc ctg aat cta cag gct gcc agg tgg ctc caa cgc gcg gag tcg gcc act atc cct ggc  
 A \*  
 S L N L Q A A R W L Q R A E S A T I P G  
 2176 gca gaa gca aga aag cgc gtg att gag aaa aca gtg gag gca tac aga aat tgt gta act  
 A E A R K R V I E K T V E A Y R N C V T  
 2236 aac gcc cca ctg tgc tcc ctt aaa tcc aaa ctg gat tgg gct ggc ttt caa gat atc  
 N A P L C S L K S K L D W A G F Q Q D I  
 2296 cgt gaa gca gtc cag tcc ctt gag cta gac gct ggt gta ggc att ccc tat atc gcg tat  
 R E A V Q S L E L D A G V G I P Y I A Y  
 2356 ggc ctc ccc aca cac cga gga tgg gtt gag gac cat aag ctt ctc cca gtg ctc act cag  
 G L P T H R G W V E D H K L L P V L T Q  
 2416 ctg acc ttt gac cga cta cag aag atg tcg gag gcc agc ttt gag gat atg agc gca gaa  
 L T F D R L Q K M S E A S F E D M S A E  
 2476 gag ctg gtt caa gaa ggg ctc tct gat cct atc aga cta ttt gtc aaa gga gag ccc cac  
 E L V Q E G L C D P I R L F V K G E P H  
 2536 aaa cag agc aaa ctc gat gaa ggc cgc tac cgc ctc atc atg tct gtt tcc ttg gtg gat  
 K Q S K L D E G R Y R L I M S V S L V D  
 2596 caa ctg gta gcc cgg gtt ctg ttc caa aat cag aac aaa agg gaa att tcc ctg tgg agg  
 Q L V A R V L F Q N Q N K R E I S L W R  
 2656 tct gtg cct tcc aaa ccc ggt ttt ggc ctt tca act gac act caa act gct gaa ttc ttg  
 S V P S K P G F G L S T D T Q T A E F L  
 2716 gag tgt ctt caa aag gtg tct gga gcg cca tct gtg gaa gaa ttg tgt gca aat cac aag  
 E C L Q K V S G A P S V E E L C A N H K  
 2776 gag tac acg cgc cca acc gac tgt tcc gac tgg tca gtc gcg tat tgg atg ctg  
 E Y T R P T D C S G F D W S V A Y W M L  
 2836 gag gat gat atg gag gtg aga aat cgc ctg aca ttt aat aac acc cag ctc acc aag cgc  
 E D D M E V R N R L T F N N T Q L T K R  
 2896 ctt cgg gcc gcc tgg ttg aag tgc ata gga aat tcc gtc ctg tgc ctg tcc gat ggc act  
 L R A A W L K C I G N S V L C L S D G T  
 2956 tta ctt gcc caa act gtt ccc ggt gtg caa aag agc gga agt tac aat aca agt tcc tcc  
 L L A Q T V P G V Q K S G S Y N T S S S  
 3016 aac tct aga atc cgg gtt atg gct gcc tat cac tgt ggc gcc gac tgg gca atg gcc atg  
 N S R I R V M A A Y H C G A D W A M A M  
 3076 ggg gac gat gct ctc gaa gcc ccc aac tcc gac ctg gag gag tat aaa aca cta ggt ttc  
 G D D A L E A P N S D L E E Y K T L G F  
 3136 aaa gtc gag gta ggt cga gaa ctc gaa ttc tgt tca cac atc ttc aga aat ccg acc ctc  
 K V E V G R E L E F C S H I F R N P T L  
 3196 gcc gtt cgg gtc aac acc aac aaa atg ctt tac aag ttg atc cat ggt tat aat ccg gaa  
 A V P V N T N K M L Y K L I H G Y N P E  
 3256 tgt ggc aat cca gaa gtg att caa aac tat ctg gct gca gta ttc tct gtg ctg cag gaa  
 C G N P E V I Q N Y L A A V F S V L Q E  
 3316 ctc cga cac gat cgt gag ctc gtt gcc aag ctc cac cag tgg ttg gtt cgg agt gcc acc  
 L R H D R E L V A K L H Q W L V P S A T  
 3376 aca aaa gaa cac tga agg agc tca cta aaa cta gcc aag cat acg cga gtt gca agc att  
 T K E H \*  
 3436 gga agt tca agt ctc gtt aca tca acc gga caa aat aga tta taa att ttt agc ggg att  
 3496 tgc ttt agg att ctc atc cgc aat ccc att ttc agt agc cgg ttt ata ttt tgt tta cct  
 3556 aaa gat ttc ctc cca cgt gcg atc aat tgt taa tga gta cgg tcg tgg tta aag gaa atg  
 M S T V V V K G N V  
 3616 tca atg gtg gtg tac aac aac caa gaa ggc gaa gaa ggc aat ccc ttc gca ggc gcg cta  
 N G V V Q Q P R R R R Q S L R R R A N  
 S M V V Y N N Q E G E E G N P F A G A L  
 3676 aca gag ttc agc cgg tgg tta tgg tca cgg ccc ctg ggc aac cca ggc gtc gaa gac gca  
 R V Q P V V M V T A P G Q P R R R R R R  
 T E F S R W L W S R P L G N P G V E D A

