# RNA IN POTATO LEAFROLL VIRUS

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## 1. Introduction

Potato leafroll virus (PLRV) is a small icosahedral and aphid-transmitted circulative virus [1–4]. It is only observed in ultrathin sections of phloem tissues of infected plants [2]. These features allow us to consider it as a possible member of the Luteovirus group [5]. The location of PLRV and the low yield of its extraction [6] explain the lack of studies relative to the genetic material of this virus. The only results so far published indicate that PLRV contains double-stranded DNA [7]. Here we show that molecules of RNA, homogeneous in size, can be extracted from purified virions.

## 2. Materials and methods

PLRV was purified from stem fibers and from leaves in 1 expt of infected potatoes (*Solanum tuberosum*) as in [6]. The final step of purification corresponds to a sucrose gradient centrifugation as illustrated in fig.1A. The homogeneity of the virion preparation was checked by CsCl equilibrium centrifugation (fig. 1B) and by electron microscopy (fig. 1C). Infectivity of the purified virus was demonstrated using the membrane feeding method in [8]. *Myzus persicae* and *Physalis floridana* were, respectively, the vector and the host plant in this test. The concentration of the virus preparations was estimated assuming that $A_{260} = 5$ is equivalent to 1 mg virus/ml [9].

The nucleic acid was extracted from purified virions by the SDS–proteinase K procedure using essentially a combination of two techniques [10,11]. Extraction incubation was carried out at 30°C for 30 min, in a 1 ml mixture containing 50–100 μg virus, 0.02 M dithioerythritol, 0.15 M NaCl, 0.015 M EDTA, 0.05 M Tris–HCl (pH 8), 0.25% SDS and 0.5 mg/ml proteinase K (Boehringer). The reaction mixture was then treated twice with an equal volume of saturated phenol and the 2 phenol phases successively washed with $H_2O$. To the combined aqueous phases were added 1/10th vol. 2 M K acetate (pH 5) and 2 vol. ethanol. The nucleic acid pellet was collected by centrifugation at 250 000 × *g* for 30 min, dried under vacuum and dissolved in water. Nucleic acid (5–10 μg) was recovered.

Hydrolyses were performed with bovine pancreatic DNase (Worthington DPFF), or bovine pancreatic RNase (Worthington RAF) pre-heated at 100°C for 5 min to destroy contaminating DNase activity, in 20 μl, at 37°C and for 45 min. Incubation mixtures contained: for RNase 0.3–1 μg RNA, 0.01 M Tris–HCl (pH 7.4), 1 mM EDTA and 1 ng heated RNase; for DNase, 0.3–1 μg DNA, 0.01 M Tris–HCl (pH 7.4), 0.01 M $MgCl_2$, 1 mM EDTA, 0.025 M 2-mercaptoethanol, 5% glycerol and 1 ng DNase.

Native or hydrolyzed nucleic acids were analyzed by electrophoresis in vertical slab gels (0.16 cm thick and 15 cm long) at pH 7.8 [12], containing 1% or 1.5% agarose, or 2.5% acrylamide–0.5% agarose. After migration, RNA or DNA bands were located by fluorescence with ethidium bromide [13] and photographed. As markers were used single-stranded linear RNAs of mengo virus (mengo RNA, $2.8 \times 10^6$ daltons), of common strain tobacco mosaic virus (TMV RNA, $2.1 \times 10^6$ daltons), of *Rhopalosiphum padi* barley yellow dwarf virus (BYDV RNA, $1.8 \times 10^6$ daltons), of *Solanum tuberosum* cytoplasmic ribosomes (25 S and 18 S, $1.3 \times 10^6$ and $0.7 \times 10^6$ daltons, respectively), of *E. coli* ribosomes (23 S and 16 S, $1.2 \times 10^6$ and $0.55 \times 10^6$ daltons, respectively) and double-

