

## EFFECTIVENESS OF CROSS PROTECTION IN THE PRESENCE OF MIXED VIRUS INFECTION

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(Submitted by Corresponding Member D. Shabanov on August 10, 1994)

Cross protection is being used successfully for the control of (tomato mosaic virus) ToMV in glasshouse and field tomato crops [1,3,4,6]. In the past decades incidences of (cucumber mosaic virus) CMV were very frequent and raised the question whether this method was effective with mixed virus infection.

The aim of the present investigation was to test effectiveness of ToMV strain B-5 with proven good protected properties in infected with CMV tomatoes.

**Material and methods.** Tomato cv. Ideal, susceptible to ToMV and CMV, 2 strains of ToMV — B-5 and GM-O, and 1 strain of CMV — CMV-MB were used. B-5 is a natural symptomless strain of ToMV, "0" Pelham's group, possessing good protective properties against many ToMV and TMV (tobacco mosaic virus) strains [5]. Both pathogenic strains causing typical ToMV and CMV symptoms are isolated from field tomato crops and are characteristic of their population in Bulgaria.

The experiments were carried out on field conditions in 1990-1992 and included 8 variants, each in 4 replications with 10 plants in a replication, by the Latin square pattern. Results from tomato yields were subjected to a variance analysis [2]. Desinfected tomato seeds were sown in pots with sterile soil and the seedlings were grown without picking off. The plants were inoculated with B-5 (concentration 0.01 mg/ml) at the cotyledon-first real leaf phase, using a separate glass spatula for each plant. After 7 days tomato plants were infected with purified virus of GM-O at the same concentration. Inoculums of the CMV-MB were prepared prior to rubbing by homogenising CMV mosaic tobacco leaves in 0.01 M phosphate buffer, pH7, diluted to 1:10 (w/v). The plants were inoculated threefold during different phases of their development: at the third-fourth leaf in 1990; beginning of flowering before planting in 1992 and the appearance of the first fruit after planting in 1991. Inoculation during the first year is carried out every second day, and during the other two years — in three successive days. Since ToMV spreads in a mechanical way, planting, removal of suckers, binding and picking of fruit were done with gloves changed for each variant. All agrotechnical requirements adopted for undeterminate tomato field cropping were observed.

**Results and discussion.** Control plants and those protected with B-5 and infected or not with GM-O remain symptomless till the end of vegetation. The ones inoculated with GM-O develop more slowly and 14-18 days after infection green mosaic appeared. In the experiment carried out in 1990 plants from all variants inoculated with CMV were extremely depressed and had symptoms typical for the virus regardless of the fact whether they had been inoculated with ToMV or not. Almost all flowers were sterile due to the early CMV symptoms. In the other two years the sterility was observed only in the flowers situated on the level of leaves with typical CMV symptoms. As

## 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 P<sub>101</sub> 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.*

СЕЛСКОСТОПАНСКА АКАДЕМИЯ

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## **CUCUMBER MOSAIC VIRUS CAUSING LETHAL NECROTIC DISEASE IN FIELD TOMATO**

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### **ABSTRACT**

The necrotic strain of cucumber mosaic virus (CMV) is a causal agent for tomato necrosis and tomato mosaic virus is an attendant infection which does not change CMV disease.

### **INTRODUCTION**

In the past decade the wide spread of a necrotic disorder in field tomato is the most important disease on this crop in Bulgaria.

According to data in literature necrotic disorder may result from double streak (4) or spread of a necrotic strain of cucumber mosaic virus (CMV) [3, 6, 7, 13, 14]. Tobacco mosaic virus (TMV) and potato virus X (PVX) or TMV and CMV caused double streak on tomato [2, 4]. Kovacevski described a CMV necrotic strain and a new virus closely related to CMV - Marmor cucumeris var. lycopersicovastans (MCL) that provoked necrotic disease on tomato plants [1, 2, 4]. Recent studies establish that CMV strains containing fifth satellite RNA (sat RNA) cause tomato necrotic disease in many regions in the world [6, 7, 13, 14].

The aim of the present study is to clarify the agent causing necrotic disease in field tomato which appears nowadays in Bulgaria.



## LINES FROM INTERSPECIFIC HYBRIDIZATION RESISTANT TO TOMATO SPOTTED WILT VIRUS

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(Submitted by Academician N. Tomov on February 1, 1995)

Tomato spotted wilt virus (TSWV) cause serious economic losses in many food and ornamental crops worldwide [6,7]. The losses in tomato market (*Lycopersicon esculentum* Mill) due to TSWV reduces production by 50-90% [2].