3736 gaa gag gag gca atc gcc gct caa gaa gaa ctg gag ttc ccc gag gac gag gct caa gcg  
R G G N R R S R R T G V P R G R G S S E  
E E E A I A A Q E E L E F P E D E A Q A  
3796 aga cat tcg tgt tta caa agg aca acc tca tgg gca act ccc aag gaa gtt tca cct tcg  
T F V F T K D N L M G N S Q G S F T F G  
R H S C L Q R T T S W A T P K E V S P S  
3856 ggc cga gtc tat cag act gtc cgg cat tca agg atg gaa tac tca agg cct acc atg agt  
P S L S D C P A F K D G I L K A Y H E Y  
G R V Y Q T V R H S R M E Y S R P T M S  
3916 ata aga tca caa gca tct tac ttc agt tcg tca gcg agg cct ctt cca cct cct ccg gtt  
K I T S I L L Q F V S E A S S T S S G S  
I R S Q A S Y F S S S A R P L P P P V  
3976 cca tcg ctt atg agt tgg acc ccc att gca aag tat cat ccc tcc agt cct acg tca aca  
I A Y E L D P H C K V S S L Q S Y V N K  
P S L M S W T P I A K Y H P S S P T S T  
4036 agt tcc aaa tta cga agg gcg gcg cca aaa ctt atc aag cgc gga tga taa atg ggg tag  
F S Q I T K G G A K T Y Q A R M I N G V E  
S S K L R R A A P K L I K R G \*  
4096 aat ggc acg att ctt ctg agg atc agt gcc gga tac tgt gga aag gaa atg gaa aat ctt  
W H D G S S E D Q C R I L W K G N G K S S  
4156 cag ata cgc cag gat cct tca gag tca cca tca ggg tgg ctt tgc aaa acc cca aat agg  
D T A G S F R V T I R V A L Q N P K X V  
4216 tag act ccg gat cag agc ctg gtc caa gcc cac aac caa cac cca ctc caa ctc ccg acc  
D S G S E P G P S P Q P T P T P T P Q K  
4276 agc acg agc gat tta ttg ctt acg ttg gca tac cta tgc taa cca ttc agg cca ggg aga  
H E R F I A Y V G I P M L T I Q A R E N  
4336 acg acg acc aga tca tat tgg gtt ctt tag gga gcc aaa gga tga aat ata tag agg acg  
D D Q I I L G S L G S Q R M K Y I E D E  
4396 aga acc aga act aca caa atg tta gtt ctg agt att act ctc aat cga gca tgc aag ccg  
N Q A N Y T N V S S E Y Y S Q S S M Q A V  
4456 tcc cta tgt att act tta atg tcc cga aag gcc aat ggt cag tcg aca tca gct gcg aag  
P M Y Y F N V P K G Q W S V D I S C E G  
4516 ggt atc aac cca cta gca gca cct ccg atc caa acc ggg gta gga gtg acg ggg tga tcg  
Y Q P T S S T S D P N R G R S D G V I A  
4576 cgt att caa acg ccg act ccg att att gga atg ttg gtg aag ccg atg gtg tca aaa ttt  
Y S N A D S D Y W N V G E A D G V K I S  
4636 cga agc tac gca acg ata aca cct acc gcc aag gtc acc cag aac ttg aaa tta act cgt  
K L R N D N T Y R Q G H P E L E I N S C  
4696 gtc att ttc gcg agg gcc aac tcc ttg aac ggg acg cta caa tta gct tcc acg ttg aag  
