Acta Biochimica Polonica

Vol. 41 No. 4/1994

QUARTERLY

Nucleotide sequence of RNA of a Polish isolate of potato leafroll luteovirus

Andrzej Pałucha, Ewa Sadowy, Alicja Kujawa, Marek Juszczuk, Włodzimierz Zagórski and Danuta Hulanicka

Institute of Biochemistry and Biophysics, Polish Academy of Sciences,
A. Pawińskiego 5a, 02–106 Warsaw, Poland

Received: 7 April, 1994

Key words:nucleotide sequence, potato leafroll luteovirus

The nucleotide sequence of the genomic RNA of a Polish isolate of a potato leafroll virus was determined. Some variations between the determined sequences were observed. A comparison of the frequency of sequence variants in particular regions of the genome is presented. The nucleotide sequence reported will appear in the EMBL Data Library under the accession number X74789.

Potato leafroll virus (PLRV), a member of the luteovirus group [1] is transmitted in a persistent manner by aphids [2], and is responsible for significant world-wide economic losses in potato crops [3]. The small isometric particles of PLRV are 25 nm in diameter and encapsidate a single stranded positive-sense RNA (M_r about 2×10^6) with a 5′-linked protein (VPg) and no significant stretches of 3′poly(A) sequence [4].

A significant burst of the infections which occurred in 1989 stimulated us to undertake studies on a Polish PLRV isolate. Several phenotypically different PLRV strains were detected by Polish phytopathologists. Classification of these strains has been presented by Syller [5].

As concerns luteoviruses, the nucleotide sequence of two isolates of barley yellow dwarf virus [6, 7], beet western yellows virus [8] and two European isolates of PLRV (Scottish — PLRV-S and Dutch — PLRV-D) [9, 10] have been published. The nucleotide sequence of one Australian and one Canadian isolates of PLRV (PLRV-A and PLRV-C, respectively) and their relationship to two European isolates have been also reported [11].

It is assumed that replication of RNA genomes results often in a spectrum of highly homologous sequences representing molecular variants of consensus genome. Such variants are called quasi-species [12]. Quasi species are subject to natural selection [13]. Selective pressure on PLRV genome in various geographical regions may differ owing to climate conditions and/or cultivar of host plants. Therefore, one can expect the predominance of specific sequence fragments in various geographic regions.

Particularities of Polish agriculture, where local cultivars of seed potato predominate as well as unexpected burst of infection in late eighties, incited us to sequence local isolate of PLRV.

This paper presents the genome structure and complete nucleotide sequence of a Polish isolate of PLRV (PLRV-P).

MATERIALS AND METHODS

Virus propagation and purification. The PLRV-P used was derived from a field isolate (Collection, Potato Research Institute, Młochów, Poland). The isolate was maintained in potato tubers and virus particles were extracted from infected potato shoots and leaves. PLRV was purified according to Tamada [14], with the modification using of pectonase and cellulase enzymes instead of drisalase.

RNA was isolated by proteinase K treatment, followed by phenol extraction. Synthesis of cDNA and cloning of double stranded cDNA were performed as described by [15].

Clones were tested by colony blotting and hybridised with ^{32}P-labelled cDNA to PLRV RNA [16].

Overlapping restriction fragments from cDNA clones were sequenced using the "dideoxy chain termination" method [17].

The 5′-end of PLRV genome was sequenced using RNA as a template [18] and AMV reverse transcriptase and two primers located 132–155 and 361–380 nucleotides from the 5′-end of the sequence (Fig. 1)

To obtain the cDNA clone representing the 3′-terminal sequence of the genomic RNA an oligonucleotide primer 20 nucleotides in length, complementary to the extreme 3′-terminus of the Scottish (PLRV-S) and the Netherlands (PLRV-D) isolates, was used to prime reverse transcription. Additionally confirmation of the nucleotide sequence of the 3′-terminus was obtained by sequencing of two independent clones constructed in random priming experiments.

Sequence analysis and alignment were done with DNASIS program.

RESULTS

Ninty eight per cent of genome sequence is based on sequencing both cDNA strands, 95% represents results from sequencing several (2–4) independent, overlapping clones.

The established length of the PLRV-P genome is 5882 nucleotides. The same length has been found for the Netherlands isolate [10] and PLRV-A [11]. The genome organisation of PLRV-P is the same as that of the other isolates [9 –11].

Sequence variants

During sequencing overlapping clones, we detected in minor sub-population of viral cDNA's, point mutations representing the variants of consensus sequence.

These mutations were localised in strategically neutral regions of the genome. None of these mutations resulted either in premature termination of viral ORF's or in creation or destruction of cysteine codons therefore preserving the overall architecture of disulfite bonds in viral proteins. None created hypothetical proteolytical cleavage sites or glycosylation sites. Finally, mutations were not creating or destroying serine or tyrosine codons, therefore not affecting hypothetical phosphorylation sites of viral peptide.

To rule out possibility of technical ambiguities as a variant we accepted the nucleotide change occurring in more than one independent clone.

Similar results were reported for the PLRV-S isolate [9] and for other RNA viruses. The recent studies of Holland *et al.* [12] showed that most of the RNA viruses form complex "quasispecies" populations which can evolve very rapidly.

We found that variants arose due to different types of mutations (Table 1). The most frequent are C⇔U and A⇔G substitutions. These mutations represent transitions — errors which occur during replication more frequently than transversions.

