

SEROLOGICAL AND ELECTROPHORETIC CHARACTERIZATION OF THE NECROTIC STRAIN CMV-NB OF CUCUMBER MOSAIC VIRUS

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Summary

Serological and electrophoretic properties of Bulgarian isolate CMV-NB causing tomato fruit necrosis were investigated. Its serogroup affiliation was studied by means of indirect ELISA with polyclonal and monoclonal antibodies as well as by immunodiffusion and immunoelectrophoresis with sera against subgroups I and II of CMV. Strain NB belongs to subgroup I but distinguishes from the other tested strains from this group in counter and rocket immunoelectrophoresis especially. The electrophoretic mobility of capsid protein and whole virions of NB was also compared with those of other virus strains. Strain NB was characterized with very bigger mobility of virus particles in electric field. It explained differences established in immunoelectrophoretic analysis. The obtained results supposed presence of mutations in CMV-NB, which led to increasing negative surface charges of virions.

Introduction

Cucumber mosaic virus (CMV) is a type representative of cucumovirus group. Its icosahedronic 30 nm diameter virions contain one kind of capsid protein with molecular weight approximately 24 kDa. CMV possesses three-component genome and a sub-genome RNA [5]. Some strains also contain another, fifth satellite RNA. It could significantly affect the induced symptoms and could cause, for example, tomato necrotic disease [12, 13, 29]. CMV is spread worldwide and it is presented by plenty of strains, which

are divided into two main serogroups or subgroups. The groups are denoted in a different way depending on the methods used: DTL and ToRS, U and N, I and II, WT and S etc. [3, 21, 22, 23, 24].

CMV is of great importance for the Bulgarian agriculture, having big influence on tomato yield especially. A series of research on the strain variety have been performed using indicator test, different serological methods and PCR [4, 9, 11, 15, 19, 27, 33]. From tomato plants cv. Carmelo, which have shown

stem and fruit necrosis, was isolated a strain denoted as CMV-NB [29]. This strain contains a satellite RNA, which results in attenuation to a different degree of the symptoms in some plant species and also in well-exhibited nec-

rosis on tomato stems and fruits [28].

The aim of the present research was a further characterization of the necrotic strain CMV-NB by means of serological and electrophoretic methods.

Materials and Methods

Viruses. The test object was the CMV-NB strain (NBIMCC 2126). The viruses used for control were: 1) Bulgarian CMV strains isolated from cucumber (Uo, 1k-92), tomato (MB, 146D), pepper (PB, 108P) and tobacco (TB, 131T) [28]. 146D and 108P strains belong to subgroup II and the rest ones to subgroup I [11, 19]; 2) American strains isolated from bean (7, subgroup Ia), pepper (26, subgroup Ib) and *Vinca major* (3, subgroup II) [2]; muskmelon (Fny, subgroup I) [25]; *Campanula rapunculoides* (GR, subgroup II). All the above-mentioned strains belong to the NBIMCC collection.

Virus multiplication. The CMV strains were propagated and maintained in *Nicotiana tabacum* cv. Nevrocop 1146 and tobacco cv. HT (containing N gene from *N. glutinosa*). Leaves with well exhibited systemic or local symptoms were used for further investigation.

Virus purification. The applied method was the one of Lot et al. [18] with some modifications. After double differential centrifugation the viruses were suspended in 0.01 M K-Na phosphate buffer, pH 7.2, containing 5 mM EDTA. The concentration was determined spectrophotometrically.

Antisera (AS). Four Bulgarian sera produced by the team were used as follows: homologous to subgroup I CMV strains - Uo [34], MB and PB and homologous to subgroup II - 146D [10]. Their titres in agar diffusion test were 1:8192, 1:512, 1:1024 and 1:4096 respectively. A German antiserum for serogroup N was applied too.

ELISA (enzyme linked immunosorbent assay). ACP-ELISA (antigen coated plate) according to Koenig [14] was performed.

1. With polyclonal antibodies (PABs) Uo and 146D. The antigen (plant sap) was

dropped in dilution of 1:25 in carbonate buffer, pH 9.6, and incubated for 16 hours at 4°C. The IgG were applied diluted 1:500 in PBS-T with 2 % PVP and 1 % OA for 2 hours at 35°C. Antirabbit peroxidase conjugate 1:1000 in PBS-T with 1 % OA was added for 2 hours at 35°C. The used substrate was orthophenylenediamine and the extinction was read at 492 nm.

