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SOME MOLECULAR CHARACTERISTICS OF PELARGONIUM LINE PATTERN VIRUS

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Pelargonium line pattern virus (PLPV), a so far ungrouped virus isolated from *Pelargonium zonale*, provokes intracellular changes very similar to those of some dianthoviruses. We found that the virus sedimented as a single component, contained a single polypeptide species with an M_r of 38,000 and an RNA molecule of 1.2×10^6 daltons. As PLPV was proved to be characterized with monopartite genome, it is impossible to propose the affiliation of this virus species to dianthovirus group, despite very similar cytopathic effect.

Introduction

Pelargonium line pattern virus (PLPV), a stable isometric virus, was described for the first time in Great Britain by Stone and Hollings (1976, 1977). Later, it was reported from Croatia by Pleše and Štefanac (1980). Štefanac and Pleše (1982) found some alterations induced by PLPV in epidermal and mesophyll cells of infected test plants. Inclusion bodies which appear as granular flakes in the light microscope, consist of virus particles scattered in an amorphous loosely distributed electron dense substance, as revealed by electron microscopy. On the basis of inclusion bodies and its other properties it was impossible to propose PLPV as a member of any known group of plant viruses. In contrast to some virus groups characterized by cytoplasmic and vacuolar bodies made up of virus particles and dark-staining substance (Christie and Edwardson 1977, Van Kammen and De Jager 1978, Di Franco et al. 1980), this virus does not provoke any additional ultrastructural features e. g. crystals, vacuolate inclusions etc. However, similar ultrastructural effects are caused by red clover necrotic mosaic

virus (R. J. B. Francki, pers. comm.) and sweet clover necrotic mosaic virus (SCNMV) (Hiruki et al. 1984) which belong to dianthovirus group (Matthews 1982). All member viruses have a bipartite genome (Hiruki 1987). Their virions contain single-stranded genomic RNAs of approximate molecular weights (M_r) 1.5×10^6 (RNA-1) and 0.5×10^6 (RNA-2) and a coat protein of M_r $38—40 \times 10^3$. The particles measure 31—35 nm in diameter and sediment at 130—135 S as a single component. This paper reports an attempt to reveal some characteristics of PLPV. especially the genome organization, in order to confirm the possible affiliation to dianthovirus group.

Material and Methods

PLPV isolated from *Pelargonium zonale* in Croatia (Pleše and Štefanac 1980) was used in our investigation. The isolate was propagated in *Chenopodium quinoa* kept in darkness for 24 hours before inoculation, using 0.066 M phosphate, pH 7.6 as inoculation buffer. Partially purified preparation was obtained by the method described for SCNMV by Hiruki et al. (1984). Locally infected leaves (stored frozen at −25° C) were homogenized in cold 0.066 M, pH 7.0 phosphate buffer (1:2, w/v) containing 0.36% ascorbic acid. The homogenate was clarified by an equal volume of chloroform-butanol (1:1) and centrifuged at 10,000 g for 20 min. After mixing with polyethylene glycol (PEG) 6,000 and sodium chloride at 8% and 0.4% (w/v), respectively, the virus was precipitated by another cycle of low speed centrifugation. It was followed by two cycles of differential centrifugation (high speed centrifugation at 70,000 g for 3 hr and 90,000 g for 2 hr and low speed centrifugation at 5,000 g for 20 min). Virus pellets were resuspended in 0.01 M, pH 7.0 phosphate buffer.

For further virus purification, linear 10—40% gradients were prepared in the same buffer by melting frozen 25% sucrose solution. After the centrifugation at 60,000 g for 3 hr in a Beckman 25.1 swinging bucket rotor, gradients were analyzed on an ISCO model 640 density gradient fractionator using UA-5 absorbance monitor.

Partially purified virus preparation, fixed in 1% formaldehyde, was stained with potassium phosphotungstate (2%, pH 6.8) and examined in a Siemens Elmiskop I electron microscope.

Virus coat protein was analyzed by polyacrylamide gel electrophoresis on 7% slab gel containing 0.1% SDS according to Weber and Osborne (1969). Molecular weight markers, supplied by Sigma as MW-SDS-70 kit, were: bovine plasma albumin (66,000 daltons), ovalbumin (45,000), porcine stomach mucosa pepsin (34,700), bovine pancreas trypsinogen (24,000), bovine milk-lactoglobulin (18,400) and egg white lysozyme (14,300).

The virus purified by density gradient centrifugation was used for RNA extraction. It was mixed with an equal volume of extraction medium (1 M Tris-HCl, 1 mM EDTA, 1% SDS, pH 7.8). Water saturated phenol was added to the mixture (1:1, v/v), vortex mixed for 2 min and centrifuged for 3 min. The aqueous-phase was subsequently treated once with phenol and two times (for a few seconds) with ether which was finally evaporated in water bath at 37° C. After 1/20 volume 4 M sodium acetate, pH 6.0 and 2.5 volume absolute ethanol were added, the sample was

stored at −25° C overnight. RNA was precipitated at 5000 g for 20 min and the pellet resuspend in TE-buffer (10 mM Tris, 1 mM EDTA, pH 7.5). Nucleic acid extraction was made also by the method of Wilcockson and Hull (1974).

