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Оригинален научен труд
Original Research Paper

ПРЕЖИВУВАЊЕ НА СОЕВИТЕ ТОБАМОВИРУС ВО ЛИОФИЛИЗИРАНИ ПРОЧИСТЕНИ ПРЕПАРАТИ

SURVIVAL OF TOBAMOVIRUS STRAINS IN LYOPHILIZED PURIFIED PREPARATIONS

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Summary

The using of freeze-dried purified preparations is suitable for conservation of the members of the *Tobamovirus* group. The effect of different variants of cryoprotection was examined after lyophilization, accelerated storage test at 100°C and 12 years of storage. The infectivity of eleven tobamovirus strains in purified preparations with equal concentration and determined spectrophotometricaly were compared. Tobacco mosaic virus (TMV) strains showed higher real infectivity than representatives of tomato mosaic virus (ToMV) which differed also to each other. The differences could not be definitely bind with the mosaic kind or pathotype of the strains but possibly are due to the presence of defective particles. TMV strains survived after freeze-drying in protected purified preparations 86-100% while ToMV ones kept between 52% and 85%. After accelerated storage test at 37°C the studied strains were divided in three groups by their stability as TMV exhibited the highest survival. Strain TMV B distinguished from other viruses with its great resistance after treatment at 45°C for 120 days while some ToMV strains were not detected by the used test. After storage in lyophilic form for a period of 12 years, TMV strains exhibited 1.3-5 times higher infectivity than ToMV. The freeze-drying and long-term preservation intensified the differences between ToMV strains as the green strains from pathotypes 0 and 1 were more viable than the tested yellow strains after storage as lyophilized purified preparations.

STRUCTURE AND FUNCTION OF THE CUCUMBER MOSAIC VIRUS GENOME

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Summary

Contemporary knowledge about the structure and functions of cucumber mosaic virus (CMV) and its satellite RNAs/cRNA is presented. The possibility for protecting cultivated plants by use of vaccine strains of CMV (with or without cRNA) or transgenic plants, expressing RNA or capside protein, is discussed.

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ELECTROPHORETIC MOBILITY OF DIFFERENT IN PATHOGENICITY STRAINS OF TOBAMOVIRUS GROUP

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Summary

Twenty four Tobamovirus strains divided according to their ability to overcome the resistance genes in tobacco and tomato as well as in their severity of systemic symptoms were tested. Significant differences between means of particle mobility of the virus groups were found especially about yellow mosaic strains which were distinguishable enough by the electrophoretic analysis.

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Antiphytoviral activity of 1-morpholinomethyl-tetrahydro-2(1H)-pyrimidinone (DD13)

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The antiphytoviral activity of 1-morpholinomethyl-tetrahydro-2(1H)-pyrimidinone (DD13) in a test system including protoplast cultures, surviving tissues and greenhouse plants was examined. The inhibitory effect was quantitatively investigated by immunofluorescence and enzyme linked immunosorbent assay. The antiviral action *in vitro* was 96%. The first 6 h after inoculation was the most sensitive period of the tomato mosaic virus (ToMV) reproduction cycle. DD13 possessed a protective effect in 97–100% plants infected with ToMV and cucumber mosaic virus (CMV).

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DETERMINATION OF PARAMETERS FOR TOBACCO MOSAIC VIRUS CRYOGENIC TREATMENT AND FREEZE-DRYING

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SUMMARY

Studies on determination of appropriate conditions for cryogenic treatment and lyophilization of tobacco mosaic virus (TMV) in infected leaf tissue, plant sap and purified preparation were carried out. The found parameters were: freezing to minus 22°C (for 1.5 hours); twelve-hours-sublimation at 50 Pa vacuum until 20°C temperature of the sample and secondary drying at 1-2 Pa for 5-6 hours. Fifteen variants of cryoprotection were applied. TMV showed a survival higher than 89% after freeze-drying in glucose, fructose, maltose, lactose, sorbitol and the combinations with dextran. A technological scheme for automatic processing and standard work was developed.