2. With monoclonal antibodies (MAbs). MAbs for subgroup I (DTL), subgroup II (BD9) and not serotype-specific (CF11) were used [6, 7]. The scheme for ACP-ELISA was the same with one exception - antimouse peroxidase conjugate was applied.

Double radial diffusion according to Ouchterlony and Nilsson [20]. It was performed in 0.7 % agarose gel in 0.075 M veronal buffer, pH 8.6, containing 5 mM EDTA. The viruses were applied in the form of purified preparations (15 µg in a well) while the antisera - in dilution of 1:2 - 1:4. The gels were stained with Blue R-250 [32].

Immunoelectrophoresis. It was carried out in 1 % agarose gel in 0.075 M veronal buffer, pH 8.6, containing 5 mM EDTA, at 150 V and 10°C for 2 – 2.5 hours. The gel staining was the same as in the immunodiffusion.

1. **Counter immunoelectrophoresis.** The antisera were applied in dilution of 1:2 - 1:16 and the virus preparations were 5 µg each.

2. **Rocket immunoelectrophoresis** [32]. The gel contained 30 µl Uo antiserum or 35 µl 146D antiserum respectively. The antigens were also applied as purified preparations.

Electrophoresis of virus particles. It was carried out under the same conditions as the rocket immunoelectrophoresis without the application of the antisera.

Electrophoresis of virus protein. SDS-polyacrylamide gel electrophoresis (SDS-

PAGE) according to Laemmli was performed [16]. The gels were stained with Blue R-250.

Results and Discussion

For initial serological differentiation of the virus isolates ACP-ELISA with PABs and MABs was performed (Fig. 1 and Fig. 2). The reactions of 8 other strains of CMV are shown for comparison. When PABs were applied CMV-NB behaved as a strain from subgroup I. The extinction values with IgG Uo were more than four times as high as the ones with IgG 146D.

The results obtained after using MAb DTL

were similar to those with IgG Uo. With MAb BD 9 the strains from subgroup I showed lower values and the ones from subgroup II (146D, 108P, 3 and GR) - high values. CMV-NB did not differ from the other tested subgroup I strains. The application of MAb CF11, however, revealed higher extinction values for NB (0.443) compared to the other strains from subgroup I (Fig. 2) but CF11 was

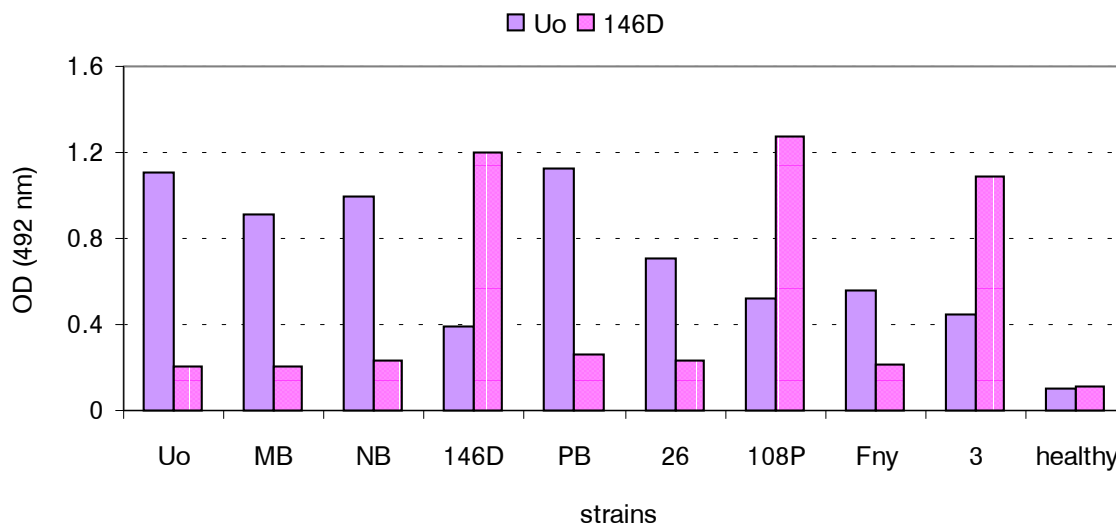


Fig.1. ACP-ELISA with polyclonal antibodies of CMV strains.