Ribonucleic acid, semidenaturated by incubation at 85° C for 2 min in the presence of 80% deionized formamide, was loaded on 1.2% agarose gel prepared in TBE-buffer system (90 mM Tris, 90 mM boric acid, 1 mM EDTA, pH 8.3). The electrophoresis was performed in a mini-submarine electrophoresis apparatus at 100 V per 10 cm gel, for 1 hr at room temperature. The RNA bands were stained with 5 μg/ml ethidium bromide and then photographed under ultra-violet light on transilluminator with a Polaroid camera. Tobacco mosaic virus RNA ($M_r = 2.1 \times 10^6$) and brome mosaic virus RNAs (1.1×10^6; 1.0×10^6; 0.7×10^6; 0.3×10^6) were used as references for molecular weight estimation.

Results and Discussion

As described previously (Pleše and Štefanac 1980), particles of PLPV were damaged when purified by the method commonly used for some stable spherical viruses and stained with potassium phosphotungstate. However, the purification method described for SCNMV, a member of dianthovirus group, resulted in satisfactory yield of PLPV. Moreover, the electron microscope analysis of purified virus produced very good quality of virus preparation (Fig. 1). The isometric virus particles had a diameter of about 30 nm. In contrast to complete stain penetration of some particles of SCNMV (Hiruki et al. 1984), particles of PLPV exhibited only slight penetration after staining with 2% potassium phosphotungstate.

It is evident from absorbance curve that virus particles sedimented as a single component in sucrose density gradient centrifugation (Fig. 2). The large absorbance peak at the top of gradient contains plant cell proteins present in partially purified preparation. Another small, slightly expressed peak, situated below the major single particles peak, probably resulted from some small virus aggregates.

In SDS-PAGE virus coat protein migrated as a single polypeptide species for which a molecular weight of c. 38000 was calculated. This data is in good agreement with that previously reported by Stone and Hollings (1977), but also with mol. wt. range given in review by Hiruki (1987) for members of dianthovirus group.

As stated above, we obtained for PLPV virion characteristics very similar to those described for dianthoviruses, and the isolate was readily purified by the method described for a member of this virus group. In addition, ultrastructural changes provoked by PLPV greatly resemble the effects caused by dianthoviruses. However, there is no similarity in the genome organization, because all members of dianthovirus group contain two pieces of RNA. We detected only one RNA molecule with an M_r of 1.2×10^6, as resulted from gel electrophoresis (Fig. 3). Both nucleic acid extraction methods, using either phenol or sodium perchlorate, revealed the same result, so we have to consider that PLPV is characterized by monopartite genome. As genome organization is one of the most important principal virus characteristics, the idea about affiliation of PLPV to dianthoviruses is no longer viable. As observed by dr.

Hiruki (pers. comm.), serological tests using purified PLPV were negative when tested to SCNMV antiserum in both gel-diffusion and ELISA.

Does PLPV belong to another known plant virus group? There are some recent proposals dealing with PLPV as a potential (R. G. Milne, pers. comm.) or even definite member (Adam et al. 1990) of carmovirus group. The results obtained in our investigation are in good agreement with member characteristics of this virus group. It seems, however, that such presumption is not generally accepted, leaving the question of PLPV taxonomic status still open.