It was of interest to compare the frequency of sequence variants in particular regions of the viral genome (Table 2). The changes in noncoding region at the 5′-end, and noncoding intercistronic region occurred slightly more frequently than in the other regions of viral genome. No change occurred in the noncoding

Table 1

Types of nucleotide changes (observed) among clones of PLRV-P

Type	Number of variants	Percent of total variants
A ⇔ C	1	3.6
A ⇔ G	9	32.2
A ⇔ U	2	7.2
C ⇔ G	2	7.2
C ⇔ U	14	50.0
G ⇔ U	0	0.0

Fig. 1. The nucleotide sequence of PLRV-P RNA.
The nucleotide changes are indicated above the continous sequence. The amino-acid sequence of the six ORFs are shown below the nucleotide sequence.

```
        10   A    20        30        40        50        60
CAAAAGAAUACCAGGGGAAAUUGCAGCUUUAGCGCAUAAACUCUACACUCAUUGCAAACG
        70        80        90       100       110       120
UUAUAGCAUAUGAUUGUAUUGACCCAGUCUGGAACCUUGCUUUUUGACCAAAGAUUUAAA
          M  I  V  L  T  Q  S  G  T  L  L  F  D  Q  R  F  K

       130       140       150       160       170       180
CUCUCAAAGUUUCUCUUCGUUGUCAUUGCAACAGGCUUUCCUCUUCUCCUGCAGCAAGCG
 L  S  K  F  L  F  V  V  I  A  T  G  F  P  L  L  L  Q  Q  A

       190       C         210       220       230       240
AGCUUAAUUUACGGCUAUAAUCAUGAACAGAUUUACCGCAUAUGCCGCUCUUUUCUUUAU
 S  L  I  Y  G  Y  N  H  E  Q  I  Y  R  I  C  R  S  F  L  Y
                       M  N  R  F  T  A  Y  A  A  L  F  F  M

       250       260       270        U        290       300
GUUCUCCCUUUGCUCAACUGCAAAAGAGGCAGGAUUUCAACAUCCGGCCUUCAACUUCCG
 V  L  P  L  L  N  C  K  R  G  R  I  S  T  S  G  L  Q  L  P
  F  S  L  C  S  T  A  K  E  A  G  F  Q  H  P  A  F  N  F  R

       310       320      A      C   340       350       360
AGGCACCUCCACUAUGAGUGCCUUGAGUGGGGAUUACUCUGCGGCACCCACCCCGCUAUA
 R  H  L  H  Y  E  C  L  E  W  G  L  L  C  G  T  H  P  A  I
  G  T  S  T  M  S  A  L  S  G  D  Y  S  A  A  P  T  P  L  Y

       370       380       390       400        C  A  A  420
CAAAUCGUGGGCCCUACCAUCGUCAUUAAACUUGACGACCCAACCACUGCCGCCGCUUAC
 Q  I  V  G  P  T  I  V  I  K  L  D  D  P  T  T  A  A  A  Y
  K  S  W  A  L  P  S  S  L  N  L  T  T  Q  P  L  P  P  L  T

       430       440    C  450       460       470       480
AGAUCGGAGCUACUACGAGUUAGUUCAAGCUCUUAUAUCCAAAAUGCGGCUGGAUUGUCA
 R  S  E  L  L  R  V  S  S  S  S  Y  I  Q  N  A  A  G  L  S
  D  R  S  Y  Y  E  L  V  Q  A  L  I  S  K  M  R  L  D  C  Q

       490       500       510       520       530       540
AACGGUUGGGGACAUGACAUGGAGGCAUUUGUCAGAAAUGCUAUUUGCCUCCUGGAACUC
 N  G  W  G  H  D  M  E  A  F  V  R  N  A  I  C  L  L  E  L
  T  V  G  D  M  T  W  R  H  L  S  E  M  L  F  A  S  W  N  S

       550       560       570       580       590       600
CGUGAAAGAAGUAUCCCUCAAAGCGGCCUCCGUGACCUUAUGGGCAAUUAUCAACAUUUG
 R  E  R  S  I  P  Q  S  G  L  R  D  L  M  G  N  Y  Q  H  L
  V  K  E  V  S  L  K  A  A  S  V  T  L  W  A  I  I  N  I  W

       610       620       630       640       650       660
GUUCGGUCUCUAUUGGACGCUUGCAAGGUUGAUCACUUUGUUCCUCUGGACUUUCAGCAU
 V  R  S  L  L  D  A  C  K  V  D  H  F  V  P  L  D  F  Q  H
  F  G  L  Y  W  T  L  A  R  L  I  T  L  F  L  W  T  F  S  I

       670       680       690       700       710       720
AGAAGCCUUAUGCUUAAUUUUGCUCGGUUGUAUAACCAGCUUGAUCUACAAGGGCGCGCU
 R  S  L  M  L  N  F  A  R  L  Y  N  Q  L  D  L  Q  G  R  A
  E  A  L  C  L  I  L  L  G  C  I  T  S  L  I  Y  K  G  A  L

       730       740       750       760       770       780
AAGUCUUUCAGAGCACUUACCGGUUUUCCUGUUUAUGUCCCCUCUGAAGAUUAUUUGGAG
 K  S  F  R  A  L  T  G  F  P  V  Y  V  P  S  E  D  Y  L  E
  S  L  S  E  H  L  P  V  F  L  F  M  S  P  L  K  I  I  W  R

       790       800       810       820       830       840
GGCAGCUUUCUCCAAAAGGAAUUACAAGAAUGAGAGGGCUGUGGAAGGAUACAAAGGGUU
 G  S  F  L  Q  K  E  L  Q  E  -
  A  A  F  S  K  R  N  Y  K  N  E  R  A  V  E  G  Y  K  G  F
```