Tomato spotted wilt virus was reported by KOVATCHEVSKI [1] in Bulgaria. This virus was persistently transmitted by thrips, which determines the great losses of tomato grown near ornamental and tobacco planting. The most promising and the most effective economic method for controlling this disease is development of tomato disease resistant cultivars and hybrids and their introduction into tomato production.

There is little information in literature about TSWV resistance of some wild species as *L. pimpinellifolium* Mill. [4], *L. peruvianum* (L) Mill., *L. chilense* Dun. and *L. pennellii* Corr [3]. Though sources of resistance to TSWV have been discovered no commercial cultivars for tomato production have been created up to now.

In the present paper data are presented for resistance to TSWV in tomato lines obtained with the participation of tomato wild species.

**Material and methods.** A series of lines obtained with the participation of *L. pimpinellifolium*, *L. pimpinellifolium* f. *galapagos* Rick, *L. cheesmanii* var. *minor* (Hook) Mull. and *L. peruvianum* var. *humifusum* Mull. of Geosem were tested for resistance to TSWV. Cultivars Merkourii and Venera, known as susceptible to TSWV were used as a control.

Lines with *L. pimpinellifolium* are determinate, vigorous in habit and leafy. The fruits are round, two lobed, good in taste and with an average weight of 80-100 g. Lines with *L. pimpinellifolium* f. *galapagos* and *L. cheesmanii* var. *minor* are determinate. The leaves are large, light green in colour. The fruits are two located, beta orange, round and with average weight of 80-90 g. They possess high  $\beta$ -caroten content.

Lines with *L. peruvianum* var. *humifusum* are indeterminate in habit. The leaves are large, dark green, very thick, almost not serrated. The fruits are round, very firm, two lobed, with a green ring, red in colour and with an average weight of 60-70 g.

Eighty seeds from each line were sown in two repetitions. The obtained plants were grown at high thrips density (Western flower thrips). It is a vector for infection with TSWV as previously established. We observed visually thrips migration in each tomato seedlings. The presence of high infection background with thrips was confirmed by the infection in over 90% of the control plants in seedling phase. Forty plants from each line were planted in the field. The reaction of each plant from the infected lines was evaluated as resistant or susceptible depending on the presence or absence of symptoms [3].

## DISEASE MODULATION ON SEVERAL PLANTS BY CUCUMBER MOSAIC CUCUMOVIRUS PLUS SATELLITE RNA ISOLATED FROM TOMATO IN BULGARIA

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(Submitted by Corresponding Member A. Mehandziev on April 10, 1996)

CMV (cucumber mosaic virus) is the most frequently found one in tomato and inflict considerable losses [1-7]. The typical symptoms provoked by CMV in tomato are: stunting, fern leaves, deformation and sterility of flowers, mosaic and small fruits [3-4].

During the period 1958-1959 Kovachevski [2] described in Sofia region the appearance of a new disease in tomato named "veinal necrosis" which was observed later (1966, 1970, 1971) also in Plovdiv region [1]. The disease is caused by a virus described as MCL (*Marmor cucumeris* var. *lycopersicovastans*), closely related to CMV [2,3]. Tomato plants with "veinal necrosis" disease have been found incidentally in 2 near-town regions on limited area. Although the affected plants are not suitable for production of market tomato, the disease is not economically important.

Since 1986 severe necrotic disorder of epidemic proportion has been observed in field tomato from many regions of the country [4]. This disease is widely spread in tomato cultivars susceptible and resistant to ToMV (tomato mosaic virus). ELISA tests have shown that the samples from disease plants possess CMV and ToMV. Surprisingly, a great number of samples from resistant to ToMV tomato have both viruses. All ToMV isolates belong to Pelham's "0" group [3] and infect systemically only susceptible to this virus tomato [4]. The symptoms described are similar to those caused by the interaction of TMV (tobacco mosaic virus) and CMV, known as "double streak" [1,2]. The study has proved that the disease is provoked by necrotic CMV strain and and ToMV as an attendant infection does not change CMV symptoms in tomato [4]. Keeping in mind that from many regions all over the world necrotic CMV strains, possessing fifth satellite RNA and causing tomato necrotic disease [6-10] have been isolated, it is necessary to perform more detailed investigation of the biological properties of the isolated Bulgarian necrotic strain and its genome.

The aim of the present study was comparative investigation of some biological properties of the necrotic CMV strain and typical mosaic strain and checking the presence of satellite RNA in the first one.