H F R E G Q L L E R D A T I S F H V E A  
4756 cgc cta ctg atg ggc gat tct ttc tcg ttg gtc ccg cta tcc aga aaa ccg caa agt ata  
P T D G R F F L V G P A I Q K T A K Y N  
4816 act ata cta tct cat acg gtg act gga ccg acc gag aca tgg aac tgg ggc tga tca ccg  
Y T I S Y G D W T D R D M E L G L I T V  
4876 tgg tgc ttg atg aac att tag aag gca ctg gtt ccg cta aca gag tgc ggc ggc ccc cac  
V L D E H L E G T G S A N R V R R P P R  
4936 ggg agg gcc aca cct ata tgg cct cgc cgc gcg aac ccg aag gaa aac ccg ttg gaa ata  
E G H T Y M A S P R E P E G K P V G N K  
4996 aac caa ggg acg aaa ccc cga tac aaa cgc agg aaa gac aac ctg atc aaa ctc cgt ctg  
P R D E T P I Q T Q E R Q P D Q T P S D  
5056 acg acg tat ccg atg ctg gtt ccg taa aca aca gcg gct caa ctg agt cgc tgc aat tgg  
D V S D A G S V N N S G S T E S L Q L E  
5116 agt tcg ggg taa act cag ata gta cct acg atg cta cag tcg atg gta cag act gcc cca  
F G V C N S D S T Y D A T V D G T D W P R  
5176 gaa ttc ctc cac caa ggc acc cac ctg aac cta gag ttt ccg gca att caa gaa ctg tta  
I P P P R H P P E P R V S G N S R T V I  
5236 ttg act ttt ctc cga aag ccg atc tat tgg aga att ggg atg ccg aac act tcg acc ctg  
D F S P K A D L L E N W D A E H F D P G  
5296 gtt att cca aag atg tcg ctg ctg cta cta tta tag cgc acg gca gta ttc aag atg  
Y S K E D V A A A T I I A H G S I Q D G  
5356 ggc gaa gta tgt tgg aga aga gag agg aaa gtg tca aga aca cct cct cct gga agc  
R S M L E K R E E S V K N K T S S W K P  
5416 ccc cgt tat cta aag ccg tga gcc cag cca tag cca aat tgc gct cga ttc gca aat ccc  
P L S K A V S P A I A K L R S I R K S Q  
5476 aac ccc tcg agg gag gga ccc tta aga aag acg cta ctg atg gtg tct cat cta ttg gca  
P L E G G T L K K D A T D G V S S I G S  
5536 gtg gtt ctc taa cag gtg gca cgc tta aga gga agg taa cta ttg aag agc gtt tac tgc  
G S L T G G T L K R K V T I E E R L L Q  
5596 aga cct taa caa ctg aac aaa ggc tgt ggt acg aga att tga aga aaa cta acc ctc cag  
T L T T E Q R L W Y E N L K K T N P A  
5656 ctg cta tcc aat ggc tgt atg aat atc agc cac ctc ccc aag tag ata gaa aca tag ctg  
A I Q W L Y E Y Q P P P Q V D R N I A E  
5716 aaa agc cat tcc aag gga gga aat gag tcg act cac gac tta aaa ctg agt gtc cgc ccg  
K P F Q G R K \*  
5776 aca tta agc gga acg aaa gcc gaa agg tga tta ggc tct caa cgc ctg cta gag acc gtc  
5836 gaa aga cgc gac tgt gta gcc aag atc ctc tta cat ggt tgt gta gtt a 5884