Fig. 1. continued

```
       850       860       870       880       890       900
UUCGGUCCCACAGAAACCGCCAAAGUCUGCCGUAAUUGAACUACAACAUGAAAACGGCAG
  S  V  P  Q  K  P  P  K  S  A  V  I  E  L  Q  H  E  N  G  S

       910       920       930       940       950       960
CCAUCUCGGGUACGCGAACUGCAUUCGCUUGUACAGUGGAGAGAACGCCUUGGUGACAGC
  H  L  G  Y  A  N  C  I  R  L  Y  S  G  E  N  A  L  V  T  A

       970       980       990      1000      1010      1020
UGAACACUGUCUAGAAGGCGCCUUCGCAACGUCGUUGAAAACUGGAAACAGGAUUCCGAU
  E  H  C  L  E  G  A  F  A  T  S  L  K  T  G  N  R  I  P  M

      1030      1040      1050      1060      1070      1080
GUCGACUUUCUUUCCCAUUUUCAAAAGUGCCCGUAAUGAUAUCUCCAUACUAGUAGGUCC
  S  T  F  F  P  I  F  K  S  A  R  N  D  I  S  I  L  V  G  P

      1090      1100      1110      1120      1130      1140
ACCCAACUGGGAAGGUCUACUAUCAGUCAAAGGAGCUCAUUUCAUUACAGCUGACAAAAU
  P  N  W  E  G  L  L  S  V  K  G  A  H  F  I  T  A  D  K  I

      1150      1160      1170      1180      1190      1200
CGGCAAAGGUCCUGCCUCUUUCUACACUCUUGAGAAAGGGGAGUGGAUGUGCCAUAGUGC
  G  K  G  P  A  S  F  Y  T  L  E  K  G  E  W  M  C  H  S  A

      1210      1220      1230      1240      1250      1260
CACCAUAGAUGGAGCCCAUCACCAGUUCGUGUCUGUUUUAUGCAACACUGGACCCGGAUA
  T  I  D  G  A  H  H  Q  F  V  S  V  L  C  N  T  G  P  G  Y

      1270      1280      1290      1300      1310      1320
UUCCGGAACAGGGUUUUGGUCUUCAAAGAAUCUGCUUGGUGUGCUUAAAGGCUUCCCACU
  S  G  T  G  F  W  S  S  K  N  L  L  G  V  L  K  G  F  P  L

      1330      1340      1350      1360      1370      1380
GGAAGAGGAGUGUAACUACAAUGUUAUGUCUGUUAUACCCUCGAUCCCAGGAAUCACUUC
  E  E  E  C  N  Y  N  V  M  S  V  I  P  S  I  P  G  I  T  S

      1390      1400      1410      1420      1430      1440
CCCAAAUUAUGUGUUUGAGUCGACCGCCGUAAAAGGCCGCGUCUUCUCGGAUGAAGCUGU
  P  N  Y  V  F  E  S  T  A  V  K  G  R  V  F  S  D  E  A  V

      1450      1460      1470      1480      1490      1500
GAAAGAGCUAGAGCGGGAAGCAUCCGAAGCCGUCAAGAAGCUUGCCAGAUUUAAAUCACU
  K  E  L  E  R  E  A  S  E  A  V  K  K  L  A  R  F  K  S  L

      1510      1520      1530      1540      1550      1560
UACCGGCAAGAACUGGGCUGAUGAUUAUGACUCCGAUGAGGAUUACGGUCUGGAGAGAGA
                                        G  L  R  S  G  E  R
  T  G  K  N  W  A  D  D  Y  D  S  D  E  D  Y  G  L  E  R  E

      1570      1580      1590      1600      1610      1620
GGCUGCAACAAAUGCGCCCGCAGAGAAAACUGCUCAAACAAACUCAGCAGAGAAGACUGC
 G  C  N  K  C  A  R  R  E  N  C  S  N  K  L  S  R  E  D  C
  A  A  T  N  A  P  A  E  K  T  A  Q  T  N  S  A  E  K  T  A

      1630      1640      1650      1660      1670      1680
UCCAUCAACUUCAGCAGAGAAAACUGCUCCAACAAACAAGCCUUUAAAUGGGCAAGCGGC
 S  I  N  F  S  R  E  N  C  S  N  K  Q  A  F  K  W  A  S  G
  P  S  T  S  A  E  K  T  A  P  T  N  K  P  L  N  G  Q  A  A

      1690      1700      1710      1720      1730      1740
ACCGUCCGCCAAAACAAACGGCAACUCCGACAUCCCCGACGUCGCUACAAGCGCACCACC
 T  V  R  Q  N  K  R  Q  L  R  H  P  R  R  R  Y  K  R  T  T
  P  S  A  K  T  N  G  N  S  D  I  P  D  V  A  T  S  A  P  P

      1750      1760      1770      1780      1790      1800
AAUGGACAAAAUGGUCGAACAGAUCAUCACAGCUAUGGUGGGGAGAAUCAAUCUCUCGGA
 N  G  Q  N  G  R  T  D  H  H  S  Y  G  G  E  N  Q  S  L  G
  M  D  K  M  V  E  Q  I  I  T  A  M  V  G  R  I  N  L  S  E
```