**Materials and methods.** Several biological properties of 2 CMV strains isolated from field tomato had been tested. CMV-MB was obtained from tomato with typical symptoms of cucumber mosaic disease and CMV-NB — from commercial tomato cv. Karmello with stem and fruit necrosis [4]. CMV-PB was isolated from pepper with seed born infection [11] and used as a marker in the determination of the number of CMV-NB RNAs.



## CYTOCHEMICAL INVESTIGATION OF TOMATO ANTHER ABNORMALITIES AFTER CUCUMBER MOSAIC VIRUS (CMV) INFECTION

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(Submitted by Corresponding Member A. Mehandjiev on October 10, 1997)

Biochemical investigations of the host responses after virus infection showed changes in photosynthesis, respiration, and secondary metabolite accumulation [1]. The alterations in the oxidative pentose-phosphate pathway (PPP) connected with virus multiplication [1,9] as well as of peroxidase (POD) activity which is involved in the plant stress responses were reported as well [5,7]. Little information is available about the metabolic shifts in floral organs and disturbances of reproductive processes after virus infection [6].

The purpose of the present research was to investigate the changes in the metabolism of anthers, meiocytes and mature pollen grains of CMV infected tomato plants by the use of cytochemical methods.

**Material and methods.** Anthers of *Lycopersicon cheesmanii typicum* plants after infection with CMV strain CMV-MB were used. The plants were inoculated mechanically in the phase of cotyledons – first true leaf.

Free floating frozen cross sections of anthers 50  $\mu$ m thick were prepared and used in the cytochemical investigation as follows: the dehydrogenases (isocitrate dehydrogenase – IDH, glutamate dehydrogenase – GDH, alcohol dehydrogenase – ADH, lactate dehydrogenase – LDH and glucoso-6-phosphate dehydrogenase – G6PDH) were demonstrated by the method of tetrazolium reductases. Cytochrome oxidase (CO) was proved by NADl reaction, POO – by the method of Graham and Karnovski with diaminobenzidine tetrahydrochloride and polyphenoloxidase (PO) – with DOPA.

Hydrolases – esterase (E) and acid phosphatase (AP), were investigated by the method of simultaneous azocopulation [4]. The viability of pollen was monitored using fluorochromatic (FCR) test [2]. The effect of CMV infection on the pollen was estimated by determination of both staining intensity and percentage of pollen grains with positive reaction. Pollen samples were assessed by observing more than 300 pollen grains.

**Results and discussion.** The cytochemical investigation of IDH in CMV infected tomato anthers showed that the cells of the middle layer of the anther wall were deeply stained compared with the non-infected controls. The meiocytes of infected tomatoes exhibited higher enzyme activity than the control. Notwithstanding, the percentage of pollen grains with positive cytochemical reaction for IDH diminished. Whereas in

## ACCELERATED STORAGE TEST OF TOBAMOVIRUSES IN FREEZE-DRIED LEAVES

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(Summary)

The preservation of 11 tobamovirus strains, belonging to tobacco mosaic and tomato mosaic viruses, after freeze-drying in leaves was investigated. Comparative quantitative studies on the strains' survival were carried out by the use of accelerated storage test (at 28 and 37°C) for a period of 120 days. The survival of the most of the strains lowered to a great extent (of 26-67%) until the 30-th day, while during the next 30 days the decrease was of no more than 13,5%. A peculiar selection of the most stable virions for each strain was realised.

The resistance after freeze-drying was strain specific and the virus preservation, when stored, had a greater importance for the general infectivity than the survival right after lyophilization. In tomato mosaic virus, a certain relation between the symptomatology and stability of the strains was observed, as yellow strains were preserved better in lyophilized form. The accelerated storage test was a convenient approach for collection activities when plant viruses were freeze-dried, as it provided a preliminary characteristic for the strains' stability. A treatment at 28-37°C for about 30 days is suitable for the tobamovirus group.