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APPLICATION OF ACCELERATED STORAGE TEST TO LYOPHILIZED PLANT VIRUSES

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SUMMARY

The preservation of nine plant virus strains of tobamovirus and cucumovirus groups after freeze-drying in different lyophilic forms was examined. Quantitative studies on survival were performed. In tobacco mosaic virus (TMV) and cucumber mosaic virus (CMV), accelerated storage test at 70 - 100°C was applied for screening 20 protecting media. A perspective medium, 5% sorbitol, 3.6% dextran, for plant viruses lyophilization with high cryo- and xeroprotective effects was found.

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Prediction of the preservation of freeze-dried cucumber mosaic virus

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Key words: accelerated storage test, lyophilization, mathematical model, prediction

Abstract

A mathematical model to predict the outcome of preserving plant viruses has been developed. The survival of two cucumber mosaic virus strains lyophilized in leaves and in protected plant sap was evaluated using an accelerated storage test at 28, 37 and 45 °C. The combinations of 5% (w/v) sorbitol and 7% (w/v) glucose with 3.6% (w/v) dextran 40000 were applied as protecting media. The predicted values to a great extent correspond to the experimental data, achieved after 1, 4 and 7 years of storage.

Tomato lines segregation for resistance to cucumber mosaic virus

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Key words: breeding, cucumber mosaic cucumovirus, disease, interspecific hybridisation, resistance, tomato

Abstract

15 lines were bred by interspecific hybridisation and I1 to I6 generation of three of them were tested for resistance to CMV. In spite of the selection by CMV resistance in the progenies the number of the resistant plants did not always increase. The progenies having 100 % symptomless plants for two or more consecutive years were not selected in the studied lines. A large spectrum of variations in the percentage of symptomless plants in the progenies per year and the presence of disease and symptomless parts in one plant were established. These results are possibly due to the complex mechanism of inheritance of CMV resistance as well as to the influence of environmental factors on the expression of the resistance observed.

Differential expression of β-glucosidase in tomato - stress stimuli systems

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Key words: abiotic and pathogenic stress, β-glucosidase, fungal pathogenesis, tomato

Abstract

In the present work the responses of β-glucosidase in leaves of tomato plants subjected to various stress factors of both pathogenic (fungi, bacteria, viruses) and abiotic origin (heat shock) were studied. Biochemical and cytochemical methods were applied. It was established that an increase of β-glucosidase activity is induced uniquely by fungal pathogens. The cytochemical tests confirm the finding. Hence, the conclusion can be drawn that β-glucosidase response is a specific character of fungal pathogenesis in tomato; probably, the enzyme is involved in plant - fungi recognition. The data are in accordance with our previous results on tobacco and wheat - stress stimuli systems.

Metabolic changes in tomato fruits and seeds after viral, bacterial and fungal infections

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Key words: *Botrytis cinerea*, cucumber mosaic virus, *Lycopersicon esculentum*, metabolic changes, *Pseudomonas syringae* pv. *tomato*, tomato

Abstract

The metabolic changes in tomato fruits and seeds separately infected with cucumber mosaic virus, *Pseudomonas syringae* pv. *tomato* or *Botrytis cinerea* were investigated cytochemically. The changes of peroxidase (E.C. 1.11.1.7) and β-glucosidase (E.C. 3.2.1.21) were investigated biochemically as well. Tomato fruits were involved in the study because of their high economic value. Tomato seeds were investigated since they have been used most extensively as a model system for studying the physiology and biochemistry of seed development. The diseases caused by the pathogens under study are of special importance for yield reduction in tomato. The three pathogens provoked local changes in the activities of enzymes under study that affect the infected pericarp tissues and neighboring seeds. It was established non-specific and specific responses. The non-specific responses of invaded tissues were expressed as a local enhancement of peroxidase activity in both pericarp tissues and seeds as well as a decrease in activities of: **i.** enzymes taking part in aerobic and anaerobic respiration, **ii.** hydrolases esterase and acid phosphatase involved in the basic metabolism as well as an enhancement of their activities in neighboring tissues. Furthermore, it was observed an enhancement of α-galactosidase activity in infected area was observed. The specific responses depending on the type of the pathogen consisted in an enhancement of glucose-6-phosphate dehydrogenase activity by virus infection and an increase of β-glucosidase activity by fungal invasion.