Fig. 1. continued

```
      1810      1820      1830      1840      1850      1860
GAUAGAGGAGAAGAUAGUGAGCAGGGUGUCUCAGAAAGCCCUGCAGAAGCCCAAACAAAA
 D  R  G  E  D  S  E  Q  G  V  S  E  S  P  A  E  A  Q  T  K
  I  E  E  K  I  V  S  R  V  S  Q  K  A  L  Q  K  P  K  Q  K

      1870      1880      1890      1900      1910      1920
GAAGCGCGGAAGGCGUGGAGGGAAGAACAAGCAAAACAGUUCACCUCCUACUUCAACGCA
 E  A  R  K  A  W  R  E  E  Q  A  K  Q  F  T  S  Y  F  N  A
  K  R  G  R  R  G  G  K  N  K  Q  N  S  S  P  P  T  S  T  Q

      1930      1940      1950      1960      1970      1980
AUCUACAAGUGGGGCGCCCAAGAAGAAGGCUGUCCCCCAGGCUUCAGGAAGUGCGGGCAC
 I  Y  K  W  G  A  Q  E  E  G  C  P  P  G  F  R  K  C  G  H
  S  T  S  G  A  P  K  K  K  A  V  P  Q  A  S  G  S  A  G  T

      1990 U    2000      2010      2020      2030      2040
AUCCCCGGCUACUACCACCCCCGCACCAGAGGCGAAACCCAGUGGGGGCAAAAACUCUGC
 I  P  G  Y  Y  H  P  R  T  R  G  E  T  Q  W  G  Q  K  L  C
  S  P  A  T  T  T  P  A  P  E  A  K  P  S  G  G  K  N  S  A

      2050      2060         G        2080      2090      2100
CAAGUUCAUCCCGAGCUGGCGGAGAAAACAACAGGAUUCGGCUGGCCAAAAGCCGGAUCU
 Q  V  H  P  E  L  A  E  K  T  T  G  F  G  W  P  K  A  G  S
  K  F  I  P  S  W  R  R  K  Q  Q  D  S  A  G  Q  K  P  D  L

      2110      2120      2130      2140      2150      2160
GAAGCUGAACUCCAAAGCCUGAAUCUACAGGCUGCCAGGUGGCUCCAACGCGCGGAGUCG
 E  A  E  L  Q  S  L  N  L  Q  A  A  R  W  L  Q  R  A  E  S
  K  L  N  S  K  A  -

      2170      2180      2190      2200      2210      2220
GCCACUAUCCCUGGCGCAGAAGCAAGAAAGCGCGUGAUUGAGAAAACAGUGGAGGCAUAC
 A  T  I  P  G  A  E  A  R  K  R  V  I  E  K  T  V  E  A  Y

      2230      2240      2250      2260      2270      2280
AGAAAUUGUGUAACUAACGCCCCACUGUGCUCCCUUAAAUCCAAACUGGAUUGGGCUGGC
 R  N  C  V  T  N  A  P  L  C  S  L  K  S  K  L  D  W  A  G

      2290      2300      2310      2320      2330      2340
UUUCAACAAGAUAUCCGUGAAGCAGUCCAGUCCCUUGAGCUAGACGCUGGUGUAGGCAUU
 F  Q  Q  D  I  R  E  A  V  Q  S  L  E  L  D  A  G  V  G  I

      2350      2360      2370      2380      2390      2400
CCCUAUAUCGCGUAUGGCCUCCCCACACACCGAGGAUGGGUUGAGGACCAUAAGCUUCUC
 P  Y  I  A  Y  G  L  P  T  H  R  G  W  V  E  D  H  K  L  L

      2410      2420      2430      2440      2450      2460
CCAGUGCUCACUCAGCUGACCUUUGACCGACUACAGAAGAUGUCGGAGGCCAGCUUUGAG
 P  V  L  T  Q  L  T  F  D  R  L  Q  K  M  S  E  A  S  F  E

      2470      2480      2490      2500      2510      2520
GAUAUGAGCGCAGAAGAGCUGGUUCAAGAAGGGCUCUGUGAUCCUAUCAGACUAUUUGUC
 D  M  S  A  E  E  L  V  Q  E  G  L  C  D  P  I  R  L  F  V

      2530      2540      2550      2560      2570      2580
AAAGGAGAGCCCCACAAACAGAGCAAACUCGAUGAAGGCCGCUACCGCCUCAUCAUGUCU
 K  G  E  P  H  K  Q  S  K  L  D  E  G  R  Y  R  L  I  M  S

      2590      2600      2610      2620      2630      2640
GUUUCCUUGGUGGAUCAACUGGUAGCCCGGGUUCUGUUCCAAAAUCAGAACAAAAGGGAA
 V  S  L  V  D  Q  L  V  A  R  V  L  F  Q  N  Q  N  K  R  E

      2650      2660      2670      2680      2690      2700
AUUUCCCUGUGGAGGUCUGUGCCUUCCAAACCCGGUUUUGGCCUUUCAACUGACACUCAA
 I  S  L  W  R  S  V  P  S  K  P  G  F  G  L  S  T  D  T  Q

      2710      2720      2730      2740      2750      2760
ACUGCUGAAUUCUUGGAGUGUCUUCAAAAGGUGUCUGGAGCGCCAUCUGUGGAAGAAUUG
 T  A  E  F  L  E  C  L  Q  K  V  S  G  A  P  S  V  E  E  L
```