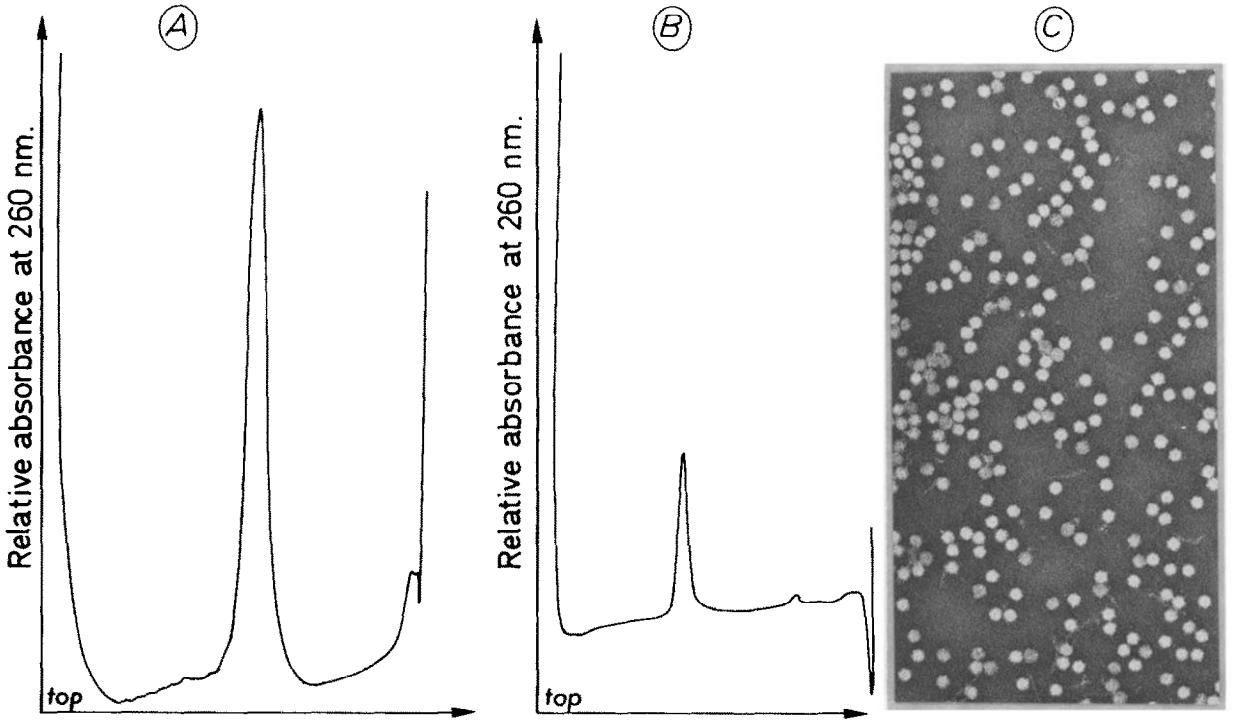


Fig.1. Purification of PLRV. (A) Preparative centrifugation in 5–20% sucrose gradient in 0.01 M Na-phosphate (pH 7.2) and 1 mM EDTA (4°C, 273 000 × $g$, 60 min). (B) Analytical equilibrium centrifugation in CsCl in 0.01 M Na-phosphate (pH 7.2), 1 mM EDTA and 0.025 M 2-mercaptoethanol (starting density = 1.38 g/cm$^3$; rotor SW 50, 4°C, 175 000 × $g$, 50 h). (C) Electron micrograph of particles stained with 2% phosphotungstate (pH 7) (× 72 000).

stranded linear DNA of bacteriophage lambda (λDNA, $30 \times 10^6$ daltons).

## 3. Results

Analysis of native nucleic acid extracted from PLRV reveals a major sharp band nearly in the middle of the gel (fig.2, slot 1). If PLRV nucleic acid is treated with 95% dimethylsulfoxide (DMSO) before electrophoresis, there is still only one band, but at a slightly retarded position owing to some shrinkage of the gel (fig.2, slot 2). After similar DMSO treatment and electrophoretic migration, genomic TMV RNA is not affected (not illustrated here) while Cauliflower Mosaic Virus double-stranded DNA is denatured [14]. Such results indicate that PLRV nucleic acid molecules do not possess hidden breaks and that they are rather homogeneous after extraction.

