

ELECTROPHORETIC DIFFERENTIATION OF SOME TOBAMOVIRUSES

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Summary

Purified preparations of 16 tobamoviruses including 3 strains of tobacco mosaic virus (TMV) and 13 strains of tomato mosaic virus (ToMV) were investigated by the means of horizontal polyacrylamide gel electrophoresis (PAGE). Among the representatives of ToMV, the strains from group "2" after Pelham demonstrated the highest particle mobility and the ones from group "1", causing yellow mosaic, migrated most slowly in electric field. Besides that all the ToMV strains differed among each other, all they moved significantly quicker than the TMV strains. The horizontal PAGE was applied for virus detection in plant saps and for identification of unknown isolates. The electrophoretic analysis of viral particles had a good reproducibility and reliability of the results and proved a possibility for fast screening of a large number of isolates.

Introduction

The electrophoretic analysis is one of the methods for physicochemical characterization of plant viruses widely applied in virus proteins and nucleic acids studies. The electrophoresis of intact viral particles is however rarely used despite the fact that the type strain electrophoretic mobility is a part of the virus description [4, 2]. A correlation is established between the electrophoretic mobility and the biological properties of the viruses [1, 3, 18]. The electrophoretic methods can be used for identification, virus purification, differentiation of several viral agents in diseased plants, etc. [5, 12, 20].

Among the members of the tobamovirus group, the TMV, ToMV and pepper mild mottle virus (PMMoV) have a particular economic importance. Pelham divides the ToMV strains into the following groups:

0, 1, 2, 2² (2^a), 1.2, according to their ability to overcome the corresponding tomato genes for resistance [9]. The determination of this characteristic is performed by infection of tomatoes possessing the respective resistance genes, which is a continuous and labour-consuming process.

The electrophoretic mobility of intact viral particles depends on the superficial charges which are related to the amino acid composition of the capsid protein. Even an individual amino acid substitutions can change the total virus charge and can reflect on its mobility in electric field.

In the present paper the possibility of using horizontal polyacrylamide gel electrophoresis (PAGE) for differentiation, characterization and preliminary identification of different TMV and ToMV strains have been studied.

Materials and Methods

Viruses. Sixteen strains from the tobamovirus group were investigated (Table 1). The strains B and № 52 were isolated from tobacco and the rest - from susceptible or possessing different resistance genes tomato plants. The TMV strains were propagated in *Nicotiana tabacum* and the ToMV ones in *Lycopersicon esculentum*. The viruses were purified by polyethylene glycol [2] and their concentration was determined spectrophotometrically by the use of an extinction coefficient

($E^{0.1\%}_{1\text{ cm}}$) 3.1 at 260 nm [4, 22]. The isolates, marked as X, Y and Z, were identified during the research process. Plant saps, obtained by homogenisation of mosaic leaves in 0.01 M phosphate buffer, pH 7.2, dilution 1:5 to 1:10 (w/v), depending on the viral concentration, were also studied. The extracts were centrifuged at 10000 rpm for 15 min. The purified tobamovirus preparations with a concentration of 1 mg/ml were freeze-dried [21].

Table 1. Tobamovirus strains used.

NBIMCC №	Virus	Strains	Pelham's group	Propagation plants ^a	Symptoms ^b	References
tobacco						
2022	TMV	B	0	cv. SamsunN'	GM	15
2077	TMV	MA	0	cv. SamsunN'	GM	13
2023	TMV	№ 52	0	cv. SamsunN'	GM	15
tomato						
2074	ToMV	ENP	0	cv. Ideal	GM	13
2078	ToMV	GM-0	0	cv. Ideal	GM	16, 17
2021	ToMV	C-65	0	cv. Ideal	SL	16, 17
2024	ToMV	B-5	0	cv. Ideal	SL	16, 17
2064	ToMV	Lat-0	0	cv. Ideal	YM	c
2076	ToMV	SD	0	cv. Ideal	YM	13
2348	ToMV	YM-0	0	cv. Ideal	YM	16, 17
2349	ToMV	GM-1	1	cv. Mobaci	GM	16, 17
2114	ToMV	V-69	1	cv. Mobaci	SL	19
2111	ToMV	SO-69	1	cv. Mobaci	YM	13
2112	ToMV	GeBI	1	cv. Mobaci	YM	13
2123	ToMV	TMVj	2	cv. Moperou	YM	c
2113	ToMV	SG-64	2	cv. Moperou	YM	13