Fig. 1. continued

```
      2770      2780      2790      2800      2810      2820
UGUGCAAAUCACAAGGAGUACACGCGCCCAACCGACUGUUCCGGUUUCGACUGGUCAGUC
 C  A  N  H  K  E  Y  T  R  P  T  D  C  S  G  F  D  W  S  V

      2830      2840      2850      2860      2870      2880
GCGUAUUGGAUGCUGGAGGAUGAUAUGGAGGUGAGAAAUCGCCUGACAUUUAAUAACACC
 A  Y  W  M  L  E  D  D  M  E  V  R  N  R  L  T  F  N  N  T

      2890      2900      2910      2920      2930      2940
CAGCUCACCAAGCGCCUUCGGGCCGCCUGGUUGAAGUGCAUAGGAAAUUCCGUCCUGUGC
 Q  L  T  K  R  L  R  A  A  W  L  K  C  I  G  N  S  V  L  C

      2950      2960      2970      2980      2990      3000
CUGUCCGAUGGCACUUUACUUGCCCAAACUGUUCCCGGUGUGCAAAAGAGCGGAAGUUAC
 L  S  D  G  T  L  L  A  Q  T  V  P  G  V  Q  K  S  G  S  Y

      3010      3020      3030      3040      3050      3060
AAUACAAGUUCCUCCAACUCUAGAAUCCGGGUUAUGGCUGCCUAUCACUGUGGCGCCGAC
 N  T  S  S  S  N  S  R  I  R  V  M  A  A  Y  H  C  G  A  D

      3070      3080      3090      3100      3110      3120
UGGGCAAUGGCCAUGGGGGACGAUGCUCUCGAAGCCCCCAACUCCGACCUGGAGGAGUAU
 W  A  M  A  M  G  D  D  A  L  E  A  P  N  S  D  L  E  E  Y

      3130      3140      3150      3160      3170      3180
AAAACACUAGGUUUCAAAGUCGAGGUAGGUCGAGAACUCGAAUUCUGUUCACACAUCUUC
 K  T  L  G  F  K  V  E  V  G  R  E  L  E  F  C  S  H  I  F

      3190      3200      3210      3220      3230      3240
AGAAAUCCGACCCUCGCCGUUCCGGUCAACACCAACAAAAUGCUUUACAAGUUGAUCCAU
 R  N  P  T  L  A  V  P  V  N  T  N  K  M  L  Y  K  L  I  H

      3250      3260      3270      3280      3290      3300
GGUUAUAAUCCGGAAUGUGGCAAUCCAGAAGUGAUUCAAAACUAUCUGGCUGCAGUAUUC
 G  Y  N  P  E  C  G  N  P  E  V  I  Q  N  Y  L  A  A  V  F

      3310      3320      3330      3340      3350      3360
UCUGUGCUGCAGGAACUCCGACACGAUCGUGAGCUCGUUGCCAAGCUCCACCAGUGGUUG
 S  V  L  Q  E  L  R  H  D  R  E  L  V  A  K  L  H  Q  W  L

      3370      3380      3390      3400      3410      3420
GUUCCGAGUGCCACCACAAAAGAACACUGAAGGAGCUCACUAAAACUAGCCAAGCAUACG
 V  P  S  A  T  T  K  E  H  -
      3430      3440         C       A 3460      3470      3480
CGAGUUGCAAGCAUUGGAAGUUCAAGUCUCGUUACAUCAACCGGACAAAAUAGAUUAUAA
    C 3490      3500      3510      3520      3530      3540
AUUUUUAGCGGGAUUUGCUUUAGGAUUCUCAUCCGCAAUCCCAUUUUCAGUAGCCGGUUU
      3550      3560      3570      3580      3590      3600
AUAUUUUGUUUACCUAAAGAUUUCCUCCCACGUGCGAUCAAUUGUUAAUGAGUACGGUCG
                                                M  S  T  V

      3610      3620      3630      3640      3650      3660
UGGUUAAAGGAAAUGUCAAUGGUGGUGUACAACAACCAAGAAGGCGAAGAAGGCAAUCCC
             M  S  M  V  V  Y  N  N  Q  E  G  E  E  G  N  P
V  V  K  G  N  V  N  G  G  V  Q  Q  P  R  R  R  R  R  Q  S

      3670      3680      3690      3700      3710      3720
UUCGCAGGCGCGCUAACAGAGUUCAGCCGGUGGUUAUGGUCACGGCCCCUGGGCAACCCA
 F  A  G  A  L  T  E  F  S  R  W  L  W  S  R  P  L  G  N  P
L  R  R  R  A  N  R  V  Q  P  V  V  M  V  T  A  P  G  Q  P

    C 3730      3740      3750      3760      3770      3780
GGCGUCGAAGACGCAGAAGAGGAGGCAAUCGCCGCUCAAGAAGAACUGGAGUUCCCCGAG
 G  V  E  D  A  E  E  E  A  I  A  A  Q  E  E  L  E  F  P  E
R  R  R  R  R  R  R  G  G  N  R  R  S  R  R  T  G  V  P  R


      3790      3800      3810      3820    G 3830      3840
GACGAGGCUCAAGCGAGACAUUCGUGUUUACAAAGGACAACCUCAUGGGCAACUCCCAAG
 D  E  A  Q  A  R  H  S  C  L  Q  R  T  T  S  W  A  T  P  K
G  R  G  S  S  E  T  F  V  F  T  K  D  N  L  M  G  N  S  Q
```