Percentage of different bases are (23% U, 25% C, 28% A and 24% G). These results are almost similar those of BWYV RNA and BYDV RNA (22% U, 24% C, 29.5%

A and 24.5% G). The Egyptian strain was compared with six PLRV sequences and the overall similarities for all genomic sequences were 100%, 98.5%, 98.4%, 98.3%, 98% and 93.5% for polish, French, Wageningen, UK, Canada, and Australian strains, respectively as shown in {Table 1 and Fig 2}. Five substantial open reading frames (ORFs) are present in PLRV RNA (Fig 2). ORF1 started from nucleotide 70 till nucleotide 813 (AA1-247), ORF2 from 203 to 2122 (AA1-639), ORF3 from 1540 to 3390 (AA 1-617), ORF 4 from 3588 to 5741 (AA1-718), while ORF5 started at 3613 and ended at 4083 (AA1-156). The overall similarities between Egyptian strain and other strains in the intergenic regions are shown in {Table 2 and Fig 3}.

Table 1. Overall percent identity of PLRV- N with different PLRV isolates

Strain	Similarity %
Egypt X Australia	93.5
Egypt X UK	98.3
Egypt X Polish	100
Egypt X French	98.5
Egypt X Canada	98
Egypt X Wageningen	98.4

Fig. 2. Phylogenetic tree determines dimensions of relationship between Egyptian PLRV and different PLRV strains

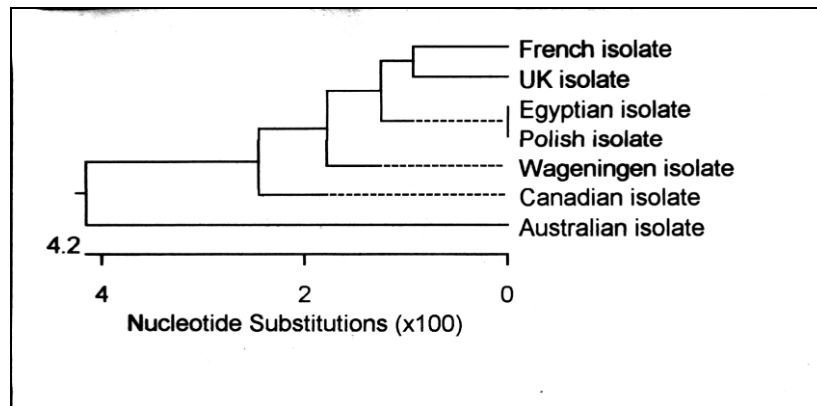


Table 2. Overall percent identity of PLRV- Egypt with different PLRV isolates in intergenic regions

Strain	Similarity %
Egypt X Australia	90.5
Egypt X UK	97.4
Egypt X Polish	100
Egypt X French	97
Egypt X Canada	97.5
Egypt X Wageningen	97.5

Fig. 3. Phylogenetic tree determines dimensions of relationship between PLRV and different PLRV isolates in intergenic regions

