

INVESTIGATIONS ON THE STRAIN VARIABILITY OF TOBAMOVIRUSES AND CUCUMOVIRUS ISOLATED FROM BULGARIA

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Summary

The biological properties of 4 tobacco mosaic virus (TMV) strains, 8 tomato mosaic virus (ToMV) strains, 1 pepper mild mottle virus (PMMV) strain and 5 cucumber mosaic virus (CMV) strains isolated from crop plants in Bulgaria have been described. TMV, ToMV (the first predominate) and CMV occurred in tobacco. The same viruses were found in pepper, and also the strain P₁₀₁ of PMMV have been isolated from cultivars possessing L gene. ToMV strains only from "0" and "1" Pelham's group and 2 CMV strains - mosaic and necrotic - occurred in tomato. CMV-NB possessing fifth satellite RNA caused yellow mosaic in tobacco, necrosis on tomato stems, leaves and fruits and was attenuated for cucumbers. Virus strains provoking green mosaic with an exception of CMV-NB were typical for the investigated cultures.

Introduction

Cucumber mosaic virus, tobacco mosaic virus and tomato mosaic virus are economically the most important phytopathogenic viruses in Bulgaria which fact determines the necessity of studying their strain variability in our country.

TMV [2, 29] is a type member of the tobamovirus group which, until 1976 combined 2 groups - of tomato and tobacco strains. The tomato strains localized by the gene N' (*Nicotiana sylvestris*) are separated as a single virus - ToMV [4]. As a result of common use of pepper cultivars with TMV and ToMV resistance gene (L), the so called pepper strains

detached as PMMV [25] appeared and got spread.

CMV as the most frequently occurring phytopathogenic virus is represented by many strains separated in 2 groups mainly on the basis of their serological properties. Investigations on the reasons causing systemic necrosis in tomato showed that it is a result of infection with CMV strains possessing satellite RNA [3].

Some biological properties of the TMV, ToMV, PMMV and CMV strains isolated from different crop plants and regions in Bulgaria are presented in this work.

Materials and Methods

Diagnostic species *N. glutinosa*, *N. sylvestris*, *N. tabacum* cv. Samsun, *Lycopersicon esculentum* cvs. Ideal and Mobaci; *Capsicum annuum* cvs. Albena and Danube, and *Cucumis sativus* cv. Gergana were used. CMV susceptible *N. tabacum* cv. HT possessing gene N of *N. glutinosa* and localising the TMV, ToMV and PMMV; cv. Samsun EN' with gene N' from *N. sylvestris* dividing TMV and

ToMV and *C. annuum* cv. Danube which is systemically infected only by PMMV were applied as differential host plants. The tomato lines Ideal (T_{m+}), Mobaci (T_{m-1}), Moperou (T_{m-2}) and Momor (T_{m-2}) were used to determine the belonging of the ToMV strains to Pelham's [10] groups.

All TMV strains were propagated in to-

bacco cv. Samsun EN' in order to avoid the contamination with ToMV strains. ToMV strains were multiplied in different tomato cultivars: "0" group - in Ideal, while "1" group - in Mobaci (T_{m-1}). The strains from "1" group were propagated in tomato possessing T_{m-1} resistance gene against TMV and ToMV because our experience showed that virus preparations, obtained from susceptible to those viruses tomato, contained also strains from "0" group. Strain P₁₀₁ of PMMV was multiplied in tomato cv. Ideal, and all CMV strains - in tobacco cv. HT.

Seeds of all plants were sown in pots with sterile soil and tobacco seedlings were picked off in sterile soil too. Tomato and pepper plants were infected at cotyledon - primary real leaf phase and the tobacco - at the 5 - 6 leaves phase using separate glass spatula for each plant.

Preparations of the tobamovirus strains were obtained after single-lesions passaged

infection. Leaves of tobacco cv. HT (gene N) were rubbed with the initial inoculum and the obtained local necrotic lesions were used to inoculate the corresponding propagation plants.

After disease development the leaves from all plants with equal symptoms identical to those obtained after inoculation with the initial isolate were collected. The tobamovirus strains purification was performed according to a standard method [3].