Fig. 1. continued

```
      3850      3860      3870      3880      3890      3900
GAAGUUUCACCUUCGGGCCGAGUCUAUCAGACUGUCCGGCAUUCAAGGAUGGAAUACUCA
 E  V  S  P  S  G  R  V  Y  Q  T  V  R  H  S  R  M  E  Y  S
G  S  F  T  F  G  P  S  L  S  D  C  P  A  F  K  D  G  I  L

      3910      3920      3930      3940      3950      3960
AGGCCUACCAUGAGUAUAAGAUCACAAGCAUCUUACUUCAGUUCGUCAGCGAGGCCUCUU
 R  P  T  M  S  I  R  S  Q  A  S  Y  F  S  S  S  A  R  P  L
K  A  Y  H  E  Y  K  I  T  S  I  L  L  Q  F  V  S  E  A  S

      3970      3980      3990      4000      4010      4020
CCACCUCCUCCGGUUCCAUCGCUUAUGAGUUGGACCCCCAUUGCAAAGUAUCAUCCCUCC
 P  P  P  P  V  P  S  L  M  S  W  T  P  I  A  K  Y  H  P  S
S  T  S  S  G  S  I  A  Y  E  L  D  P  H  C  K  V  S  S  L

      4030      4040      4050          U     C         4080
AGUCCUACGUCAACAAGUUCCAAAUUACGAAGGGCGGCGCCAAAACUUAUCAAGCGCGGA
 S  P  T  S  T  S  S  K  L  R  R  A  A  P  K  L  I  K  R  G
Q  S  Y  V  N  K  F  Q  I  T  K  G  G  A  K  T  Y  Q  A  R

      4090      4100      4110      4120      4130      4140
UGAUAAAUGGGGUAGAAUGGCACGAUUCUUCUGAGGAUCAGUGCCGGAUACUGUGGAAGG
 -
M  I  N  G  V  E  W  H  D  S  S  E  D  Q  C  R  I  L  W  K

      4150      4160      4170      4180      4190      4200
GAAAUGGAAAAUCUUCAGAUACCGCAGGAUCCUUCAGAGUCACCAUCAGGGUGGCUUUGC
G  N  G  K  S  S  D  T  A  G  S  F  R  V  T  I  R  V  A  L

      4210      4220      4230      4240      4250      4260
AAAACCCCAAAUAGGUAGACUCCGGAUCAGAGCCUGGUCCAAGCCCACAACCAACACCCA
Q  N  P  K  -  V  D  S  G  S  E  P  G  P  S  P  Q  P  T  P

      4270      4280      4290      4300      4310      4320
CUCCAACUCCCCAGAAGCACGAGCGAUUUAUUGCUUACGUUGGCAUACCUAUGCUAACCA
T  P  T  P  Q  K  H  E  R  F  I  A  Y  V  G  I  P  M  L  T

      4330      4340      4350      4360      4370      4380
UUCAGGCCAGGGAGAACGACGACCAGAUCAUAUUGGGUUCUUUAGGGAGCCAAAGGAUGA
I  Q  A  R  E  N  D  D  Q  I  I  L  G  S  L  G  S  Q  R  M

      4390      4400        U       4420      4430      4440
AAUAUAUAGAGGACGAGAACCAGAACUACACAAAUGUUAGUUCUGAGUAUUACUCUCAAU
K  Y  I  E  D  E  N  Q  N  Y  T  N  V  S  S  E  Y  Y  S  Q

      4450      4460      4470 C    4480      4490      4500
CGAGCAUGCAAGCCGUCCCUAUGUAUUACUUUAAUGUCCCGAAAGGGCAAUGGUCAGUCG
S  S  M  Q  A  V  P  M  Y  Y  F  N  V  P  K  G  Q  W  S  V

      4510      4520      4530      4540      4550      4560
ACAUCAGCUGCGAAGGGUAUCAACCCACUAGCAGCACCUCGGAUCCAAACCGGGGUAGGA
D  I  S  C  E  G  Y  Q  P  T  S  S  T  S  D  P  N  R  G  R

        A       4580      4590 U    4600      4610      4620
GUGACGGGGUGAUCGCGUAUUCAAACGCGGACUCCGAUUAUUGGAAUGUUGGUGAAGCGG
S  D  G  V  I  A  Y  S  N  A  D  S  D  Y  W  N  V  G  E  A

      4630      4640      4650      4660      4670      4680
AUGGUGUCAAAAUUUCGAAGCUACGCAACGAUAACACCUACCGCCAAGGUCACCCAGAAC
D  G  V  K  I  S  K  L  R  N  D  N  T  Y  R  Q  G  H  P  E

      4690      4700      4710      4720      4730      4740
UUGAAAUUAACUCGUGUCAUUUUCGCGAGGGCCAACUCCUUGAACGGGACGCUACAAUUA
L  E  I  N  S  C  H  F  R  E  G  Q  L  L  E  R  D  A  T  I

      4750      4760      4770      4780      4790      4800
GCUUCCACGUUGAAGCGCCUACUGAUGGGCGAUUCUUUCUCGUUGGUCCCGCUAUCCAGA
S  F  H  V  E  A  P  T  D  G  R  F  F  L  V  G  P  A  I  Q
```