		Percent Identity								
		1	2	3	4	5	6	7		
Divergence	1	█	97.0	97.5	97.5	97.5	26.9	36.5	1	Wageningen isolate
	2	3.1	█	97.5	96.5	97.5	26.9	36.5	2	Canadian isolate
	3	2.5	2.6	█	97.0	100.0	26.9	35.5	3	Egyptian isolate
	4	2.6	3.6	3.1	█	97.0	26.4	36.5	4	French isolate
	5	2.5	2.6	0.0	3.1	█	26.9	35.5	5	Polish isolate
	6	278.3	275.5	278.3	301.1	278.3	█	25.4	6	UK isolate
	7	140.6	140.8	147.6	140.8	147.6	350.0	█	7	Australian isolate
		1	2	3	4	5	6	7		

Fig. 4A. Similarities between Luteoviruses RNA sequences in the intergenic regions, the sequence are following nucleotides 3368 (BWYV), 3473 (PLRV-EG) and 2745 (BYDV). Numbers in brackets are the length of intervening sequences in nucleotides, \* indicates a match with PLRV-RNA sequences, - indicates that a gap has been inserted to enhance the degree of matching.

```

BWYV- GAUUAC-AAAUUCCUAGC-AGGCUUCG. (40) .UAUCUAUUCUACUACC-U-AAGA (28). AUG
      *****  *****  ****  * * * * *      * * * * *
PLRV- GAUUAU-AAAUUUUUAGC-GGGAUUUG. (40) .UUUAGGAUUCTCATCC-U-AAGA (27). AUG
      ***  ****  *****  * * * * *      **  ** * ****
BYDV- UUACCAAU-CUUAGCUGGG-UUG.. (44) ....UACUUUAUUUACAAUAAGU (26). AUG
    
```

Fig. 4B. Sequence repeat in PLRV-RNA, the sequence start at 1577 (topleft) and ended at 1657 (bottom right). \* indicated the same nucleotide in all three repeats. ! Indicates the same nucleotide in two of the three repeats

```

[*!*****!*****!*!***!!
CCGCAGAGAAAACUGCUCAACAAAC
UCAGCAGAGAAGACUGCUCCAUAACU
UCAGCAGAGAAAACUGCUCCAACAAAC
    
```

### DISCUSSION

Number of different sources reported a very close values to our strain sequence as mentioned by Keese *et al* (1990) in Australia, Van der wilk *et al.*, (1989) who determined the full genomic as 5882 nucleotide, while it was reported as 5885 nucleotides by Guyader and Durcay (2002). On the other hand, Mayo *et al* (1989)

mentioned that 5987 nucleotide is the full genomic sequence of PLRV UK strain. All the sequences of mentioned strains are similar to the value of 6 kb estimated by Rowhani and Stace-Smith (1979) and 6.1 kb obtained by restriction mapping of cDNA. The putative 3' terminal sequence is coterminal with that obtained by Prill *et al* (1988) and differs from that determined by Mayo *et al* (1989) strain in that our strain has C at position 5820 instead of U at position 5871 instead of G at position 5976.

Comparing our results with those of Mayo *et al* (1989), similar base ratios were obtained (23% U, 25% C, 28% A and 24% G), of PLRV RNA Egypt, these results are similar to those of BWYV RNA and BYDV RNA (22% U, 24% C, 29.5% A and 24.5% G).

The sequence of PLRV Egyptian strain was compared the sequence of six different PLRV strains and the overall similarities for all genomic sequence are 100%, 98.5%, 98.4%, 98.3%, 98% and 93.5% for polish, French, Wageningen (Dutch), UK, Canada, and Australia strains respectively. These results are in harmony with those mentioned by Keese *et al* (1990) who compared the nucleotides sequences of an Australian and Canadian strains of PLRV *Luteoviruses*.

Results showed that five substantial open reading frames (ORFs) are present in PLRV RNA Egyptian strain. ORF1 started from nucleotide 70 till nucleotide 813 (AA1-247), ORF2 from 203 to 2122 (AA1-639), ORF3 from 1540 to 3390 (AA 1-617), ORF 4 from 3588 to 5741 (AA1-718), while ORF5 started at 3613 and ended at 4083 (AA1-156). In our sequence during cloning ORF4 it doesn't split into two ORFs as in case of those stains of (Guyader and Ducary, 2002 and Mayo *et al* 1989).