^a tobacco cv. Samsun N' possess resistance gene N' (from *N. sylvestris*); tomato cv. Ideal is a susceptible one and cvs. Mobaci and Moperou have resistance genes Tm-1 and Tm-2, respectively.

^b GM - green mosaic, YM - yellow mosaic, SL - symptomless.

^c supplied by H. Laterrot, France.

Electrophoresis. The native horizontal PAGE was done in a 3 % gel [11]. Ten microliter samples with a virion concentration of 1.5 – 2 mg/ml were applied. The gels were stained with Coomassie Brilliant Blue R - 250 [10]. The viruses with a lower concentration, particularly in saps, were found after densitometric scanning of the dried gels with Shimadzu thin-layer scanner CS-930. The $R_f = S/F$ was counted, where S was the distance passed by the sample and F

was the distance between the start and the front. The relative mobility of each of the strains was determined in relation to R_f of ToMV ENP which was accepted as 100 % and was applied as a control in each gel. All the experiments were performed in 3 replications and average R_f was obtained. Standard deviations for each strain and virus group were estimated. For strain TMV B ten replications on one gel were made and the value of both standard deviation and standard error were calculated [14].

Results

Electrophoretic analysis of the purified preparations of 16 strains and two unidentified isolates from the tobamovirus group were carried out. Significant differences were found between the mobilities of the TMV and ToMV particles (Fig. 1). The green and the symptomless

ToMV strains from both of the groups "0" and "1", as well as the yellow ones from group "0", were not distinguished by their R_f . The virions of the "1" strains, causing yellow mosaic, moved more slowly and those of the "2" group strains exhibited the highest electrophoretic mobility.