List of abbreviations: CMV - cucumber mosaic virus; *Pst* - Pseudomonas syringae pv. tomato; *Bc* - Botrytis cinerea; IDH - isocitrate dehydrogenase, GDH - glutamate dehydrogenase; ADH - alcohol dehydrogenase; LDH - lactate dehydrogenase; G6PDH - glucose-6-phosphate dehydrogenase; CytO - cytochrome oxidase; Est - esterase; AP - acid phosphatase, α-gal - α- galactosidase; β-gl - β-glucosidase; PO - peroxidase

Introduction

The increasing pressure of different infections intensified research on the mechanisms of plant survival. It was revealed that during the host-parasite relationship the host defense reactions are expressed as non-specific responses to the biotic stress (Mauch *et al.* 1988) and specific responses depending on the character of the pathogens (Edreva *et al.* 1999). Usually, this kind of investigations was carried out on vegetative organs. The metabolic responses of fruits and seeds to the

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A model for longevity prediction of freeze-dried tobamoviruses

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Key words: freeze-drying, prediction, preservation, protectant, tomato mosaic virus

Abstract

An accelerated storage test was carried out on freeze-dried samples of five variants of tomato mosaic virus – from leaf material, plant sap and purified preparation with and without protectant, at 28, 37 and 45 °C. A model for longevity prediction of lyophilized tobamoviruses was developed. The preservation of tomato mosaic virus decreased to 10% after 0.7–2.8 years in unprotected variants and after 7.5–11 years in protected samples under real storage conditions. Experimental data have confirmed the predicted values.

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ORIGINAL PAPER

Accelerated leaf senescence takes part in enhanced resistance in cucumber mosaic virus inoculated pepper leaves

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Abstract Altered photosynthetic reactions in cucumber mosaic virus (CMV) inoculated leaves of virus resistant lines L113 and L57 and susceptible pepper (*Capsicum annuum* L.) plants cv. Albena grown in controlled environment and in the field were investigated. The CMV inoculated leaves of virus resistant lines developed different symptoms—necrotic local lesions on L113 and chlorotic spots on L57 while the same leaves of susceptible cv. Albena were symptomless. The changes in Photosystem II (PSII) and PSI electron transport were evaluated by chlorophyll fluorescence, and far-red (FR) light induced leaf absorbance $A_{810-860}$. CMV infection caused a decrease in maximal PSII quantum yield, F_v/F_m, in susceptible leaves. Increased non-photochemical fluorescence quenching in CMV-inoculated leaves of both resistant lines were observed. In CMV-inoculated leaves of all tested plants FR light induced P700 oxidation was decreased. In the present study, the viral-infected pepper plants grown in controlled environment to avoid the effects of abiotic factors were used as model system that allow us to investigate the differences in leaf senescence in CMV-inoculated leaves of susceptible and resistant pepper lines expressing different symptoms. Earlier leaf falls of inoculated leaves as a result of accelerated leaf senescence is important for building successful secondary virus resistance strategy following fast responses such as hypersensitive reaction.

Keywords Chlorophyll fluorescence · Cucumber mosaic virus · Disease resistance · Leaf senescence · Photosynthesis

Introduction

Viruses invade rapidly growing tissues and cause characteristic changes in plant phenotype, i.e., disease symptoms. Viral pathogenesis is accompanied by a variety of changes in plant gene expression that likely reflect the ways that viruses generically and specifically interact with host cells (Maule et al. 2002; Yang et al. 2007). The virus-infected plants possessing resistance, i.e. abilities to overcome virus attack, develop marked metabolic changes leading to variety of defense reactions (Hammond-Kosack and Jones 2000; Vidhyasekaran 2004).

