Criteria for Identification of *Cauliflower Mosaic Virus*'s of the Far Eastern Strains

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Abstract

On the base of the present-day principles to classify plant viruses the identification of *Cauliflower mosaic virus* (CaMV), a new virus for the Russian Federation, is carried out. Biological properties of 7 isolates have been studied. Symptomatology, range of host-plants and physical properties of virions of studied strains differ. The least thermostable strain is CaMV-B3 (TIP – 75°C) and the highest TIP (85°C) is CaMV-B1. The highest virus concentration in sap was observed for CaMV-B2 (DEP – 10^{-6}), and lowest – CaMV-R1 (10^{-1}–10^{-2}). CaMV-B2 and CaMV-C2 lost infection during 4 days in room conditions, CaMV-B3 – 1 day. A virus proteins were isolated (42 and 44 kD). The native nucleic acid of CaMV have been extracted. The DNA was separated into mixtures of circular and linear molecules. Size of the DNA is about 8000 base pairs.

Keywords: *Cauli ower mosaic virus*; *Brassicaceae*; identification

INTRODUCTION

The fundamental investigations are essential for study of properties virus-specific proteins and nucleic acids of phytoviruses and their strains not only described in literature but also recent for description to a new species according to the latest requirement of classification. Just fundamental investigations of viruses and their strains by using classical methods of virology are put in first place inasmuch viruses with similar biological characteristics (range of host-plants, symptomatology, pathogeny, cytopathology, and formation of intracellular inclusions, physical properties of virions, mode of transmission and so on) and antigenic characteristics have similar type of nucleic acid, morphological and physical-chemical properties. Antigenic and biological properties are characteristics to be a base for genera formation.

In this article the principal criteria were examined, that was taken for the grounds of identification of the virus and their strains – *Cauli ower mosaic virus* – not described in Russia earlier. The strains were found out on plants family *Brassicaceae* on agrocoenoses of south Primorye Territory. CaMV is the first DNA-containing phytovirus, discovered in the territory of Russia.

MATERIALS AND METHODS

The virus isolates with virus-like symptoms were used, isolated from cauli ower (CaMV-C1, CaMV-C2), white cabbage (CaMV-B1, CaMV-B2, CaMV-B3), and radish (CaMV-R1, CaMV-R2).

The range of host-plants and symptomatology were studied on 51 of species and kinds of plants from *Amaranthaceae* Juss., *Brassicaceae* Burnett., *Chenopodiaceae* Vent., *Cucurbitaceae* Juss., *Fabaceae* Lindl., *Resedaceae* Gray, *Solanaceae* Juss., *Scrophulariaceae* Juss. families.

The thermal inactivation point (TIP), dilution end point (DEP), longevity *in vitro* (LIV) under the room temperature, and the virus transmission by insects (different species of aphids) were determined by a procedure similar to that reported by GIBBS and HARRISON (1976).

The samples for electron microscopy were contrasted for 30 s with 2% uranyl acetate on water. The

grids were observed in JEOL JEM 100 S electron microscope.

For the reveiling a virus inclusion bodies the sections of lower epidermis of infected radish plants were stained with toluidine blue and observed in light microscope.

The antiserum to the cucumber mosaic virus was used as control test on presence ones in infected plants using reaction double diffusion.

CaMV-C1 was purified by a modification of the procedure of MORRIS *et al*. (1980). The SDS-polyacrylamide gel electrophoresis was performed on a vertical slab gel (LAEMMLI 1970). The antiserum to CaMV-C1 was prepared according to GNUTOVA (1985).

Virus DNA was isolated from the infected plants by procedure of RICHINS and SHEPHERD (1983). In order to estimate the total genome length of the *Hind*III endonuclease the fragments of *E. coli* λ phage DNA were used.