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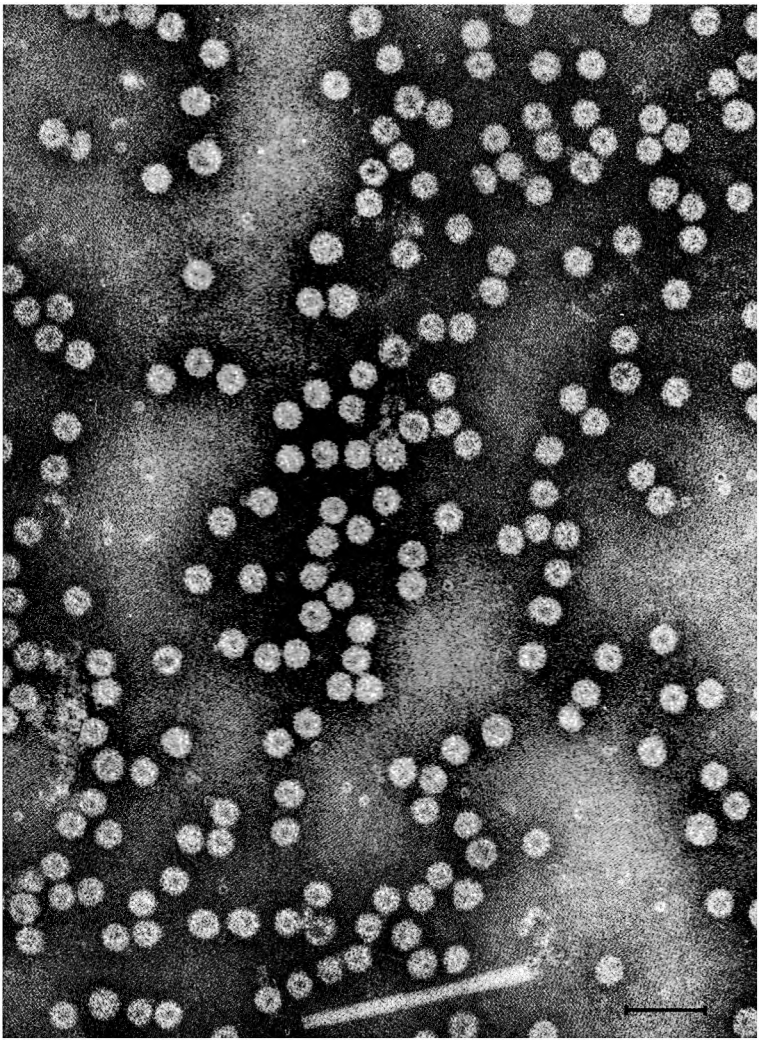

Fig. 1. Electron micrograph of partially purified PLPV stained with 2% potassium phosphotungstate after fixation in 1% formaldehyde. Tobacco mosaic virus particles were used as internal standard. Bar represents 100 nm.

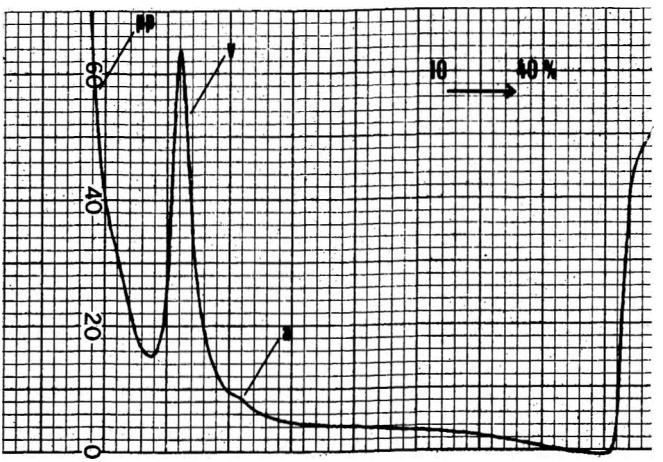

Fig. 2. Sedimentation profile obtained by UV-monitoring of partially purified PLPV after density gradient centrifugation (pp — plant protein peak, v — virus particles peak, a — virus aggregates peak).

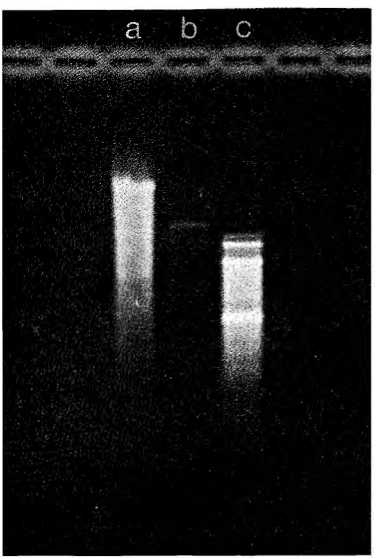

Fig. 3. Electrophoretic mobility in agarose gel of RNAs extracted from: a) tobacco mosaic virus, b) PLPV, c) brome mosaic virus. Experimental conditions described in the text.

SAŽETAK

NEKA MOLEKULARNA SVOJSTVA VIRUSA LINIJSKOG MOZAIKA PELARGONIJE

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Virus linijskog mozaika pelargonije (pelargonium line pattern virus — PLPV) jedan je od virusa koji do sada nisu uvršteni ni u jednu od ustanovljenih skupina biljnih virusa. Budući da je po citopatološkom učinku sličan diantovirusima, odredili smo neka svojstva tog virusa s namjerom da predložimo njegovo uvrštavanje u navedenu virusnu skupinu. Virus smo uspješno purificirali po metodi opisanoj za diantoviruse. U gradijentu gustoće virus je sedimentirao kao jedna nukleoproteinska komponenta, a molekularna masa kapsidnog polipeptida iznosila je 38000. Elektroforezom smo ustanovili da virusne čestice sadržavaju samo jednu molekulu RNA molekularne mase $1{,}2 \times 10^6$. Pošto je osnovna značajka diantovirusa genom podijeljen na dvije molekule RNA, pretpostavka o pripadnosti PLPV-a diantovirusima neodrživa je usprkos podudarnosti u ostalim navedenim svojstvima.

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