The diagnostic plants were infected either with preparations of the tobamovirus strains in concentration 0.01mg/ml or with CMV inoculums obtained after homogenization of mosaic leaves of tobacco infected with the corresponding strain in 0.01M potassium-sodium phosphate buffer, pH 7.0, supplemented with 0.1% Na₂SO₃ and 0.1% ascorbic acid, diluted 1:3 (w/v). The symptoms were recovered 7, 14 and 28 days after inoculation.

Results and Discussion

As a result of several years of investigations (1985-1989) only tobamovirus strains causing green mosaic were found out in tobacco. Regardless of the statements of Èivàchevski et al. [8] that the yellow strains occur more frequently in tobacco as compared with tomato, we did not succeed to isolate them. All tested isolates induced uniform symptoms in the diagnostic plants. The results obtained after inoculation of *N. sylvestris* that separates TMV and ToMV showed predominant spreading of TMV.

TGM strain of TMV was isolated from tobacco cv. Dzhebel 81, Plovdiv region and ToGM of ToMV - from tobacco cv. Plovdiv 7, cultivated in the region of Pazardzhik. Both strains were typical for TMV and ToMV populations appeared in tobacco. They caused green mosaic and depending on the host, the plant growth was depressed to a different extent (Table 1).

No52 was a TMV strain received from tobacco cv. Harmanliyska Basma 163, Plovdiv region. Practically all plants of this tobacco were infected with TMV but two ToMV isolates were also found. No52 caused mild green mosaic in tomato, tobacco and pepper, without influencing the plant development. Using series of passages in different host plants the attenuated strain MT-6 had been obtained. It was symptomless for tomato, to

bacco and sometimes caused mild mosaic in pepper (Table 1).

Tobamoviruses occurred frequently in pepper grown in polyethylene and glasshouses and were relatively rare for the field pepper. The samples from TMV and ToMV susceptible cultivars, mainly chilly pepper and cultivars possessing gene L₁ - Danube and Brainsma Wonder, were gathered in the period 1979 - 1986.

All isolates obtained from pepper provoked green mosaic in the systemically infected plants and suppressed their development to different extent depending on the species. Isolates of yellow strains were not found. TMV predominated in the glasshouse pepper susceptible to TMV and ToMV while ToMV isolates in most cases were found in plants infected also with TMV. TMV was obtained from pepper with systemic necrosis in the fruits in all cases [1]. ToMV strains predominated in the field pepper susceptible to both viruses. Mosaic, stem and fruit necrosis caused by TMV and ToMV strains appeared during April - June in pepper cultivars possessing resistance gene L₁. These strains were systemically multiplied in pepper with L₁ gene because this resistance is thermosensitive. Only green isolates of TMV and ToMV in relatively equal quantities were found in cv.