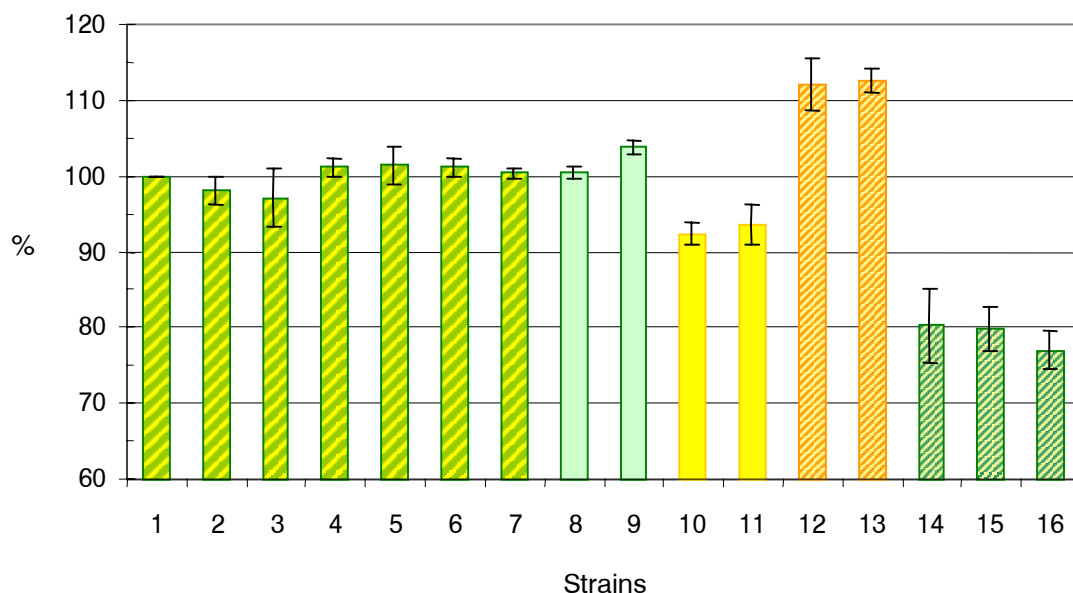


Fig. 1. Relative electrophoretic mobility of the tobamovirus strains. The average values of three experiments, as well as the standard deviation, are presented. R_f of ToMV ENP is accepted as 100%. Lines: 1, ENP; 2, GM-0; 3, SD; 4, C-65; 5, B-5; 6, Lat-0; 7, YM-0; 8, GM-1; 9, V-69; 10, SO-69; 11, GeBI; 12, TMVj; 12, SG-64; 14, B; 15, №52; 16, MA.

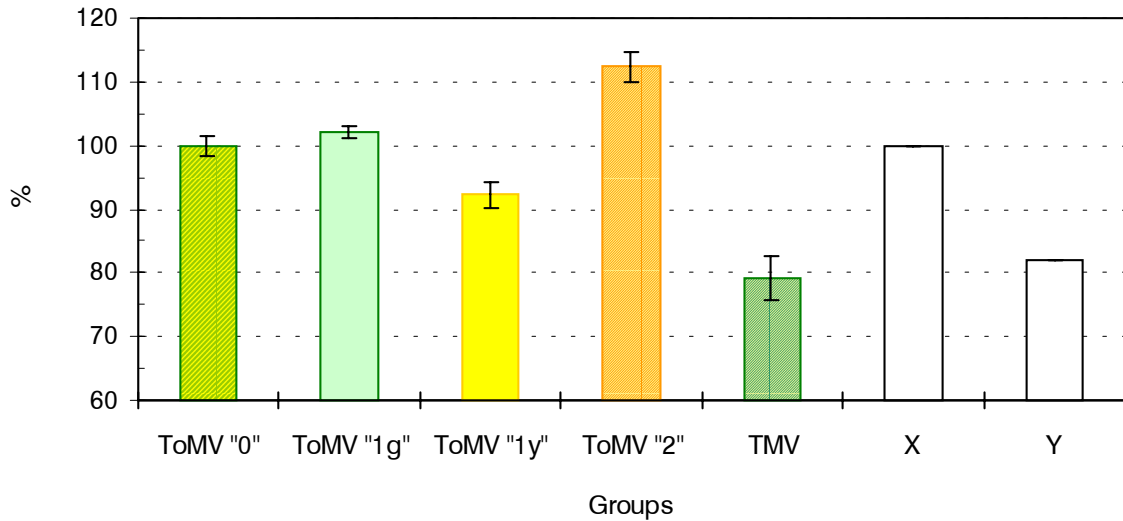


Fig. 2. Relative electrophoretic mobility of the ToMV groups and TMV. The presented values are average for all the replications and strains in each group. Standard deviations are shown. Rf of ToMV ENP is accepted as 100%. X and Y are unknown isolates.

Fig. 2 shows the average Rf - values for all the investigated Pelham's groups. Group "1" was divided into two subgroups: "1g" including the green and the symptomless strains and "1y" including the yellow strains. The established

differences between the electrophoretic mobilities were well enough expressed in order to distinguish the groups, except for the "0" and "1g" strains. The longest distance was between TMV and group "2" (Fig. 3A).