# **EFFECT OF CRYOPROTECTION ON PRESERVATION OF LYOPHILIZED IN SAP TOBAMOVIRUSES**

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*(Summary)*

*Four strains of tobacco mosaic virus (TMV) and seven ones of tomato mosaic virus (ToMV) were freeze-dried in plant sap, without and with the protecting medium - 5% sorbitol and 3,6% dextran 40 000. Accelerated storage test at elevated temperatures was applied and the survival of differentiating in biological properties tobamoviruses was tested. The relative infectivity, respecting only the influence of storage, as well as the real infectivity in relation to nonlyophilized virus samples, was determined. The strain survival right after lyophilization did not certainly correlate with the resistance during the storage period. ToMV strains causing yellow mosaic proved to be the most stable after storage, whereas the isolated from tomatoes TMV strains were among the most susceptible ones. The application of protecting medium was of significant importance for the resistance of freeze-drying increase and for the longevity of the viruses in lyophilized saps. A quantitative valuation of the effect of the cryoprotectant, freeze-drying and storage was made. The importance of the three factors was strain specific and the protecting medium and the storage conditions were the most essential for the virus infectivity after lyophilic preservation in plant saps.*



## 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.*

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# TOBAMOVIRUS STRAIN P101 ISOLATED FROM PEPPER IN BULGARIA

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## ABSTRACT

*The tobamovirus strain P101 was isolated from pepper growing in greenhouse in Bulgaria. It does not differ from the other tobamoviruses in virion morphology, stability in sap, preparations, dried and frozen leaves as well as in the thermal inactivation point. The ratio  $A_{260}/A_{280}$  of P101 purified preparation is 1.3 and it is higher than ones for other viruses from the group. The strain P101 has close electrophoretic mobility to paprika mild mottle virus (PaMMV, strain P11). When the homologous antiserum is used, P101 and other typical virus strains from the tobamovirus group arrange in the following series of serological relationship:  $P101 \geq PaMMV > \text{tobacco mild green mosaic virus} > \text{pepper mild mottle virus} > \text{tobacco mosaic virus} > \text{tomato mosaic virus}$ . P101 and strain P11 of PaMMV are strongly related but not serologically identical. Both strains overcome the L1 resistance gene (pathotype P1) but at least 28 accessions of *Capsicum chinense* are resistant to P101 and susceptible to P11. The Bulgarian strain differs from P11 by the reaction of tomato and *Nicotiana tabacum* cv. Samsun N'N'. However, the established differences between both strains are not enough to propose P101 as a separate virus and it can be define as a strain of PaMMV.*

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# INFLUENCE OF VIRUS CONCENTRATION ON EXPRESSION OF CUCUMBER MOSAIC VIRUS RESISTANCE IN PEPPER

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## ABSTRACT

Pepper lines L57 and L113 and cv.Pico are inoculated by 1, 5, 10, 25, 75 µg/ml cucumber mosaic virus (CMV) in inoculum. Both lines are resistant to the virus and cv.Pico is used as a susceptible control. The lines respond with the development of chlorotic (L57) and necrotic (L113) spots on the inoculated leaf. The virus is established only in the inoculated leaves and their petioles of the resistant lines but cv.Pico is inoculated systemically. The virus existence on the rubbed leaves is an indicator for the effectiveness of inoculation. Inoculum with virus concentration 1 µg/ml does not inoculate the leaves, with CMV concentration of 5 µg/ml inoculates 66,7% of the rubbed Pico and L57 leaves and 50% of the L113 ones. The higher concentrations (25 and 75 µg/ml) inoculate all the rubbed leaves. The mosaic symptoms on inoculated Pico plants are developed up to 14 days post inoculation. All L57 plants which are inoculated by 75 µg/ml virus in inoculum and 50% of these which are inoculated by 50 µg/ml are infected systemically. The resistance is overcome at 66,7% of L113 plants which are rubbed by 75 µg/ml CMV. The data suggest that the expression of CMV resistance in pepper depends on virus concentration in inoculum and it is different for the both lines.



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# PREDICTION OF THE PRESERVATION OF LYOPHILIZED TOBAMOVIRUSES

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## ABSTRACT

*Eleven tobamovirus strains were lyophilized in the form of leaves, plant sap with and without protectant, as well as protected purified preparation. Accelerated storage test at 28-81 °C for 30 to 240 days was applied to the freeze-dried samples. The infectivity was tested on a local host. The survival decrease was represented as pseudo-first order reaction and predictions for stability of lyophilizates at real storage conditions (4 °C) were developed by means of Arrhenius equation. The preservation depended on the lyophilic form and was strain specific. Some strains were expected to loose 90% of the survival after 1-3 years while others - after more than 25 years in unprotected variants. The preservation as protected purified preparations will be more than 60-70 years. Among the tested strains of tomato mosaic virus the highest longevity for the strains of group "2" after Pelham was predicted. The accelerated storage test and the prediction facilitate the maintenance of a plant virus collection.*