Nucleic acid of PLRV was incubated with either RNase or DNase in the absence (not presented here) or in the presence of λ DNA as internal control. Figure 3 shows that after RNase treatment the band corresponding to PLRV nucleic acid disappears while the band of λ DNA is not affected (slot 3a) and that DNase hydrolyzes λ DNA but not PLRV nucleic acid (slot 3b). A mixture of TMV RNA and λ DNA treated in similar conditions was used in control experiments (slot 4a, 4b). The results of these experiments strongly suggest that PLRV contains RNA. Considering the finding [7] that PLRV contains DNA we undertook other experiments to exclude the possibility that DNA has been lost during viral nucleic acid extraction and also to ensure that the RNA we have observed corresponds to nucleic acid formerly engaged in a specific complex with capsid proteins.

Prior to SDS–proteinase K incubation and subsequent phenol treatments, λ DNA in amount close

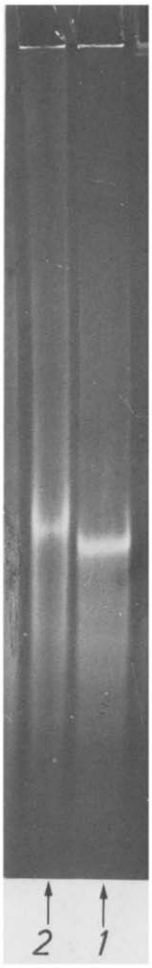


Fig.2. Analysis of PLRV nucleic acid by electrophoresis in a 1% agarose gel. (1) Native nucleic acid. (2) DMSO-treated nucleic acid.

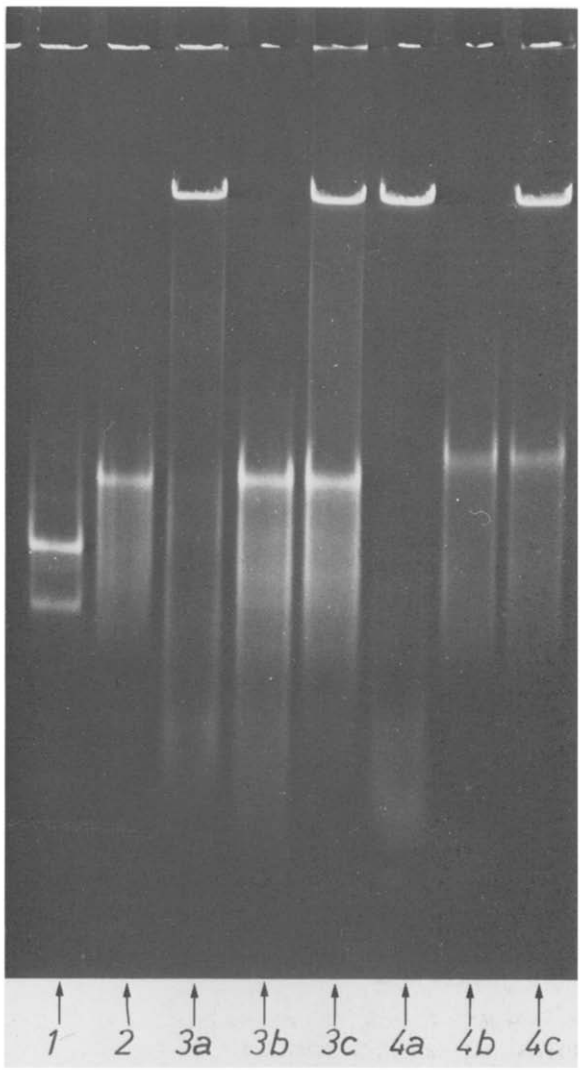


Fig.3. RNase or DNase treatments of PLRV nucleic acid monitored by electrophoresis in a 1% agarose gel. (1) *E. coli* rRNA. (2) BYDV RNA. (3) Mixture of PLRV nucleic acid and λ DNA: (a) RNase-treated; (b) DNase-treated; (c) without nuclease. (4) Mixture of TMV RNA and λ DNA: (a) RNase-treated; (b) DNase-treated; (c) without nuclease.