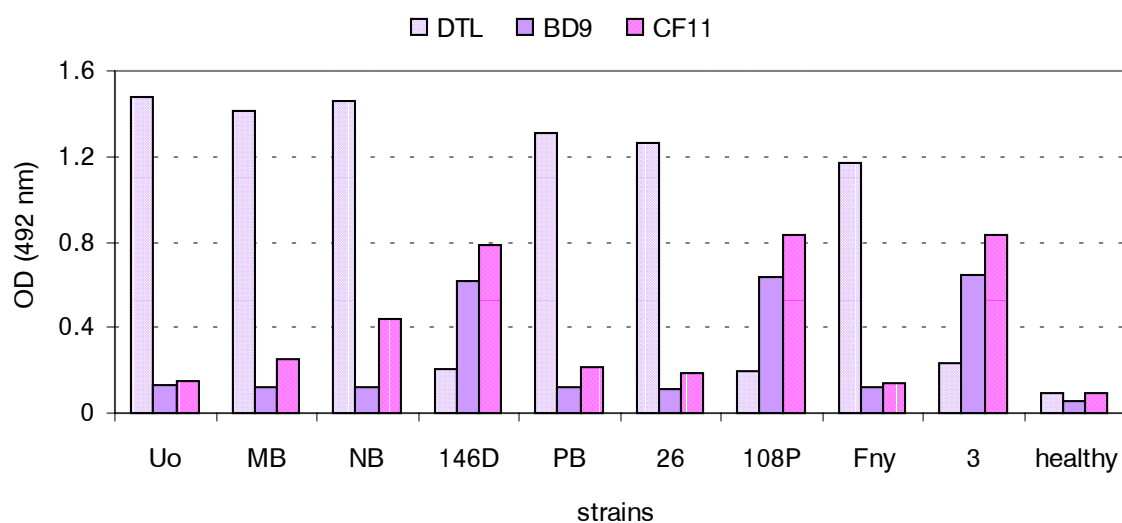


Fig. 2. ACP-ELISA with monoclonal antibodies of CMV strains.

not serotype-specific. Both MoAbs (BD9 and CF11) have been prepared for different CMV epitopes and have been used in combination for detection of serotype N [6, 7].

The serological relationship between NB and other CMV strains, belonging to subgroups I and II [11], was also investigated.

Antisera against MB, PB, Uo (subgroup I), 146D and N (subgroup II) were used. The data obtained from the double radial immunodiffusion, and also from the counter immunoelectrophoresis (for some of the sera) are summarised in Table 1. Part of the serological reactions is illustrated in Fig. 3.

Table 1. Serological relationship between CMV-NB and other CMV strains.

CMV strains subgroup		CMV-NB				
		AS MB	AS PB	AS Uo	AS 146D	AS N
Uo	I	+ ^a	+	+ ^b / ^c	+/(+)	-/+
4K-95	I	+			+	-/+
MB	I	+		+	+	-/+
146D	II	++	++	++, x/+	+/+	+/+
PB	I		+		+	-/+
108P	II	++	++		+/+	+
TB	I			+/+	+	-
7, 26	I		+	+	+	-/+
3	II	++	++	++, x/+	+/+	+/+
Fny	I	+	+	+	+	-/+
GR	II	++	++	++,x/+	+	+

^aspur formation from the control strains (+), spur formation from CMV-NB (++) , fusion of the precipitation lines (-), crossing of the precipitation lines (x); ^bimmunodiffusion; ^ccounter immunoelectrophoresis.

While tested with AS MB and AS PB the precipitation lines of NB formed spurs with ones of the strains 146D, 108P, 3 and GR from subgroup II. On the other hand, the tested strains from subgroup I produced spurs when NB was placed in adjacent well. The immunodiffusion with AS Uo also resulted in differentiation between NB and the other strains from subgroup I. CMV-NB demonstrated a well-expressed spur to subgroup II strains and, in some cases, crossing of the precipitation lines were observed. The immunoelectrophoretic analysis with AS Uo revealed different patterns. All the tested strains formed spurs towards NB.