In other sequences and in our ORF4 (3588-4214) AA 1-208 and ORF 6 (4215-5741) AA (1-508), suggesting a conflict with the conceptual translation at amino acid 204. There are three non-coding regions in PLRV RNA. These are the 5' terminal (70 nucleotides), the 3' terminal (143 nucleotides) and 200 nucleotides between ORF3 and ORF4&5. The intergenic regions present in our strain are similar to those reported by (Van der wilk *et al* 1989, and Guyader and Ducray, 2002).

There are also some similarities in the intergenic regions of PLRV RNA, and BWYV RNA and BYDV RNA (Veidt *et al* 1988). These similarities suggested that these stretches may be functionally significant. The right-hand stretch contain repeated UnA sequences followed by AAGA, two features suggested by Marsh *et al* (1988) to play a role in the formation of subgenomic RNA. The abundant subgenomic fragments of PLRV RNA lies between nucleotides 4085 and 4795 or 4695 and 5196 approximately, so it may be subgenomic mRNA for ORF4 sequence and sequence reported by Mayo *et al* (1989).

Ribosomal framshfiting can be used by different organisms to produce some kinds of protein form overlapping readingframes. This can be done in both directions. A shift in 3' direction (+1) framshfit as mentioned by Bbelcourt and Farabaugh (1990). The



other shift in 5' direction (-1 framshift) has proven for Luteoviruses (Prufer *et al.*, 1992, Garcie *et al.*, 1993). In case of PLRV, the RNA dependent RNA polymerase is expressed by -1 ribosomal framshifting in the region of overlapping between ORF2 and 2b as reported by Mayo *et al.*(1989).

The signal responsible for efficient framshfitng in PLRV is composed of the slippery sequence uuuAAAU followed by a sequence that has the potential to adopt two alternative folding pattern, either a pseudoknot, or simple tem-loop structure as mentioned by Kujawa *et al.*(1993) who confirmed that in PLRV-P, the -1 framshift in the overlap region depends on the slippery site and on the downstream positioned sequence. A proposed pseudoknot is needed for efficient framshfitng in the present study, the slippery sequences TTAAAT (uuuAAAU) is located on position 1662 nt in the overlapping region between ORF2 and ORF3. This result is in agreement with those reported for BWYV by Garcie *et al.* (1993). In retrospect, could be concluded that the identified PLRV Egypt is 100% genetic identity with PLRV Polish.

## REFERENCES

1. Beck, S. 1993. DNA Sequencing by Chemiluminescent Detection. In H. and A. Griffin (Ed.). Methods in Molecular Biology Vol. 23: DNA Sequencing Protocols. pp. 235-242. Humana Press Inc., Totowa, NJ.
2. Belcourt, M. F., and P. J. Farabaugh. 1990. Ribosomal frameshifting in the yeast retrotransposon TY. tRNA induce slippage on a 7 nucleotides minimal site. Cell b2: 339-352
3. Garcie, A., Duin, J. and Van Pleij, and C. W. A. 1993. Differential response to frameshift signal in Eukaryotic and Prokaryotic translational systems. Nucl. Acids Res. 21:401-406
4. Gamal Eldin, A. S., A. A. Sallam., H. M. Abdelmaksoud, and E. K. Fahimy. 2004. Complete Nucleotide Sequence of Potato leaf roll virus infects Potato in Egypt. Abst. (83) World Potato Congress, Kunming - China
5. Guyader, S. and D. G. Ducray. 2002. Sequence analysis of potato leaf roll virus isolates reveals genetic stability, major overlapping events and differential selection pressure between overlapping reading frame products. J. Gen. Virol. 83 (pt 7): 1799-1807
6. Keese, P., R. R. Martin, L. M. Kawchuk, P. M. Waterhouse and W. L. Gerleach. 1990. Nucleotide sequence of an Australian and Canadian isolate of potato leaf roll Luteovirus and their relationship with two European isolates. J. Gen. Virol. 71 (pt 3): 719-724
7. Kujaw, A. B., G. Drugeon, D. Hulanicke and A. L. Haenni. 1993. Structural requirments for efficient translational frameshifitng in the synthesis of the