RESULTS

The strains were transmitted successfully only on fam. *Brassicaceae*'s plants by mechanical inoculation and the aphid *Myzus persicae* Sulz. For the comparative symptomatology and description of experimental range of host-plants 20 species and kinds of plants from *Brassicaceae* family were used. It has been found that in this case the characteristics of the isolates differed. So, CaMV-C1 and CMV-C2 infected rather wide range of *Brassicaceae*'s plants, in comparison with different 5 strains, while CaMV-B3 and CaMV-R1 infected the least number of species.

Physical properties of virions of studied strains also differ. The least thermostable strain is CaMV-B3 (TIP – 75°C) and the highest TIP (85°C) is CaMV-B1. The highest virus concentration in sap of infected plants was observed for CaMV-B2 (DEP – 10^{-6}), and lowest – CaMV-R1 (10^{-1}–10^{-2}). CaMV-B2 and CaMV-C2 lost infection during 4 days in room conditions, CMV-B3 – 1 day.

The base on the results we can to conclude that isolates are independent strains of CaMV. CaMV-C1 was refered to common strain because its biological properties correspond to literary data (SHUKLA *et al*. 1972).

In cytoplasm of infected *Raphanus sativus* cells roundish inclusion bodies across diameter 2–4.5 mmk are found, typical for caulimovirus's species.

From the infected radish plants purified virus preparation has been obtained with the typical spectrum of nucleoprotein ultraviolet absorption with the maximal absorption at 260 nm and minimal – at 240 nm. The yield of virus was 3–6 mg/kg of infected leaves (depending on season). The ratio of 260 to 280 nm UV absorbance. The spherical virions of 50 nm in

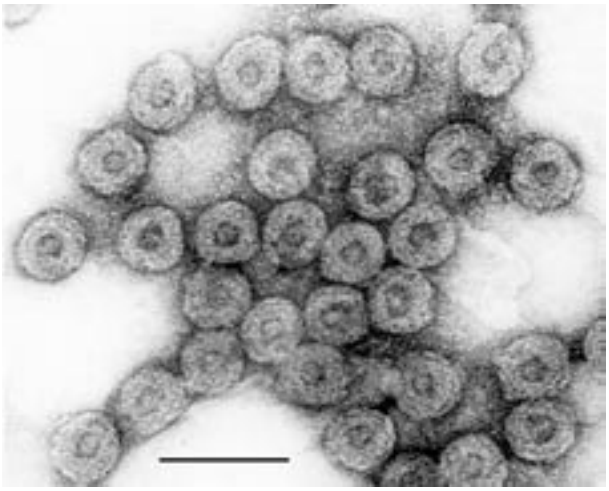

Figure 1. Electron microscopy of CaMV-C1 preparation. The scale marker is 100 nm

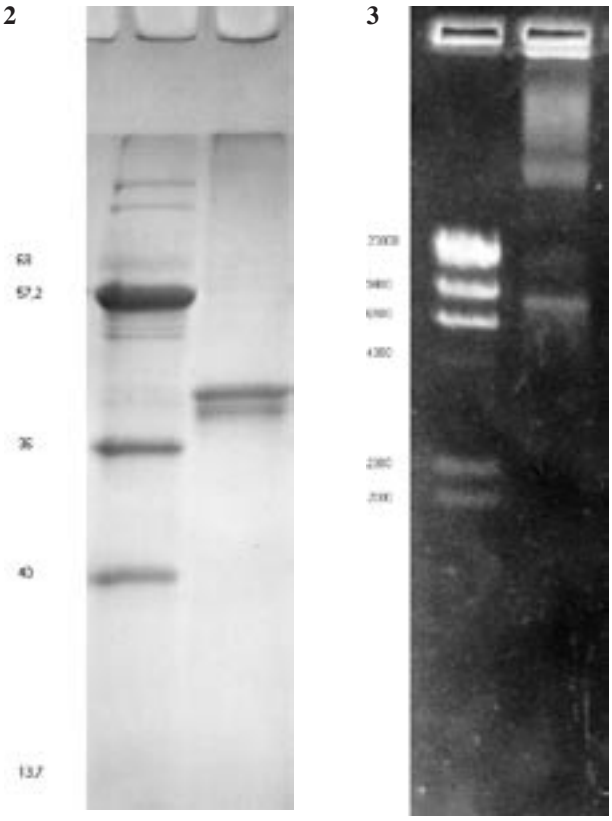

Figure 2. Polyacrylamide gel electrophoresis of coat protein of CMV-C1. Coomassie blue stain