to that presumed for RNA in the virion, was added to PLRV purified from either stems or leaves. Such an experiment is presented in fig.4. It is clear that λ DNA is quantatively recovered as well as PLRV RNA (slot 1a or 2a). A very fine band at the position of λ DNA and a smear starting from this position appear in the control slots 1b, 2b. They reflect the presence of a nucleic acid very heterogeneous in size and of higher molecular weight than PLRV RNA. Its amount is larger in PLRV extracted from leaves than from stems (compare slot 2b to 1b). It varies from one virus preparation to another and, based on the data of densitometric scanning of the gel pictures, it can represent as much as 2–3-fold the amount of PLRV RNA.

Information connected with the nature of the nucleic acid responsible for the smear was provided by the following experiment: purified PLRV virions were preincubated with either DNase or RNase before

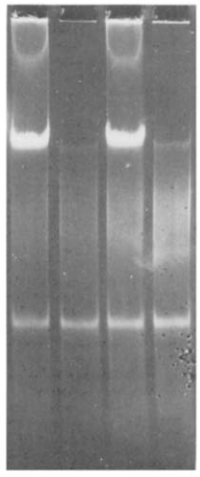


Fig.4. Extraction of PLRV RNA in the presence of exogenous DNA and analysis by electrophoresis in a 1% agarose gel. (1) PLRV RNA from stems: (a) with λ DNA; (b) control. (2) PLRV RNA from leaves: (a) with λ DNA; (b) control.

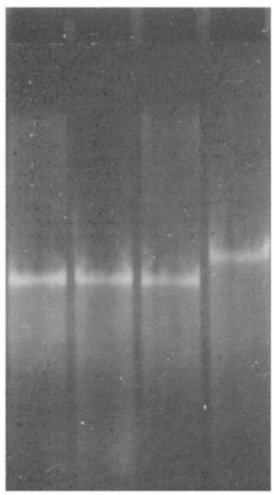


Fig.5. Preincubation of PLRV with DNase or RNase and direct analysis of nucleic acid, after SDS–proteinase K treatment, by electrophoresis in a 1.5% agarose gel. Preincubation: (1) with DNase; (2) with RNase; and (3) without nuclease. (4) TMV RNA as a marker of migration.

SDS–proteinase K treatment and directly analyzed by agarose gel electrophoresis, without phenol extraction and ethanol precipitation (fig.5). The smear observed in control (slot 1) disappears after preincubation with DNase (slot 2) whereas it is not affected by RNase treatment (slot 3). It is also important to mention that without any pretreatment of the virion with nucleases, or after such treatments, PLRV RNA subsists and that its pattern is identical to that obtained when isolation involves the phenol and ethanol steps. In the same conditions of electrophoretic analysis of intact virions, the DNase-sensitive material is observed in the gel but PLRV RNA is not (not illustrated here).

Preincubation of virions with RNase prior to SDS–proteinase K treatment was also carried out in the presence of host plant cytoplasmic rRNA. Figure 6

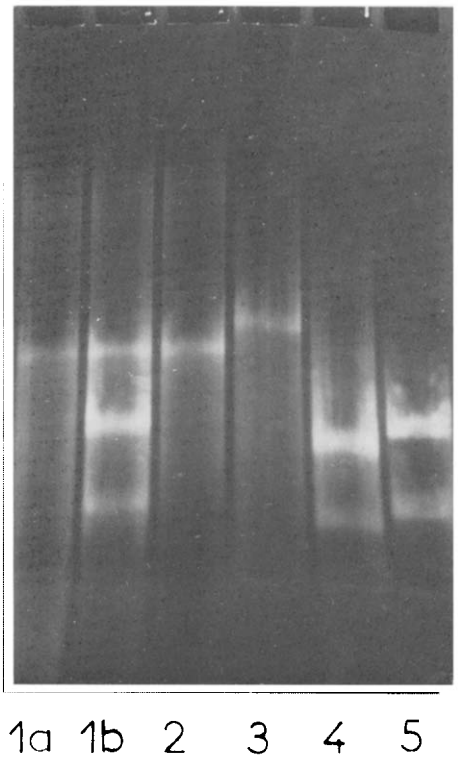


Fig.6. Preincubation of PLRV with RNase in the presence of host plant cytoplasmic rRNA and analysis as in experiment of fig.5. (1) Mixture of PLRV and rRNA preincubated: (a) with RNase; (b) without RNase. (2) PLRV preincubated without RNase. Markers of migration: (3) TMV RNA; (4) *E. coli* rRNA; (5) *S. tuberosum* rRNA.