The immunodiffusion with AS N determined NB as a strain from subgroup I. The precipitation lines fused when in the neighbouring wells Uo, 4K-95, MB, PB, TB, 7 and Fny were applied. The spurs with subgroup II strains were observed. In counter immunoelectrophoresis, however, all the tested strains produced spurs towards NB (Fig. 3).

The testing of NB with AS 146D revealed that the precipitation lines of 146D, 108P, 3 and GR formed well-exhibited spurs when both methods were carried out. A smaller spur was made from the subgroup I strains and it was better expressed in the double immunodiffusion.

The testing of the CMV control strains of one to another gave uniform results (Fig. 3). After placing AS I the precipitation lines of subgroup I strains formed spurs while after applying AS II the reaction was the opposite (the data are described in details in separate publications [11, 14]).

The obtained results show that CMV-NB belongs to subgroup I but distinguishes from all the other tested strains from this group. Depending on the titres of the added sera there are better or worse expressed serological differences between NB and the other CMV strains.

There is a possibility for this strain to belong to another (third) CMV serogroup. Devergne and Carding describe third serogroup (Co), which is closer to ToRS (subgroup II) than to DTL (subgroup I) [3]. Some

researchers include into third serological group the CMV strains, which do not differentiate from the representatives of either subgroup I or subgroup II according to immunodiffusion tests but behave as subgroup II strains in hybridization and ELISA [31]. The investigated Bulgarian strain NB can not be referred to any of these serotypes.

For further characterization of the NB serological relationship rocket immunoelectrophoresis with AS Uo and AS 146D was performed (Fig. 4 a, b). CMV strains did not divide into subgroups at the applied concentrations of the virus preparations. The American strains from subgroup II (3 and GR) showed weaker relationship (longer "rockets") with AS Uo but with AS 146D the precipitation figure was as those of Fny and 7. With the second antiserum the Bulgarian strains 146D and