## Population changes of tobacco mosaic virus strains after serial passages in tolerant to tobamoviruses tomato

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**ABSTRACT.** Three similar in biological characterization tobacco mosaic virus (TMV) strains — U1, TGM and MA, were inoculated to serial passages in a tomato cultivar carrying the tolerance Tm-1 gene. The population changes were investigated by the response of test plants, virus electrophoretic mobility and serological properties. The results obtained through the three methods have been confirmed mutually. TGM and U1 multiplied systemically in tolerant tomato. The emergence of mutants from tomato mosaic virus (ToMV) was obscured in the first strain while the second one was replaced only by ToMV from group “1”. In TGM population changes occurred after the advent of a TMV group “1” isolate. On the contrary, MA did not infect systemically tomato at all and this strain was replaced initially by ToMV virions from group “0” and after that by ToMV from group “1”. A predomination of strains from group “1” that could be related to toMV or TMV was reached at the last stage after multifold passages in tolerant to tobamoviruses tomato plants. The present investigation shows that the tested TMV strains differ in the genetic diversity level of their population within the initial host and they alter in a different way.

**Key words:** gene Tm-1, *Lycopersicon esculentum* Mill., *Nicotiana sylvestris* L., tobacco mosaic virus, tomato mosaic virus.

**Abbreviations:** CP — coat protein; HR — hypersensitive reaction; PEG — polyethylene glycol; Rf — electrophoretic mobility; ToMV — tomato mosaic virus; TMV — tobacco mosaic virus.

## INFLUENCE OF THE INOCULATION NUMBER AND PLANT STAGE ON THE EXPRESSION OF CUCUMBER MOSAIC CUCUMOVIRUS RESISTANCE IN PEPPER LINES

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**Key words:** cucumber mosaic cucumovirus, CMV, overcome, resistance, pepper lines, ELISA.

**Abstract:** Two pepper lines resistant to cucumo mosaic cucumovirus /CMV/ were tested. Young L57 plants inoculated with 25 and 50µg/ml were found to be the resistant to the lower CMV concentration and susceptible to the higher one. L113 plants respond in the same way to 50 and 75 µg/ml respectively. The L57 and L113 plants inoculated at the later plant stage /5<sup>th</sup>-6<sup>th</sup> leaf/ are resistant to 50µg/ml and 75 µg/ml virus respectively. The data suggest that resistance is overcome more easily in the young plants. Two-fold inoculation of the L113 plants with 50µg/ml and of the L57 lines with 25µg/ml causes systematic CMV movement.



## SERIAL PASSAGES OF TWO TOBAMOVIRUSES IN TOMATO POSSESSING TM-1 GENE

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**Key words:** tomato mosaic virus, tobacco mosaic virus, tolerance, electrophoretic mobility, serological relationship.

**Abstract:** Three strains of tomato mosaic virus (ToMV) and a strain of tobacco mosaic virus (TMV), belonging to "0" group and isolated from tomato, were investigated. Serial passages were carried out in tolerant to tobamoviruses tomato carrying Tm-1 gene. Three types of changes were observed. The strains causing yellow symptoms did not at all multiply in tolerant tomato and mutants did not establish. The ToMV green strain propagated systemically and symptomless but was replaced by virus particles from "1" group. The TMV strain did not systemically infect tomato with Tm-1 gene but accumulated in enough quantities in the inoculated leaf to allow the appearance of ToMV mutant from "0" group, that caused systemic symptomless infection. At the late stage ToMV virions from "1" group aroused and they dominated in the virus population. These changes were confirmed by test plants and electrophoretically. The TMV replacement by ToMV was also proved by serological analysis.

## Characterization of tobamovirus strains by electrophoretic mobility

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**ABSTRACT.** Fifteen strains of tobacco mosaic virus (TMV) and tomato mosaic virus (ToMV) were analyzed by the electrophoretic mobility of the virus particles. Vertical polyacrylamide gel electrophoresis (PAGE) and agarose gel electrophoresis were applied. The green ToMV strains from pathotypes P0 and P1 exhibited higher relative mobility than the type TMV strain by 32-33% in PAGE and 11-12% in agarose gel, respectively. Essential changes in the virion mobility of the yellow strains of TMV and ToMV pathotypes P0 and especially P1 were established. The yellow ToMV strains from P2 migrated in electric field similarly to the green ToMV ones. The tested strains were divided in subgroups according to their electrophoretic mobility, pathotype and difference in the mosaic symptoms. The results revealed considerable number of various mutations in the capsid protein of the yellow strains. The application of different electrophoresis conditions gives a possibility for additional characterization of the tobamovirus strain variability.