Fig. 1. continued

```
      4810      4820      4830      4840      4850      4860
AAACCGCAAAGUAUAACUAUACUAUCUCAUACGGUGACUGGACGGACCGAGACAUGGAAC
K  T  A  K  Y  N  Y  T  I  S  Y  G  D  W  T  D  R  D  M  E

      4870      4880      4890      4900      4910      4920
UGGGGCUGAUCACCGUGGUGCUUGAUGAACAUUUAGAAGGCACUGGUUCGGCUAACAGAG
L  G  L  I  T  V  V  L  D  E  H  L  E  G  T  G  S  A  N  R
      4930      4940      4950      4960      4970      4980
UGCGGCGGCCCCCACGGGAGGGCCACACCUAUAUGGCCUCGCCGCGCGAACCGGAAGGAA
V  R  R  P  P  R  E  G  H  T  Y  M  A  S  P  R  E  P  E  G

      4990      5000      5010      5020      5030      5040
AACCGGUUGGAAAUAAACCAAGGGACGAAACCCCGAUACAAACGCAGGAAAGACAACCUG
K  P  V  G  N  K  P  R  D  E  T  P  I  Q  T  Q  E  R  Q  P

      5050      5060      5070      5080      5090      5100
AUCAAACUCCGUCUGACGACGUAUCCGAUGCUGGUUCGGUAAACAACAGCGGCUCAACUG
D  Q  T  P  S  D  D  V  S  D  A  G  S  V  N  N  S  G  S  T

      5110      5120      5130      5140      5150      5160
AGUCGCUGCAAUUGGAGUUCGGGGUAAACUCAGAUAGUACCUACGAUGCUACAGUCGAUG
E  S  L  Q  L  E  F  G  V  N  S  D  S  T  Y  D  A  T  V  D

      5170      5180      5190      5200      5210      5220
GUACAGACUGGCCCAGAAUUCCUCCACCAAGGCACCCACCUGAACCUAGAGUUUCCGGCA
G  T  D  W  P  R  I  P  P  P  R  H  P  P  E  P  R  V  S  G

      5230      5240      5250      5260      5270      5280
AUUCAAGAACUGUUAUUGACUUUUCUCCGAAAGCCGAUCUAUUGGAGAAUUGGGAUGCCG
N  S  R  T  V  I  D  F  S  P  K  A  D  L  L  E  N  W  D  A

      5290      5300      5310      5320      5330      5340
AACACUUCGACCCUGGUUAUUCCAAAGAAGAUGUCGCUGCUGCUACUAUUAUAGCGCACG
E  H  F  D  P  G  Y  S  K  E  D  V  A  A  A  T  I  I  A  H

      5350      5360      5370      5380      5390      5400
GCAGUAUUCAAGAUGGGCGAAGUAUGUUGGAGAAGAGAGAGGAAAGUGUCAAGAACAAAA
G  S  I  Q  D  G  R  S  M  L  E  K  R  E  E  S  V  K  N  K

      5410     GG      C  5430      5440      5450      5460
CCUCCUCCUGGAAGCCCCCGUUAUCUAAAGCGGUGAGCCCAGCCAUAGCCAAAUUGCGCU
T  S  S  W  K  P  P  L  S  K  A  V  S  P  A  I  A  K  L  R

      5470      G         5490      5500      5510      5520
CGAUUCGCAAAUCCCAACCCCUCGAGGGAGGGACCCUUAAGAAAGACGCUACUGAUGGUG
S  I  R  K  S  Q  P  L  E  G  G  T  L  K  K  D  A  T  D  G

      5530      5540      5550      5560      5570      5580
UCUCAUCUAUUGGCAGUGGUUCUCUAACAGGUGGCACGCUUAAGAGGAAGGUAACUAUUG
V  S  S  I  G  S  G  S  L  T  G  G  T  L  K  R  K  V  T  I

      5590      5600      5610      5620      5630      5640
AAGAGCGUUUACUGCAGACCUUAACAACUGAACAAAGGCUGUGGUACGAGAAUUUGAAGA
E  E  R  L  L  Q  T  L  T  T  E  Q  R  L  W  Y  E  N  L  K

      5650      5660      5670      5680      5690       G
AAACUAACCCUCCAGCUGCUAUCCAAUGGCUGUAUGAAUAUCAGCCACCUCCCCAAGUAG
K  T  N  P  P  A  A  I  Q  W  L  Y  E  Y  Q  P  P  P  Q  V

      5710      5720      5730      5740      5750      5760
AUAGAAACAUAGCUGAAAAGCCAUUCCAAGGGAGGAAAUGAGUCGACUCACGACUUAAAA
D  R  N  I  A  E  K  P  F  Q  G  R  K  -

      5770      5780      5790      5800      5810      5820
CUGAGUGUCCGCCGGACAUUAAGCGGAACGAAAGCCGAAAGGUGAUUAGGCUCUCAACGC

      5830      5840      5850      5860      5870        5882
CUGCAUGAGACCGUCGAAAGACGCGACUGUGUAGCCAAGAUCCUCUUACAUGGUUGUGUAGU
```

Table 2
Nucleotide changes detected in particular regions of PLRV-P genome

Region	Number	Length	%
5′nc	1	69	1.5
ORF1	8	744	1.1
ORF2	9	1920	0.47
ORF3	3	1851	0.16
nc-inc	3	197	1.5
ORF4	4	627	0.64
ORF5	4	471	0.85
ORF6	9	1530	0.59
3′nc	0	141	0.0

region of the 3′-end in the 4 sequenced independent clones.