Figure 3. Gel electrophoresis of the native DNA of CaMV-C1

diameter were found under the electronic microscope (Figure 1). Such morphology and size of the virus particles also are a typical feature for CaMV (XIONG *et al*. 1982).

The SDS-PAGE electrophoretic analysis showed 2 bands with molecular weight of about 42 and 44 kDa in the preparation CaMV-C1 (Figure 2). This electrophoresis profile was consistent with previous results, described in literature (BRUNT *et al*. 1975; AL ANI *et al*. 1979).

The antisera to CaMV-C1 have been produced and one gave precipitates out to dilutions of 1:64 (against partially purified virus preparations). Antigen relationship between the isolates was demonstrated while using the antisera

The native CaMV-C1 DNA have been isolated. In 0.9% agarose gel the DNA migrates as multiple bands (mixture of circular and linear molecules). The length of the DNA was about 8 kbp (Figure 3). These data are in accordance with ones, described in literature for another CaMV strains (HULL & COVEY 1983).

DISCUSSION

We would recommend to use several fundamental virology methods for identification of the new virus pathogenes. This is particularly important in case absence in country (in region) of the diagnostics kits. At the first stage of morphology and range of susceptible host plants were used. This stage is also importance as let us to prove virus aetiology of disease and to remove possible virus mixture. At the next stage properties of nucleic acid and virion protein (s), cell changes, antigenic characteristics are studied.

Basing on this point of view we identified of the isolates from cabbage and radish as *Cauliflower mosaic virus* – species of caulimovirus genera first not only in Far-Eastern territory, but in Russia.

Acknowledgements: We are most grateful D. V. FOMIN for help with the electron microscopy.

References

AL ANI R., PFEFFER P., LEBEURIER G. (1979): The structure of cauli ower mosaic virus. II. Identity and location of the viral polypeptides. Virology, **93**: 188–197.

BRUNT A., BARTON R., TREMAINE J., STACEMITH R. (1975): Composition of cauli ower mosaic virus protein. J. Gen. Virol., **27**: 101–106.

GNUTOVA R.V. (1985): Immunological Investigations in Phytovirology. Moscow, Nauka.

GIBBS A., HARRISON B. (1976): Plant Virology. The Principles. Edward Arnold, London.

HULL R., COVEY S. (1983): Characterisation of cauliflower mosaic virus DNA forms isolated from infected turnip leavel. Nucleic Acids Res., **6:** 1881–1895.

LAEMMI U.K. (1970): Cleavage of structural proteins during the assembly the head of bacteriophage T4. Nature, **227**: 680–685.

MORRIS T., MULIN R., SAHLEGEL D. *et al*. (1980): Isolation of Caulimovirus from strawberry tissue infected with Strawberry vein banding virus. Phytopathology, **70**: 156–160.

RICHINS R., SHEPHERD R. (1983): Physical maps of the genome of Dahlia mosaic virus and Mirabilis mosaic virus – two members of the Caulimovirus group. Virology, **124**: 208–214.

SHUKLA D.D., WOLF P., SCHMELZER K. (1972): Studies on viruses and virus diseases of cruciferous plants. VI. Effect of cabbage black ring and cucumber mosaic virus in mixed infection on seed production of radish. Acta Phytopathol. Acad. Sci. Hung., **7**: 315–324.

XIONG C., BALAZS E., LEBEURIER G. *et al*. (1982): Comparative cytology of 2 isolates of cauli ower mosaic virus. J. Gen. Virol., **6**1: 75–81.