Table 1. Biological properties of Bulgarian strains of TMV, ToMV, PMMV and CMV

| strain | virus | isolated from | symptoms ^a | | | | | | p ^b | NBIMCC No | Ref. |
|------------------|-------|---------------|-----------------------|----------------------|------------------------------|------------------|-------------------|----------------------|----------------|-----------|---------------|
| | | | <i>N. glutinosa</i> | <i>N. sylvestris</i> | <i>N. tabacum</i> cv. Samsun | tomato cv. Ideal | pepper cv. Albena | cucumber cv. Gergana | | | |
| TGM | TMV | tobacco | LL | GM, St | GM, St | GM | GM, St, Df | | 0 | 3294 | 22,23 |
| No 52 | TMV | tobacco | LL | MGM | MGM | MGM | MGM | | 0 | 2023 | 24 |
| MT-6 | TMV | | LL | SL | SL | SL | (MM) | | 0 | 3295 | 24 |
| B | TMV | pepper | LL | GM | GM, St | GM | GM, St, Df, (N) | | 0 | 2022 | 5,24,27,28 |
| ToGM | ToMV | tobacco | LL | LL | GM, St | GM | GM, St, Df | | 0 | 3296 | |
| GM-0 | ToMV | tomato | LL | LL | GM, St | GM | GM, St, Df | | 0 | 2078 | 14,24,27,28 |
| YM-0 | ToMV | tomato | LL | LL | YM, St | YM | Y, St, Df | | 0 | 2348 | 9,24,28 |
| No 29 | ToMV | pepper | LL | LL | GM, St | GM | GM, St, Df | | 0 | 3297 | 5,11 |
| C-65 | ToMV | pepper | LL | LL | SL | SL | MGM | | 0 | 2021 | 5,6,11,13,24 |
| B-5 | ToMV | | LL | LL | (MM) | SL | MGM | | 0 | 2024 | 16,18,19,24 |
| GM-1 | ToMV | tomato | LL | LL | GM, St | GM | GM, St, Df | | 1 | 2349 | 14, 24 |
| YM-1 | ToMV | tomato | LL | LL | YM, St | YM | Y, St, Df | | 1 | 2347 | 24 |
| P ₁₀₁ | PMMV | pepper | LL | NI | NI | SL | MGM | | | 2115 | 5,11,13,15,24 |
| CMV-TB | CMV | tobacco | GM, St | GM, St | GM, St | Fl, Sf, Mf | Y, D, Mf, Df | GM, St, Mf, Df | | 3298 | 22,23 |
| CMV-MB | CMV | tomato | GM, St | GM, St | GM, St | Fl, Sf, Mf | Y, D, Mf, Df | GM, St, Mf, Df | | 2296 | 16,17,20,21 |
| CMV-NB | CMV | tomato | YM, St | YM, St | YM, St | N | Y, St, (Mf), (Df) | YS | | 2126 | 17 |
| CMV-PB | CMV | pepper | GM, St | GM, St | GM, St | Fl, Sf, Mf | N, Y, D, Mf, Df | GM, St, Mf, Df | | 2156 | 26 |
| CMV-CB | CMV | cucumber | MGM | MGM | MGM | Fl, Sf, Mf | Y, D, Mf, Df | GM, St, Mf, Df | | 3299 | |

^a-LL - local lesion, SL - symptomless, GM - green mosaic, MGM - mild green mosaic, MM - mild mosaic, YM - yellow mosaic, YS - yellow spots, Y - yellowing, Fl - fern leaves, N - necrosis, St - stunting, D - dwarfing, Sf - sterility of flowers, Mf - mosaic fruits, Df - deformation on fruits, NI - no infection, () - sometimes symptoms appeared;^b- Pelham's group: strains of "0" and "1" groups cause mosaic on susceptible tomato and "1" groups with the resistance gene T_{m-1} [10].

Danube [11].

TMV strain B was obtained from chilly pepper cv. Dzhouljunska Shipka with necrotic fruits grown in glasshouses, Pazardzhik region, and ToMV No28 strain - from cv. Danube plant with mosaic and necrotic symptoms (Table 1) grown in the glasshouses of Slivo pole village, Rousse region. The last strain did not differ from the ToMV isolates which are spread in susceptible pepper cultivars.

More than 80% of the plants of glasshouse pepper cv. Bruinsma Wonder revealed mosaic symptoms in 1979 [5]. All isolates obtained from mosaic plants did not differ in their biological properties and were described as strain P₁₀₁. Green isolates of TMV and ToMV that did not differ from strains B and No28 and P₁₀₁ isolates were found out in symptomless plants too. The isolate No65 obtained also from a symptomless plant Bruinsma Wonder differed significantly in its biological properties from the rest isolates and was described as strain C-65 of ToMV [5].

Tobacco cv. Samsun and tomato cv. Ideal plants subjected to mas and single-lesion-passaged inoculation with strain C-65 remain symptomless while in pepper plants inoculated in the same way mild green mosaic appeared. The inoculated tomato cultivars with different resistance genes were also symptomless and virus was found only in cvs. Ideal and Moperou (T_{m-1}). A pathogenic mutant of strain C-65 was not found out after numerous single-lesion-passaged and mas inoculations and continuing growth of the infected tomato plants of the same cultivars. Single-lesion isolates had been obtained and with each of them tomato plants cvs. Ideal and Moperou were inoculated. Thirty nine of the 50 tested local lesions infected cv. Ideal plants as 27 of them infected also cv. Moperou plants and 11 necroses were not reproductive. All plants remained symptomless until the end of the experiment [11].

The belonging of the strains ToMV to the Pelham's groups was determined by the symptom expression not by the ability for systemic virus multiplication but it is known that some strains from "0" group can propagate systemically in tomato possessing T_{m-1} gene without any mosaic. Keeping in view the above mentioned we put C-65 in "0" group (Table 1) because not all local lesions infected tomato cv. Ideal infected tomato cv. Moperou. Also, the virus concentration in the

second cultivar was very low.