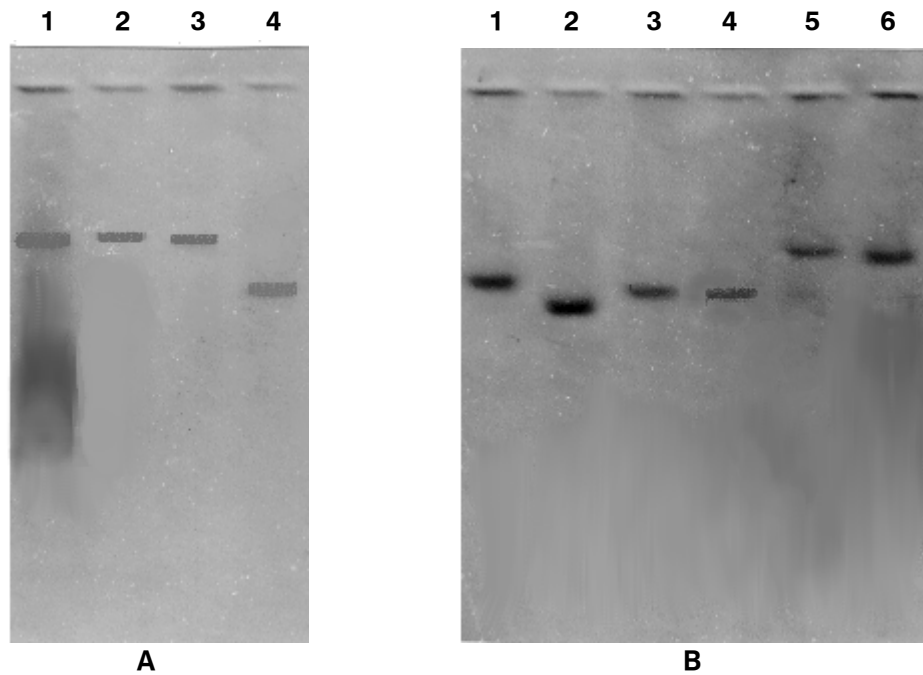


Fig. 3. Electrophoretic patterns of strains from tobamovirus group.
 (A) Lines: 1, TMV B in clarified sap; 2, TMV B after partial purification with polyethylene glycol; 3, purified TMV B; 4, purified ToMV ENP.
 (B) Purified preparations of: 1, ToMV SO-69; 2, ToMV SG-64; 3, isolate X; 4, ToMV ENP; 5, isolate Y; 6, TMV B.

The native PAGE was applied for rapid identification and differentiation of new isolates from the tobamovirus group. The purified preparation of two tobamoviruses causing yellow (X) and green (Y) mosaic were studied (Fig. 3B). The electrophoretic analysis of strain X and strain Y identified them as ToMV "0" and TMV respectively, as compared with the standards TMV - B and ToMV - ENP, SO-69 and SG-64.

The above mentioned experiments were carried out with purified virus preparations. The electrophoresis of whole viral particles can be applied for accelerated detection of

viral infection using plant extracts or semi-purified preparations. Most of the tobamoviruses accumulate in a significant degree in the plants. TMV- B, for example, was easily found in plant sap, as well as after a partial purification with polyethylene glycol (Fig. 3A). In case of an early stage of infection or low virus concentration a densitometric scanning is needed. The identification of an unknown isolate Z in sap from green mosaic plant is presented in Fig. 4. The standards used were freeze-dried preparations of TMV- B, ToMV-GeB1 and ENP. The isolate Z was determined as TMV.