Virus-infected plants display variety of morphological and physiological changes in plant organs and tissues that is manifested as disease symptoms, however some infections are symptomless. Leaf-expressed diseases with symptoms, such as chlorosis and necrosis are associated with the changes in chloroplast structure and function

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BIOLOGIE
Génétique

SOURCES OF RESISTANCE TO THE CUCUMBER MOSAIC VIRUS IN THE GENUS LYCOPERSICON

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(Submitted by Corresponding Member D. Shabanov on March 23, 1992)

The cucumber mosaic virus (CMV) caused one of the most important tomato diseases, particularly in regions where the climate favours development of leaf aphids, the vector of this virus. Investigations connected with the search of CMV resistance in wild species of the genus Lycopersicon were conducted, discovering resistance in *L. peruvianum* and *L. pimpinellifolium* [5, 4, 3] and tolerance in the Japanese variety [9]. Stamova et al. [7] found individual resistant plants in lines developed with the participation of *L. chilense*. Testing of tomato lines (F_6) from the three-genome hybrid (*L. esculentum* — *L. chilense* — *L. peruvianum* var. *humifusum*) revealed a considerable number of CMV resistant plants [8].

The aim of the present investigation was to look out for sources of resistance in various accessions of the sub-genus Eulycopersicon and Eriopersicon of genus Lycopersicon.

Material and methods. Various accessions from wild species of the genus Lycopersicon were tested for resistance to CMV. The plants were inoculated mechanically with separate glass spatula in the phase of cotyledons — first true leaf after being previously sprayed with carborundum. The inoculum was prepared from lyophilized CMV infected tobacco leaves by homogenization in 0.06M, K-Na phosphate buffer pH 7.0 diluted 1:50 w/v. The CMV strain was isolated from tomatoes and caused mosaic and fern leaves. The cucumber of mosaic plants was assessed twenty days after inoculation. The mosaic plants destroyed while all symptomless plants were reinoculated in the described way. After another period of 20 days the number of mosaic symptomless plants was again recorded and up to 4 of the latter from each accession were pricked. Each plant was tested for presence of CMV by indirect ELISA. The leaf samples were collected during fruit production and were homogenized in a carbonate buffer pH 9.6 diluted 1:10 w/v.

Results and discussion. All plants of *L. humboldti, L. pimpinellifolium* PI 305216, *L. cheesmanii* var. *minor* and *L. chilense* La 460 proved symptomless. The same results were found also in *L. esculentum* var. *cerasiforme* 1567/80 and *L. pimpinellifolium* LA 1633, but the number of tested plants was very small to allow reaching a conclusion about the degree of the population's homogeneity for the character tested. All the symptomless plants of the chosen accessions tested did not contain the virus, i. e. they were resistant. No symptomless plants were found in the accessions *L. esculentum* var. *succentariatum*, *L. pimpinellifolium* LA 1594, *L. peruvianum* var. *humifusum* LA 2151 and *L. parviflorum* LA 1326 (Table 1). In the remaining accessions tested were found both mosaic (susceptible) and symptomless plants. Part of the latter were tested for presence of the virus and among them were found both plants with the virus (tolerant) and without virus (resistant). The number of symptomless plants tested for presence of the vi-

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BIOLOGIE
Virologie

ANTIPHYTOVIRAL EFFECT OF TETRAHYDRO-2(1H)-PYRIMIDINONE DERIVATIVES ON TMV, ToMV AND CMV IN TOBACCO

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(Submitted by Corresponding Member D. Shabanov on October 6, 1992)

Numerous studies on the antiviral effect of various plant extracts and substances of known chemical composition have been carried out. The effectiveness of the latter is tested predominantly by means of spraying of whole plants which is a slow, labour-consuming and expensive method. In our previous studies [1, 4] on large-scale screening of antiviral substances protoplast cultures were successfully used. Of 23 tetrahydro-2(1H)-pyrimidinone derivatives tested, only DD12 and DD13 showed good activity. The same compounds have a selective effect, as they are not toxic for the plant cell in concentrations 10 times higher than ED_{max}.