## ГЕНЕТИКА НА УСТОЙЧИВОСТТА КЪМ ВИРУСНИ БОЛЕСТИ

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## *GENETICS OF PLANT VIRUS DISEASE RESISTANCE*

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### **Abstract**

Genetic resistance is one of a number of approaches to protect crops to viral diseases that cause serious economic losses in many major crops by reducing yield and quality. Different forms of plant virus disease resistance are compared in this review. Various forms of non-specific and especially specific resistances (extreme resistance, gene-for-gene resistance and hypersensitive response), genes for resistance and current knowledge regarding their identity and inheritance are discussed.



## SEROLOGICAL CHARACTERIZATION OF PAPRIKA MILD MOTTLE TOBAMOVIRUS STRAIN P101

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### Summary

*The serological properties of the Bulgarian strain P101 of paprika mild mottle virus (PAMMV) were investigated. Counter immunoelectrophoresis and double diffusion test were applied. The serological relationship with tobacco mosaic virus (TMV), tomato mosaic virus (ToMV), tobacco mild green mosaic virus (TMGMV), pepper mild mottle virus (PMMoV) and the Dutch strain P11 of PaMMV was studied using antisera against P101 and ToMV. The Bulgarian strain P101 was serologically very closely related to the PaMMV strain P11 originated from the Netherlands. The performed immunodiffusion tests with homologous and heterologous antisera allow P101 to be arranged in different series of serological relationship with typical tobamovirus strains. PaMMV is more related to TMGMV and PMMoV than to TMV and ToMV.*

**Key words:** *paprika mild mottle virus, tobamoviruses, immunodiffusion, immunoelectrophoresis, serological relationship.*

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## ЛИНИИ ПИПЕР, УСТОЙЧИВИ НА ИКОНОМИЧЕСКИ ВАЖНИ БОЛЕСТИ

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## Pepper Lines Resistant to Economical Important Diseases

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### Abstract

Pyramiding of genes for high quantity of  $\beta$ -carotene (provitamine A), vitamin C and dry matter content, anthocyaninlessness, early ripening, high yield and complex resistance to *P. capsici*, cucumber mosaic virus, tobacco and tomato mosaic virus in pepper lines were performed. All three lines possess good nutrition and savour qualities and considerably surpass in earliness and yield the Bulgarian standard cultivars Albena and Zlaten medal. At botanical maturity the kapiya type fruits of OKalR are orange while those of L14 and L16 are red. All lines are suitable for early and middle early field pepper production and may be efficiently used in pepper breeding programs.

RESISTANCE OF TOMATO LINES TO CLAVIBACTER MICHIGANENSIS SUBS.  
MICHIGANENSIS, CUCUMBER MOSAIC VIRUS AND TOBACCO MOSAIC VIRUS

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ABSTRACT. The present communication reports the results about resistance of tomato lines obtained by hybridization with tomato wild species to Cmm, CMV and TMV. The selected lines could be used in breeding tomato programme for obtaining varieties and hybrids possessing complex resistance to diseases.

## NEW HYBRIDS IN SWEET PEPPER (*CAPSICUM ANNUUM* L.) DEVELOPED ON THE BASIS OF GENIC MALE STERILITY.

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### Abstract

18 hybrid combinations for early field production and 13 one for cultivation under plastic house conditions were tested during 1993-1994. The line Zlaten medal *ms-8* that proved to be the best source for genic male sterility was used as female parent. 6 hybrids for early field production and 4 for plastic house production are very promising exceeding the check variety with regard to earliness with 25-36 % and in total yield with 22-72 %. The fruits of these combinations are 2-3 lobbed, long, "kapia" type, with yellow green, light green, green or dark green immature colour and red one at maturity.



# CYTOCHEMICAL INVESTIGATION OF TOMATO AND CUCUMBER POLLEN AFTER CUCUMBER MOSAIC VIRUS INFECTION

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## 1. Abstract

It was established that CMV infection of tomato and cucumber plants is associated with disturbances in respiratory pathways in pollen - decrease in activities of enzymes taking part in aerobic respiration and increase in activities of enzymes involved in anaerobic respiration. A rise of peroxidase activity as a response to the biotic stress was pointed out. In cucumber pollen enhancement of G6PDH activity was found which is supposed to indicate "de novo" biosynthesis of virus RNA. A slight intensification of hydrolytic processes and decline of PPO activity in pollen of both plant species was observed. These metabolic disturbances were connected with drastic drop in pollen viability.