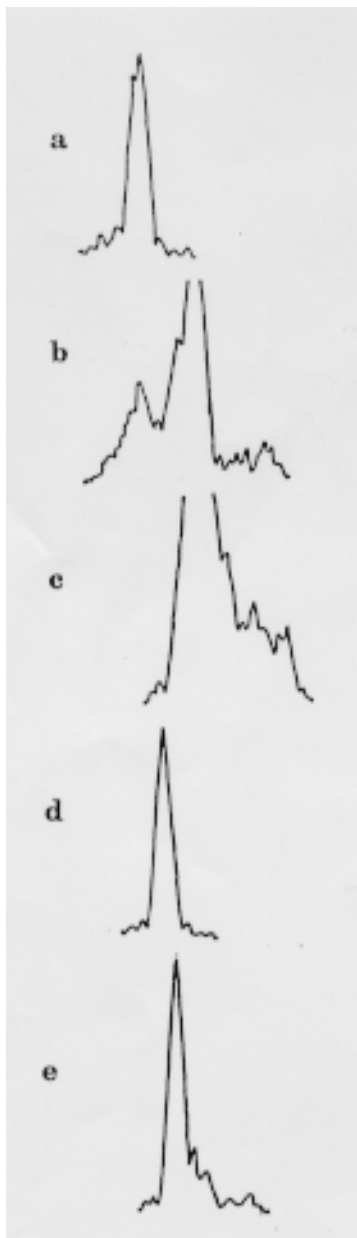


Fig. 4. Identification of unknown isolate Z by electrophoretic analysis of virus particles. Scanning at 600 nm:

- a. Purified TMV B;
- b. Isolate Z in clarified sap;
- c. Sap from healthy plant;
- d. Purified ToMV ENP;
- e. Purified ToMV GeBl.

The X, Y and Z isolates were tested on indicator plants and also by immunodiffusion for to establish their belonging to a certain virus and Pelham's group. According to the reaction on *N. sylvestris*, it was found out that X caused local lesions and Y and Z - mosaic symptoms. Therefore, the X isolate was determined as ToMV and the Y and Z ones were TMV. When

double immunodiffusion test was applied, the same conclusions were achieved (data not shown). The X isolate induced mosaic symptoms only in susceptible to TMV and ToMV tomato cultivars, and consequently it belonged to "0" Pelham's group. These results confirmed the data that we obtained by the electrophoretic analysis of viral particles.

Discussion

The investigated strains of TMV and ToMV groups had a distinct mobility and for that reason the PAGE could be applied for their rapid preliminary identification. The three replications of the electrophoretic analysis of the studied strains showed that the method was highly reliable. Among the different electrophoreses there were insignificant differences in Rf values for each strain. The ten replications of strain TMV - B on one polyacrylamide gel were done in order to see if the movement influencing factors had the same effect on the whole gel. The results for the distance run by TMV showed that a certain strain moved in one and the same way independently of the start situation. The calculations of both the standard deviation ($\sigma = \pm 0.003$) and the standard error ($m = \pm 0.010$) at average Rf of 0.311 for TMV B proved this conclusion. The use of freeze-dried preparations did not lead to any changes in the strains' electrophoretic mobilities.

The ToMV belonging to a given Pelham's group is a result of mutations in the genes coding for the RNA-dependent-RNA-polymerase ("1") or for the transport protein ("2") [6, 7, 8]. When we, however, found out reliable differences in the electrophoretic mobility of the yellow strains from the different Pelham's groups, we supposed that there were additional mutations in the gene coding for the capsid protein. The electrophoretic analysis also

allows to distinguish serologically identical strains [18]. These results correlated with the investigations of Garcia-Luque et al. (1990) on PMMoV [1]. They had also found that PMMoV-S and PMMoV-I overcoming the corresponding resistance genes in pepper (L^2 and L^3), were indistinguishable by host range and immunodiffusion test, but expressed different electrophoretic mobility.

The electrophoretic method requires small quantities of plant material and low virus concentrations can be detected after partial purification of samples or sap concentration by the means of lyophilization. The possibility for long-term storage of the freeze-dried purified virus preparations allows standard strains to be always available when necessary. The electrophoresis of whole tobamoviruses has a good reproducibility and authenticity of the results and makes possible the fast screening of a large number of isolates. Thus, the work with numerous plant species and sorts for preliminary identification of a given viral infection can be avoided. The isolates with established distinguishable electrophoretic mobilities can be put to investigations by the means of test plants as a final confirmation of the results. The advantage of the electrophoretic analysis over the serological and molecular genetic methods is that it does not require obtaining of sera or using of unique and expensive equipment and chemicals.