The present paper is aimed at comparative study in vivo and in vitro of the antiphytoviral effect of DD12 and DD13 on TMV, ToMV and CMV in tobacco.

Materials and methods. The antiviral substances 1-morpholinomethyl-tetrahydro 2(1H)-pyrimidinone (DD13) and 1-morpholinomethyl-tetrahydro-2(1H)-pyrimidinthione (DD12) [3] were studied. Typical tobacco mosaic virus (TMV), tomato mosaic virus (ToMV) and cucumber mosaic virus (CMV) strains which induce green mosaic disease in tobacco were used. TMV and ToMV were applied on *Nicotiana tabacum* cv. Samsun and CMV was applied on cv. Samsun NN. ToMV was purified with polyethylene glycol [2]. In experiments with plants, inoculums were prepared immediately prior to infection by homogenizing mosaic tobacco leaves infected with a corresponding virus in 0.01M phosphate buffer, pH7 diluted to 1:10 (W/v).

Mesophyll protoplasts from tobacco cv. Samsun were obtained and infected with a purified ToMV preparation [4]. The substances were added to the nutrient medium following infection of the protoplasts to study the antiviral activity in a dose-response experimental set-up. The infection was determined by indirect immunofluorescence[4].

The greenhouse plants of tobacco cv. Samsun at a five-six leaf stage were treated by spraying with the substances tested at a concentration of 1 mg/ml. One leaf of each plant was mechanically infected by a corresponding virus following 24 hours. In the experiment presented in Fig. 2*b* the plants are additionally infected with TMV 24 hours prior to treatment with an inhibitor. The first estimation was carried out after appearance of mosaic symptoms in all control (not treated with antiviral substances but infected with a corresponding virus) plants. The following estimations were performed after 15, 30 and 60 days (when over 50% of the plants were in blossom).

Results and discussion. The results from the comparative study of the antiviral activity of DD12 and DD13 in protoplasts cultures are presented in Table 1. The lowest DD12 concentration studied has no effect, while DD13 shows 15.6% inhibition of the virus. ED_{50} for DD13 is reached after a double increase of the initial concen-

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INFLUENCE OF CUCUMBER MOSAIC VIRUS ON SOME PHYSIOLOGICAL AND BIOCHEMICAL INDICES OF RESISTANT AND SUSCEPTIBLE TO VIRUS PEPPER

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ABSTRACT

Two lines (L113 and Okal) of *Capsicum annuum* with different susceptibility to cucumber mosaic virus (CMV) and influence of pathogenesis on some indices were studied. CMV infection of *Capsicum annuum* leaves provokes a strong increase in peroxidase activity and low MDA level of resistant line 113 in comparison with susceptible line Okal. CMV elicitation of the peroxidase in L113 through its involvement in the lignification process might be considered as a part of the defense mechanism of *Capsicum annuum* against viral infection. The increased production of hydrogen peroxide in pathogen-infected tissues of L113 (at the 3rd day post inoculation (dpi)) was explained with the appearance of hypersensitive response which is a rapid-defense response in plants against pathogens. On the other hand, low peroxidase activity and high level of lipid peroxidation in inoculated and systemic leaves of susceptible line Okal are results of the systemic spreading of virus.

LOCAL AND SYSTEMIC RESPONSES OF ANTIOXIDANTS TO CUCUMBER MOSAIC VIRUS INFECTION IN PEPPER PLANTS

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ABSTRACT

Two lines (Okal and L57) of Capsicum annuum with different susceptibility to Cucumber mosaic virus (CMV) and influence of pathogenesis on peroxidase and catalase activities, carotenoid content and symptom appearance were investigated. Visible chlorotic spots on inoculated leaves of resistant line 57 appeared approximately 17d after CMV infection. The inoculated leaves of susceptible line Okal were symptomless. The increase of carotenoid content in Okal was observed. A relationship between changes of peroxidase and catalase activity in infected plants and their degree of susceptibility to CMV is evidence.