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## BREEDING SWEET PEPPER (*CAPSICUM ANNUUM* L.)-LINES WITH COMPLEX RESISTANCE TO CUCUMBER MOSAIC VIRUS AND *PHYTOPHTHORA CAPSICI* LEONIAN

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### Abstract

In Bulgaria as in many countries cucumber mosaic virus (CMV) and *P. capsici* are the widest spread pathogens causing severe yield loses. The aim of the present investigation is to develop lines and hybrids with complex resistance to these diseases. A local Bulgarian line L57 and a Dutch line L113 resistant to CMV; the line Alfi resistant to *P. capsici* and the male sterile line Zlaten medal ms-8 were used to achieve the breeding goals. The CMV resistance tests were performed after artificial inoculation under glasshouse conditions and then the symptomless seedlings were planted in the field. The inoculations with *P. capsici* were carried out 2-3 weeks after planting the seedlings in the field. Male sterile and fertile lines resistant to both sources of CMV resistance were obtained. It was established that the Dutch line L113 is resistant to *P. capsici* as well. Hybridization in order to combine resistance to both diseases and the male sterile gene ms-8 in one the same genotype has been initiated.



## **TOMATO MOSAIC VIRUS INDUCED SYSTEMIC RESISTANCE AGAINST BACTERIAL SPOT AND SPECK DISEASES IN TOMATO**

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### **ABSTRACT**

Tomato plants primary inoculated with attenuated B-5 strain of tomato mosaic virus (ToMV) and virus free tomato plants were challenge inoculated with strains of races - R0 and R1 of *Pseudomonas syringae* pv. *tomato* (Pst) and strains XvT1, XvT3 and XvPT pathotypes of *Xanthomonas vesicatoria* (Xv). The virus inhibits the development of Xv and Pst infections in systemically infected with ToMV tomato. It was assumed that the virus activates plant's unspecific defense mechanisms which restrict the development and propagation of the bacteria t. i. systemic acquired resistance has developed in the tomato plants.

# RESISTANCE OF TOMATO MOSAIC VIRUS TO FREEZE-DRYING

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**Abstract:** *The survivals after purified preparations freeze-drying of five tomato mosaic virus strains were investigated. Lyophilized samples were undergone to accelerated storage test at 28, 37 and 45°C and the infectivity declining was followed for 120 days. Comparative studies on different viruses' preservation were performed. The resistance to lyophilic conservation to a great extent was due to the strain specificity.*



## ПРИЛАГАНЕ НА УСКОРЕНО СТАРЕЕНЕ ЗА ПРОГНОЗИРАНЕ ЗАПАЗВАНЕТО НА ЛИОФИЛИЗИРАНИ ТОБАМОВИРУСИ

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## APPLICATION OF ACCELERATED AGEING FOR PREDICTION OF THE PRESERVATION OF FREEZE-DRIED TOBAMOVIRUSES

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### ABSTRACT

Three tobamovirus strains were freeze-dried in leaves and were undergone to accelerated storage test at 28, 37 and 45°C for 240 days. The sample infectivity was tested on local host by the method of the leaf halves. The stability of the lyophilized viruses after accelerated ageing was strain specific. The trends in the survival decrease at the three temperatures were used for extrapolation of the dying speed at 4°C. After mathematical processing of the results the preservation of the tobamoviruses was predicted at real storage conditions. It was expected the survival would low to 10% compared to the initial one after freeze-drying, after 8 to 30 years depending on the strain. The prediction facilitated the collection activity in maintenance and testing of plant viruses.

Тестът за ускорено стареене при повишени температури може да се приложи за прогнозиране запазването на вируси и микроорганизми, като намаляването на жизнеността се сведе до реакция от първи порядък и се използва уравнението на Арениус [2, 3, 4]. В предпипни наши изследвания е проучена устойчивостта на тобамовируси, консервирани лиофилно под различна форма - листен материал, растителен сок и пречистен препарат [7, 8, 9, 10]. Създаден е модел за прогнозиране на дълготрайността на лиофилизирани проби растителни вируси. Важно е да се подберат подходящи температури и период на тестиране, за да се получат стойности, описващи се достатъчно добре с уравнението на Арениус [11, 12].

Цел на настоящата работа е да се предвиди запазването в реални условия на съхранение (4°C) на три шема тобамовируси след вакуумно-сублимационно сушене, като се приложи математическо моделиране.