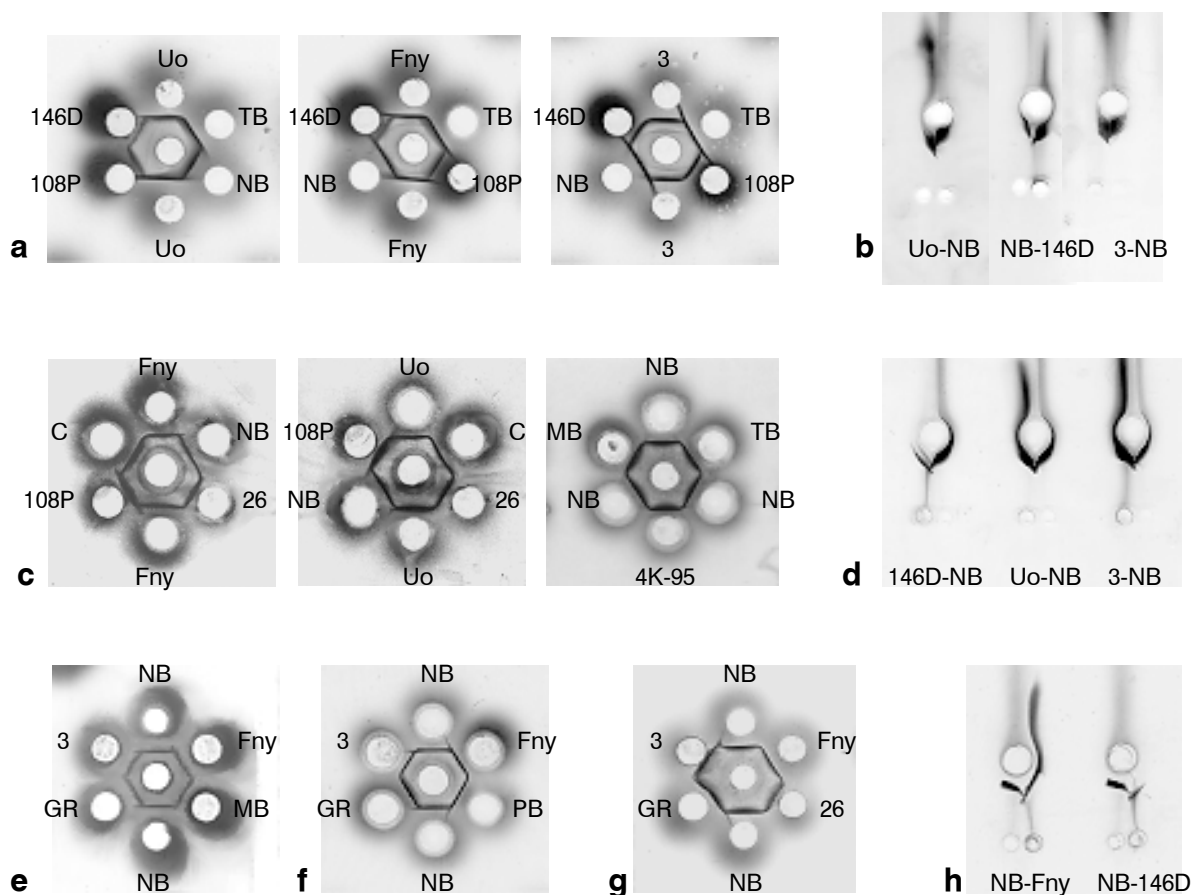


Fig. 3. Serological relationship between NB and other CMV strains with the application of AS Uo (a, b), AS 146D (c, d), AS MB (e), AS PB (f) and AS N (g, h). Immunodiffusion (a, c, e, f and g) - antisera in the central well; counter immunoelectrophoresis (b, d and h) - antisera in the upper wells.

108P from subgroup II exhibited the strongest reaction. The other viruses did not differentiate significantly. Both experiments, however, revealed big differences in the precipitation figures of NB - its "rockets" were twice as long. The electrophoretic patterns obtained as a result from the tests with both sera suggest that the weakest relationship exists between NB and the corresponding strains homologous to these sera.

The rocket immunoelectrophoresis data directed investigations to the testing electrophoretic mobility of the NB virus particles (Fig. 4, c). These results showed significant difference between the mobility of NB and the other tested viruses. The NB strain moved much faster than the other strains, which did not differ significantly. Only with Uo smaller electrophoretic mobility was observed. This

analysis turned out to be of big importance for the NB testing because in case of such big differences with the other strains the comparative immunoelectrophoretic analyses are considered to be unsuitable. This explains the NB behaviour upon rocket immunoelectrophoresis as well as the different position of the precipitation lines in the immunodiffusion and counter immunoelectrophoresis experiments. In the latter all the strains, regardless of their belonging to a certain group, produced spurs towards NB as a result of their smaller electrophoretic mobility.

In some other papers very small differences (up to several mm) in the CMV virion mobility are reported. According to Lot and Kaper the intact virions from subgroup II move faster than CMV-I [17]. In Hanada

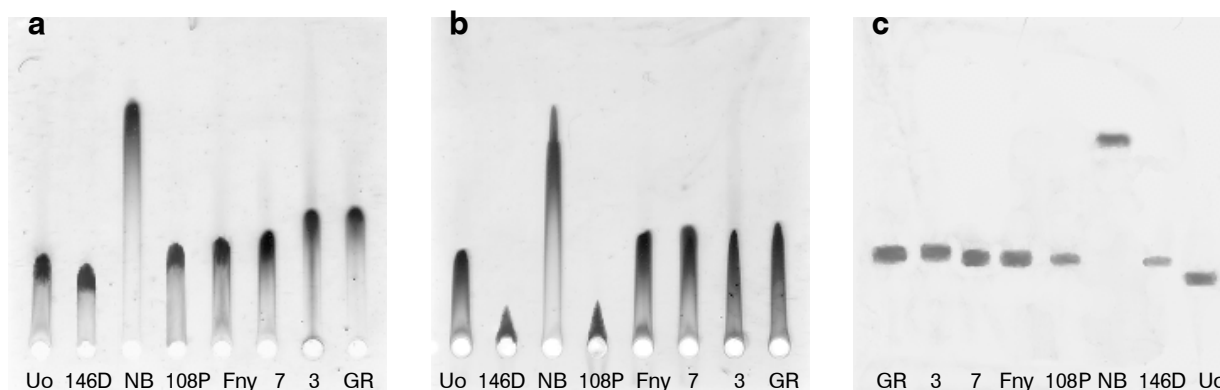


Fig. 4. Electrophoretic analysis of virus particles of CMV strains in agarose gel: rocket immunoelectrophoresis with AS Uo (a) and AS 146D (b); electrophoresis without antiserum (c).

experiments, however, the mobility of the virus particles does not correlate with their serological specificity [8]. In our research the migration of the virions in electric field is not connected with strain subgroup belonging.