Keyword: *Capsicum annuum,* CMV, catalase, carotenoid, resistance, peroxidase

Introduction

Infection of plants by necrogenic pathogens often results in enhanced protection against a challenge infection not only in the inoculated leaves but also systemically, in the healthy leaves located above in the inoculated site.

An increased production of reactive oxygen derivatives has been observed in various pathogen-infected tissues (3). However, little information is available about the role of protective antioxidative systems in plant response to pathogen attacks. Plants use enzymatic and non-enzymatic antioxidant defence mechanisms to scavenge reactive oxygen species (ROS) (5, 12)). Peroxidase (POX) and catalase (CAT) belong to the importance antioxidative enzymes removing reactive oxygen species in plants. Plant peroxidases (EC 1.11.1.7) are enzymes related to physiological changes in plant-pathogen interaction. They play an important role in lignification and suberization, catalyzing the formation of an impermeable barrier by deposing aliphatic and aromatic polymers (10). The catalases (EC 1.11.1.6) play the role in the protection of cell from the toxic effect of hydrogen peroxide (11). Carotenoids are considered as one of the most effective non-enzymatic quenchers of ROS in the cells.

To investigated the possible role of antioxidants in the pepper-CMV interaction we examined the changes in activities of guaiacol peroxidase and catalase, as well as changes in carotenoid content in the both the upper and lower leaves of *Capsicum annuum* plants after CMV inoculation of the lower leaves.

Materials and methods

Plants and pathogen inoculation

The plants material used in this experiment was two lines *Capsicum annuum* - a resistant (L57) line and a susceptible (Okal) line to *Cucumber mosaic virus* (CMV). L57 was developed through continuous selection in resistance to CMV. Seeds of pepper were planted and grown under normal greenhouse condition at 25-35°/12-18°C (day/night) and 16/8 h (light/dark). The third and fourth leaves of plants were inoculated with a suspension of CMV-PB. The virus was maintains in *N. tabacum* plants and then inoculated as crude sap extract with 0,01M phosphate buffer. Healthy plants, mock-inoculated with phosphate buffer and carborund only were used as control. For each experiment 30-d old plants were used. Inoculated (local) leaves on 1^{st}, 3^{rd}, 7^{th} and 17^{th} and uninoculated (systemic) leaves on 7^{th}, 17^{th} and 30^{th} day post inoculation (dpi) were analyzed. The readings of symptoms on local and systemic leaves of pepper plants were performed on the 7^{th}, 17^{th} and 30^{th} dpi.

Pigment analysis

Total carotenoids were extracted from the leaves with 80% acetone and determined according to Arnon (2).

Enzymatic analysis

PEROXIDASE AND β-GLUCOSIDASE RESPONSES OF TOMATO FRUITS TO VIRAL, BACTERIAL AND FUNGAL INFECTIONS

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Abstract

The changes of peroxidase and β-glucosidase activities were investigated in tomato fruits (cv. Ideal) infected with cucumber mosaic virus, *Pseudomonas syringae* pv. *tomato* or *Botrytis cinerea*, respectively, by applying biochemical and cytochemical approaches. A similarity was found in the response of peroxidase to the invasion of the three types of pathogens. The non-specific response of peroxidase was expressed as a local enhancement of the activity in both invaded pericarp tissues and seeds. Contrariwise, a specific response of fruits depending on the type of the pathogen was observed consisting in increase of β-glucosidase activity only in cells penetrated by or near the hyphae of the fungal pathogen. The implication of the metabolic shifts in plants as a response to the invasion by different types of pathogens was discussed.