Frequency of nucleotide changes in different ORF's is almost identical. This nearly random spreading of variations suggests that molecular variations might result from faulty RNA replication. Therefore, the reported consensus sequence in this paper represents the best approximation to the sequence of virus population studied.

Not all observed variations in the nucleotide sequence resulted in changes of amino-acid sequence. Data presented in Table 3 show number of total and significant point mutations.

The percentage differences for the pairwise comparison with other PLRV isolates are shown in Table 4 and indicate that the PLRV-P, PLRV-D, PLRV-S and PLRV-C are closely related to each other differing in each case by 2% whereas for the PLRV-A isolate the difference is about 7%. Similar relations were observed by Keese *et al.* [11] between PLRV isolates A, C, D, and S.

Table 3
Frequency of amino-acid changes in particular ORFs of different clones

	Total number of changes	Significant changes
ORF1	8	3
ORF2	9	4
ORF3	3	1
ORF4	4	1
ORF5	4	2
ORF6	9	3

Table 4
Percentage difference for pairwise comparison genome

PLRV-P and PLRV-D	2
PLRV-P and PLRV-S	2
PLRV-P and PLRV-A	7
PLRV-P and PLRV-C	2

REFERENCES

1. Matthews, R.E.F. (1991) *Plant Virology*, pp. 226–229, Academic Press, New York.
2. Harrison, B.D. (1984) Potato leafroll virus. CMI/AAB Description of Plant Viruses, no. 291.
3. Rochow, W.F. & Duffus, J.E. (1981) Luteoviruses and yellows diseases; in *Handbook of Plant Virus Infections and Comparative Diagnosis* (Kurstak, E., ed.) pp. 147–170, Elsvier/North Holland, Amsterdam.
4. Mayo, M.A., Baker, H., Robinson, D.J., Tamada, T. & Harrison, B.D. (1982) Evidence that potato leafroll virus is positive-stranded, is linked to a small protein and does not contain polyadenylate. *J. Gen. Virol.* **59**, 163–167.
5. Syller, J. (1985) Comparison of some isolates of potato leafroll virus in Poland. *Phytopatologische Zeitschrift* **113**, 17–23.
6. Miller, W.A., Waterhouse, P.M. & Gerlach, W.L. (1988) Sequence and organization of barley yellow dwarf virus genomic RNA. *Nucleic Acids Res.* **16**, 6097–6111.
7. Ueng, P.T., Vincent, J.R.,Kawata, E.E., Lei, C.-H., Lister, R.M. & Larkins, B.A. (1992) Nucleotide sequence analysis of the genomes of MAV-PS1 and P-PAV isolates of barley yellow dwarf virus. *J. Gen. Virol.* **73**, 487–492.
8. Veidt, I., Lot, H., Leiser, M., Scheidecker, D., Guilley, H., Richards, K. & Jonard, G. (1988) Nucleotide sequence of beet western yellows virus RNA. *Nucleic Acids Res.* **16**, 9917–9932.
9. Mayo, M.A., Robinson, D.J., Jolly, C.A. & Hyman, L. (1989) Nucleotide sequence of potato leafroll luteovirus RNA. *J. Gen. Virol.* **70**, 1037–1051.

10. van der Wilk, F., Huisman, M., Cornelissen, B.J.C., Huttinga, H. & Goldbach, R. (1989) Nucleotide sequence and organization of potato leafroll virus genomic RNA. *FEBS Lett.* **245**, 51–56.
11. Keese, P., Martin, R.R., Kawchuk, L.M., Waterhouse, P.M. & Gerlach, W.L. (1990) Nucleotide sequences of an Australian and a Canadian isolate of potato leafroll luteovirus and their relationships with two European isolates. *J. Gen. Virol.* **71**, 719–724.
12. Holland, J.J., De la Torre, J.C. & Steinhauer, B.A. (1992) RNA virus population as quasi species. *Curr. Topics Microbiol. Immunol.* **176**, 1–20.
13. Eigen, M. (1993) The origin of genetic information: viruses as models. *Gene* **135**, 37–47.
14. Tamada, T., Harrison, B.D. & Roberts, I.M. (1984) Variation among British isolate of potato leafroll virus. *Ann. App. Biol.* **104**, 107–116.
15. Ausubel, F. & Brent, R. (1987) *Current Protocols in Molecular Biology.* Editorial Office, Greene Publishing Associates, Brooklyn, N.Y.
16. Maniatis, T., Fritsch, E.F. & Sambrook, J. (1982) *Molecular Cloning: a Laboratory Manual.* New York: Cold Spring Harbor Laboratory.
17. Sanger, F., Nicklen, S. & Coulson, A.R. (1977) A DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463–5467.
18. Lane, D.J., Field, K.G., Olsen, G.J. & Pace, N.R. (1988) Reverse transcriptase sequencing of ribosomal RNA for phylogenetic analysis. *Methods Enzymol.* **167**, 138–144.