- putative viral RNA dependent RNA polymerase of potato leaf roll virus. *Nucl. Acids Res.* 21(a): 2185-2171
8. Marsh, L. S., T. W. Dreher and T. C. Hall. 1988. Mutational analysis of the *coat* and modulator sequence of the BMV RNA3 subgenomic promoter. *Nucl. Acids Res.* 1: 1385-1397
  9. Palucha, A., E. Sadowy, A. Kujawa, M. Juszczuk, W. Zagorski, and D. Hulanicka. 1994. Nucleotide sequence of RNA of polish isolate of potato leaf roll Luteovirus. *Acta Biochim. Pol.* 41 (4): 405-414
  10. Pruffer, D., E. Tacke, J. Schmitz, B. Kull, A. Kaufmann, and W. Rodhe. 1992. Ribosomal frameshifting in plants: a novel element directs the -1 frameshift in the synthesis of the putative viral replicase of potato leaf roll Luteovirus. *EMBO J.* 11 (3): 1111-1117
  11. Sangar, F., S. Nicklen and A.R. Coulson. 1977. DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA* 74, 5463-5466
  12. Sambrook, J., E. F. Fritsch and T. Maniatis. 1989. *Molecular cloning: A laboratory manual.* Cold Spring Harbor Laboratory press, cold Spring Harbor. New York.
  13. Rowhani, A., and R. Stace-Smith. 1979. Purification and Characterization of Potato leaf roll virus. *Virology* 98: 45-54
  14. Waterhouse, P. M., F. E. Gildow and G. R. Johnstone. 1988. Luteovirus group. *AAB Descriptions of Plant Viruses* No. 339

## التعريف الجزيئي والتحليل الجيني السلالي لفيروس التفاف اوراق البطاطس في مصر

هشام محمد عبد المقصود، ايمن محمد مندور، احمد شوقي جمال الدين

قسم بحوث الفيروس - معهد بحوث امراض النباتات - مركز البحوث الزراعية - الجيزة

تم عزل فيروس التفاف الاوراق في البطاطس من حقول البطاطس المصرية وتم تعريفه على اساس المدى العائلي، الاعراض الظاهرية، الانتقال الحشري، الميكروسكوب الإلكتروني، تفاعل البى سى ار، و البى سى ار اليزا. تم اجراء التحليل النيكلوتيدى الكامل للفيروس عن طريق مكملات الحمض النووى دى ان اية وتم وضعة وحفظة في بنك الجينات الدولي تحت رقم تسلسلي AY138970. اظهرت نتائج التحليل النيكلوتيدى ان الفيروس يحتوي على 5884 قاعدة نيكلوتيدية تترجم 5 مناطق مفتوحة للبروتين ما عدا منطقة مميزة تعارضيه للحمض الأميني رقم 209. تم اجراء التحليل النيكلوتيدى المقارنى بالسلالات الدولية الاخرى من فيروس التفاف اوراق البطاطس والمحفوظة لدى بنك الجينات الدولي واظهرت هذه التحليلات تتطابق عام بين كاف السلالات بنسبة 97.02% وطابق خاص بكل سلالة دولية كتالي 100% (السلالة البولندية)، 98.5% (السلالة الفرنسية)، 98.4% (السلالة الهولندية)، 98.3% (السلالة الكندية)، واخيرا 93.5% (السلالة الأسترالية).