The electrophoretic mobilities of the capsid proteins of the NB and the other CMV strains were also compared (Fig.5). The observed differences were rather strain specific. NB was from the strains with bigger electrophoretic mobility of the capsid protein. Close values were observed for GR and 3 (subgroup II) and for PB and Uo (subgroup I). Literature data for the capsid protein mobility

are contradictory. According to Daniels and Campbell the capsid protein of the isolates from subgroup II moves faster than the one from subgroup I [2]. According to other authors the results are the opposite [17]. These differences can be explained with the usage of different purification procedures. A bigger number of strains are required for such comparative research.

Based on the results of the electrophoretic analysis it can be concluded that there are single amino acid substitutions with acidic residues in the capsid protein of the NB strain. They are situated on the surface of

the virus particles. As a result negative charges accumulate on the intact virion surface, which changes significantly their electrophoretic mobility in the presence of the applied buffers (alkaline medium).

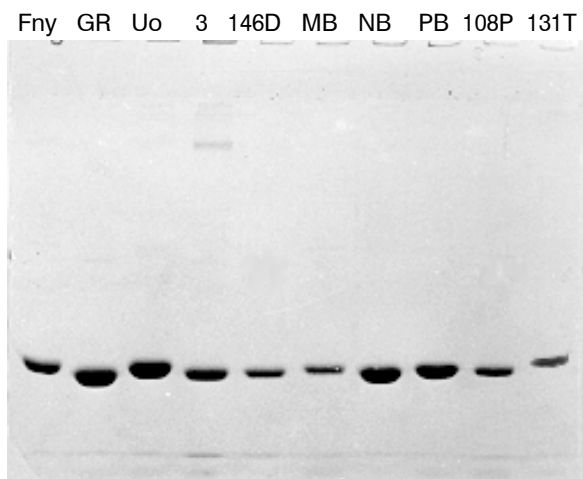


Fig. 5. SDS-PAGE of the capsid protein of CMV strains.

The research of the NB strain shows that it significantly differs from the other CMV strains. According to ELISA and the immunodiffusion tests it belongs to subgroup I, as well as according to RFLP-PCR [19] but it also shows a number of specific serological and electrophoretic properties.

The comparison between NB and the other

Bulgarian strain - 146D, isolated from tomato and causing tomato necrosis [11, 34], is of big interest. Both strains differ significantly not only serologically and electrophoretically but also with the symptoms they induce in indicator plants [27, Christova, unpublished data]. There are two tomato necrosis syndromes caused by CMV: tomato necrosis (or tomato lethal necrosis) and fruit necrosis [1, 30]. The first syndrome is caused by subgroup II strains while the second one involves some subgroup I strains, which normally don't provoke symptoms on the vegetative parts of the plants or induce weak symptoms. The presence of satellite RNA is also connected with the fruit necrosis [1]. 146D strain (subgroup II) provokes the first mentioned above necrosis syndrome while NB, containing satellite RNA [28] - fruit necrosis.

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СЕРОЛОГИЧНА И ЕЛЕКТРОФОРЕТИЧНА ХАРАКТЕРИСТИКА НА НЕКРОТИЧНИЯ ЩАМ СМV-NB НА ВИРУСА НА КРАСТАВИЧНАТА МОЗАЙКА

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Резюме

Изследвани са серологичните и електрофоретични свойства на българския изолат СМV-NB, предизвикващ некроза по плодовете на домати. Проучена е субгруповата му принадлежност чрез индиректна ELISA с поликлонални и моноклонални антители, както и чрез имунодифузия и имуноелектрофореза със серуми срещу I и II субгрупи на СМV. Щам NB се отнася към I субгрупа, но се отличава от всички тествани щамове от тази група, особено при насрещна и ракетна имуноелектрофореза. Сравнена е електрофоретичната подвижност на капсидния белтък и на цели вириони спрямо тази на групи щамове на вируса. Щам NB се отличава с много по-голяма подвижност на вирусните частици в електрично поле, което обяснява различията, установени при имуноелектрофоретичния анализ. Резултатите предполагат наличие на мутации при СМV-NB, довели до увеличаване на отрицателните повърхностни заряди на неговите вириони.