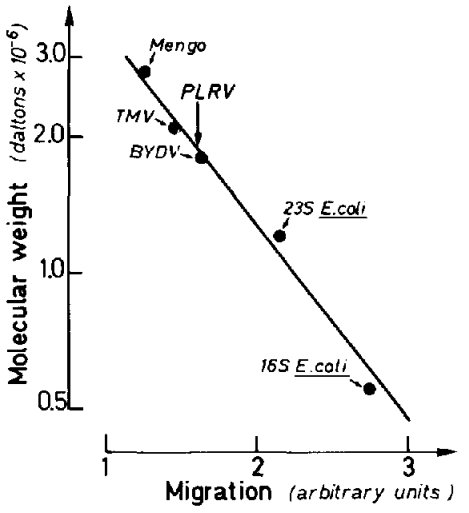


Fig.7. Electrophoretic mobility of PLRV RNA in composite 2.5% acrylamide–0.5% agarose gel compared to those of the standards quoted in section 2. Semi-logarithmic plot: molecular weight of RNAs versus migration.

(slot 1a) shows that only the 2 added rRNAs are hydrolyzed and that PLRV RNA appears at a position very distinct from those of rRNAs (slot 1b).

From all these experiments, it clearly results that:

1. PLRV preparations can be contaminated by a large amount of heterogeneous, DNase-sensitive material, especially in the case of isolates from leaves;
2. Only RNA is extracted from purified PLRV as nucleic acid molecules homogeneous in size;
3. The RNA in the virion is resistant to RNase activity in conditions where exogenous RNA is hydrolyzed.

We have no definite information concerning the structure of PLRV RNA molecules. Nevertheless, their sensitivity to RNase in the presence of 2 X SSC (not shown here) would reflect a single-stranded backbone, either linear or circular. If we assume that these molecules are single-stranded and linear, their molecular weight corresponds to $1.8–1.9 \times 10^6$ as judged by their mobility relative to RNA molecules of known size, in non-denaturing gels (fig.7).

## 4. Discussion

From < 100 μg purified PLRV virions, we were able to extract the nucleic acid and to identify it as RNA, possibly single-stranded and corresponding to homogeneous molecules of $\sim 1.85 \times 10^6$ daltons. It should be pointed out that analysis by electrophoresis has several advantages, in particular if only very low amounts of unlabelled material are available. Indeed, it is sensitive (0.1–0.3 μg RNA or DNA are easily detected) and provides useful information on the integrity and size of the molecules examined. Moreover it allows to check the purity of virus preparations and to analyze the genome directly, without intermediate steps such as phenol extraction and ethanol precipitation during which the genome could be lost.

Double-stranded DNA has been described in PLRV preparations from leaves and stems [7]. Its mol. wt $0.56 \times 10^6$ was calculated from data of sedimentation at equilibrium and its existence as homogeneous molecules was not demonstrated. More recently its molecular weight has been estimated as $1.3 \times 10^6$. By gel electrophoresis we have not observed nucleic acid in the regions corresponding to these molecular weights, using restriction DNA fragments as standards. The DNA previously reported in PLRV could correspond to a contamination by a specific fraction of sheared host plant DNA with physical properties slightly different from those of the total cellular DNA. PLRV has been reported to contain RNA [16].

The presence of RNA in PLRV is a good argument to classify this virus in the group of the Luteovirus which already includes BYDV and beet western yellow virus. These two latter viruses contain single-stranded RNA molecules of $2 \times 10^6$ daltons [17,18].

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