The attenuated strain B-5 which effectively protected tomato from the pathogenic strains of ToMV and TMV had been obtained after series of passages of C-65 in tomato [16,18, 19].

P₁₀₁ described as a pepper strain of ToMV [5, 15] is referred to PMMV according to the new distribution of the viruses in tobamovirus group (Table 1). P₁₀₁ did not differ from TMV and ToMV strains in virion morphology, stability of the virus in sap and preparation, and in temperature inactivation but after treatment with HNO₂ it is inactivated 4 times faster than the strain B and No28 [12, 15]. P₁₀₁ multiplied in *N. glutinosa* and tobacco cv. HT considerably slower than TMV and ToMV strains. When leaf-halves of tobacco cv. HT were simultaneously infected with P₁₀₁ and strains of TMV or ToMV the local lesions of the first strain were smaller and appeared on the 6th day while those of TMV and ToMV - on the 3rd one. The lack of symptoms and systemic infection of tobacco cvs. Samsun, Samsun EN' and *N. sylvestris* after inoculation with P₁₀₁ differentiated this strain not only from TMV and ToMV but also from the well-known up to now strains PMMV [11, 25]. The main property which characterized it as a PMMV was its ability to overcome the resistance of L₁ allele of gene L in pepper. P₁₀₁ was localized by the rest alleles of the same gene [7, 11, 12].

An investigation of glasshouse and field tomato has been done in the regions of Vidin, Vratza, Kyustendil, Plovdiv, Pazardzhik, Sofia and Haskovo [14, 17] during 1980 - 1993. The analyses showed that all isolates were ToMV which fact confirmed the data of Kovachevski et al. [8]. These isolates caused green mosaic in ToMV and TMV susceptible tomato, pepper and tobacco cultivars. Isolates of "0" group which did not differ from strain GM-0 had been isolated incidentally from resistant to this virus tomato in mixed with CMV infection [13, 17, 19].

GM-O was isolated from tomato cv. Triumph grown in the town of Bregovo, Vidin region (Table 1) and possessed the same biological properties as the rest of the isolates. No significant differences in the biological properties of GM-O and ToGM were registered. The strain YM-O obtained from a single plant with yellow mosaic of cv. Robin seedling

was produced from Dutch seeds in private glasshouse in Rashka Grashitza village, Sofia region in 1991. The yellow mosaic developed in the infected with this strain tomato, tobacco and pepper and the delay in the plant growth was more weakly expressed in comparison with those infected with GM-O and ToGM strains.

Tomato cvs. Vihra, Bella, Venera and Lira had been investigated during 1989 - 1992 and only isolates of ToMV "1" group causing green mosaic had been obtained. Strain GM-1 was isolated from cv. Venera, town of Knezha, Montana region and was a typical representative of the studied populations. Green mosaic developed in tobacco cv. Samsun, tomato cvs. Ideal (T_{m+}) and Moperou (T_{m-1}) and pepper cv. Albena after infection with this strain, while necrosis appeared in *N. sylvestris* (Table 1).

A plant with yellow mosaic from which ToMV YM-1 was isolated had been found in different wild species accessions of genus *Lycopersicon* in the experimental field of the Institute of Genetics. This strain diversified from YM-O only in its ability to cause yellow mosaic also in tomato cv. Moperou (T_{m-1}) which fact determined it as a strain from "1" group (Table 1).

The distribution of CMV in field tomato in the same regions where the TMV and ToMV samples were gathered had been studied during 1986-1993. Isolates of CMV from tomato with typical for the virus symptoms (fern leaves and stunting) had been obtained many times. No difference in their biological properties had been found and an isolate from field tomato, cv. Balka grown in the village of Bardarski geran, Vratza region had been named as CMV-MB.