## PEPPER LINES WITH COMPLEX RESISTANCE TO VIRUS AND FUNGAL DISEASES

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15 pepper lines resistant to *Phytophthora capsici*, tobacco and cucumber mosaic viruses were developed. The plants of these lines are 60 – 80 cm high with 2 – 3 main branches. Fruit are pending with 10 – 15 cm length and 80 – 110 g weight. The colour is dark green with glossy and pericarp thickness – 6 – 8 mm. According to the fruit shape the lines are divided in 3 groups: first one – mainly two lobbed (so called "kapia" type), second one – three lobbed and the third one – prolonged cylindrical and trapezoid (so called "lamio" type).

# **ФЕНОТИПНА ХАРАКТЕРИСТИКА НА УСТОЙЧИВОСТТА КЪМ КРАСТАВИЧНОМОЗАИЧНИЯ ВИРУС НА ЛИНИИ ПИПЕР**

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лова**

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## **PHENOTYPE CHARACTERIZATION OF RESISTANCE TO CUCUMBER MOSAIC VIRUS IN PEPPER LINES**

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### **ABSTRACT**

Phenotype characteristics of resistance to cucumber mosaic virus (CMV) in two pepper lines have been investigated. The lines differ essentially in their response after CMV inoculation. Leaf inoculated with CMV developed different symptoms - chlorotic (L57) and necrotic (L113) spots. These spots appeared comparatively late on the 10<sup>th</sup>-15<sup>th</sup> day after the inoculation if the day temperature is not low then 25°C. Plants growing at a constant temperature slow up the appearance of symptoms and even prevents it on the inoculated leaves. Most of these leaves fall down and rarely necrotic stripes could be formed from places where leaves have been attached to the stem. The check of virus distribution in plants shows that only in the inoculated leaves and their stems contain virus. CMV resistance in our pepper lines shows dominant penetrant inheritance.

# **ВИРУСНИ И БАКТЕРИЙНИ БОЛЕСТИ ПО ПИПЕРА В БЪЛГАРИЯ**

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## **VIRUS AND BACTERIAL DISEASES SPREAD ON PEPPER IN BULGARIA**

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### **ABSTRACT**

The samples collected from pepper plants with virus and bacterial diseases symptoms, cultivated in different Bulgarian regions were tested. Tobacco mosaic virus (TMV); tomato mosaic virus (ToMV); pepper mild mottle virus (PMMoV), pathotype P1.2 and *Xantomonas vesicatoria* pathotype PT were identified. CMV and *X. vesicatoria* prevailed in field pepper and tobamoviruses – in plastic greenhouse pepper.



# ФЕНОТИПНА И БИОХИМИЧНА ЕКСПРЕСИЯ НА УСТОЙЧИВОСТТА НА РАСТЕНИЯ ПИПЕР С РАЗЛИЧНА ЧУВСТВИТЕЛНОСТ КЪМ КРАСТАВИЧНОМОЗАИЧНИЯ ВИРУС

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## PHENOTYPIC AND BIOCHEMICAL EXPRESSION OF THE RESISTANCE OF PEPPER PLANTS WITH DIFFERENT SUSCEPTIBILITY TO CUCUMBER MOSAIC VIRUS

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### ABSTRACT

Two line peppers (L113 and Okal) of *Capsicum annuum* with different susceptibility to *Cucumber mosaic virus* (CMV) and influence of pathogenesis on the biochemical and phenotypic expression were studied. Necrotic local lesions accompanied by the rapid accumulation of  $H_2O_2$  and proline and low catalase activity on the inoculated leaves of resistant line L113 were observed. The correlation between changes of biochemical parameters and susceptibility of pepper line to CMV was established.

**Key words:** CMV, resistance, pepper plants, catalase activity,  $H_2O_2$ , proline

**АНТИОКСИДАНТНА ЗАЩИТА НА РАСТЕНИЯ ПИПЕР В ХОДА НА ИНФЕКЦИЯТА  
ОТ КРАСТАВИЧНОМОЗАИЧНИЯ ВИРУС**

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**ANTIOXIDANT DEFENCES IN PEPPER PLANTS DURING CUCUMBER MOSAIC VIRUS  
INFECTION**

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**ABSTRACT**

The effects of *Cucumber mosaic virus* (CMV) infection on the activities of guaiacol peroxidase (PO), catalase (CAT) and carotenoid content in two lines *Capsicum annuum* plants were investigated. The increase of carotenoids in the susceptibility line Okal was observed, while carotenoid content in the resistant line 113 was the same in infected and control plants. The activities of peroxidases, catalases and carotenoid content changed according to the susceptibility of investigated pepper lines to *Cucumber mosaic virus*. The correlation between the antioxidant enzymes and susceptibility of pepper plant to CMV was observed.

*Key words: CMV, peroxidase activity, catalase activity, carotenoid content*