This strain caused green mosaic and stunting in *N. glutinosa*, *N. sylvestris* and *N. tabacum* cv. Samsun (Table 1). Weak chlorosis and deformations mainly distortion of the leaves, latter appearance of fern leaves and sterile flowers as a consequence of the changes in flowers morphology and vitality of pollens [9] appeared on the tomato plants after infection with CMV-MB. The fruits of the disease plants were small and often mosaic. CMV-MB caused chlorosis, dwarfing and appearance of deformed and mosaic fruits in pepper. In infected cucumbers, this strain induced green mosaic and deformations of leaves, yellow mottle in fruits and stunting (Table 1).

Several necrotic disorders in tomato often killing whole plants or part of them and causing black necrosis on fruits of the affected plants were observed in many regions of Bulgaria in 1986. This epiphytoty recurred on a larger scale in 1989 and since then it had become annual. It is found out that the disease was caused by necrotic strain of CMV [17]. The isolate obtained during the same year from tomato cv. Karmello grown in the field of Banitza village, Vratza region, was named CMV-NB. It provoked yellow mosaic and suppressed plants in weaker extend than CMV-MB in all tobacco species and cultivars. The mechanical inoculation of tomato with CMV-NB was considerably more difficult than the other CMV strains and the typical for the strain symptoms appeared later. Often the infected plants were symptomless but black necrosis in the fruits appeared just before ripening. CMV-NB did not cause sterility of tomato flowers. Pepper symptoms were milder than those in the other CMV strains and rarely effected the fruits. CMV-NB was attenuated for cucumber where single yellow spots appeared on the leaves (Table 1).

CMV-TB was isolated from tobacco cv. Neurokopska Basma B-12 grown in the village of Kozarsko, Plovdiv region in 1991. It caused green mosaic in tobacco, tomato and cucumber and chlorosis in pepper (Table 1).

CMV-PM obtained from seed born infection of pepper plant cv. Zlaten Medal did not differ significantly from CMV-MB in its biological properties but was especially pathogenic for pepper and often killed it due to systemic necrosis. If resistant to the virus pepper is grown at temperature higher than 35°C necrotic local lesions will be formed on the infected leaf which was not observed in the rest CMV strains (Table 1).

Twelve CMV isolates with equal symptoms in the diagnostic and differential host plants have been obtained from seed-producing region of cucumber cvs. Svezhest and Dessislava in Experimental Station for Vegetable Crops, Gorna Oriahovitza in 1995. CMV-CB was isolated from mosaic fruit cv. Svezhest. It caused relatively mild mosaic in tobacco without suppressing the plant development which distinguished it from the rest of the CMV strains (Table 1).

The strain variability of viruses of the tobamovirus and cucumovirus groups in tomato have been studied for 14 years and all tomato cultivars, grown in glasshouses and fields in

different regions of Bulgaria have been investigated. Among viruses of tobamovirus group only green isolates from "0" and "1" Pelham's group of ToMV have been found. The two yellow isolates were obtained either from import seeds with seed infection of ToMV (YM-O) or from experimental field where wild tomato species with different origin of the seeds (YM-1) were been grown. Consequently, they were not typical for the virus population in Bulgaria. Among viruses of cucumovirus group the isolates of 2 CMV strains - mosaic and necrotic possessing satellite RNA - have been registered.

In pepper, 3 viruses of tobamovirus group were spread. TMV and ToMV isolates causing green mosaic were pathogens often found in the glasshouse pepper susceptible to these viruses. The TMV isolates predominated in our investigations, whereas according to Kostova [5] who performed the same studies also in other cultivars 3-4 years later, ToMV strains predominated. In pepper cultivars resistant to the two viruses PMMV had been observed while TMV and ToMV caused sys-

temic necrosis in the plants at increased temperatures. ToMV occurred more frequently than TMV in the field pepper but were considerably rare in the glasshouse pepper.

TMV and ToMV isolates causing green mosaic were found in tobacco and the second ones were rare. No tobamoviruses were isolated from cultivars possessing resistance gene N.

The investigations of CMV in pepper, tobacco and cucumber were made only once and this fact did not bring us to conclusions about the strain variability of the virus in these cultures. However, the cited strains were isolated from typical for the culture regions, from considerable area and at a large scale virus distribution (tobacco); from seed-producing glasshouse (cucumber) or from commercial seeds (pepper).

All described in the present study TMV, ToMV, PMMV and CMV strains are deposited in the National Bank for Industrial Microorganisms and Cell Cultures (NBIMCC) [1] and can be obtained from there.

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