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Original Research Paper
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HARACTERIZATION OF CUCUMBER MOSAIC VIRUS RESISTANCE IN TWO PEPPER LINES RESISTANT TO THIS VIRUS

E. Stoimenova, G. Marinova, B. Mihailova *

SUMMARY

Two pepper lines - L57 and L113 resistant to Cucumber mosaic cucumovirus (CMV) were developed through continuous selection. CMV reproduced only in the inoculated leaf and its stem. The resistance was determined as a result of the restriction of virus movement and the absence of CMV spread in the non-inoculated plant parts. The data suggested that CMV resistance exceeded more oftenly when the plants were inoculated: in the early stage; with a high virus concentration in the inoculum for many times. The higher growing temperature influenced the rapid phenotype expression of CMV resistance and faster fall down of the inoculated leaves. The CMV caused the hypersensitive reaction (HR) on the L113 inoculated leaves. HR resulted in the appearance of necrotic local lesion on the inoculated leaves. The L113 was suitable source to the CMV resistance in the breeding pepper programs. There was no HR and no chlorotic spots developed on the L57 inoculated leaves. The CMV resistant L57 had dark green tree and lobbed "kapia" fruits. This line possesses very good agronomic characteristics and is suitable for middle early and late field pepper production.

Key words: *CMV, resistance, DAS-ELISA, inoculation, plant diseases, HR*

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Original Research Paper
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RESISTANCE OF PEPPER ACCESSIONS AND LINES TO ECONOMICALLY IMPORTANT DISEASES

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SUMMARY

Macedonian and Bulgarian pepper accessions and lines were tested for resistance to cucumber mosaic virus (CMV), tobacco mosaic virus (TMV), tomato mosaic virus (ToMV), paprika mild mottle virus (PMMoV) - (P1 pathotype), pepper mild mottle virus PMMoV - (P1.2 and P1.2.3 pathotypes) and *Xanthomonas vesicatoria* pepper-tomato pathotype (XvPT). The L57 was resistant to CMV and L15, L16 and L113 possessed complex resistance to CMV, TMV, ToMV and *P. capsici* and L64 were resistant to CMV, TMV, ToMV, PaMMV, PMMoV (pathotype P1.2) and *P. capsici.* The lines L16, L64 and L113 were additionally moderately resistant to XvPT. The Macedonian line MK6 showed strongly expressed high antocyanine having the lowest infection index and lacking defoliation.

Sources of resistance to XvPT (MK6) and sources of complex resistance to tobamoviruses, CMV, *P. capsici* and tolerant to XvPT have been established. The lines L16 and L64 possess complex resistance to CMV, tobamoviruses and *P.capsici*, tolerant to XvPT and with desired fruit form. These lines own valuable agronomic characteristics, e.g. potential yield, high vitamin C and dry matter content and may be used by the plant breeders in pepper improvement programs.

Key words: *cucumber mosaic virus, tobacco mosaic virus, paprika mild mottle virus, pepper mild mottle virus, Xanthomonas vesicatoria pepper-tomato pathotype, resistance, disease, pepper*

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UDC 632.38: 632.938: 635.64

Original Research Paper
Оригинален научен труд

VIRUSES INDUCED SYSTEMIC RESISTANCE AGAINST BACTERIAL SPOT IN PEPPER

СИСТЕМСКА ВИРУСНА ИНДУЦИРАНА ОТПОРНОСТ НА БАКТЕРИСКАТА ДАМКАВОСТ НА ПИПЕРКАТА

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Summary

L113 pepper plants with the L1 gene-mediated resistance to tobacco mosaic virus (TMV) were inoculated primary with TMV B strain and PaMMV P101 overcomes L1 gene. After 10 days mock and virus inoculated plants were subjected to secondary (challenge) inoculation with *X. vesicatoria* pepper-tomato pathotype. The tobamoviruses inhibit the development of *X. vesicatoria* infection in virus inoculated pepper. The systemically spread virus strain suppressed bacterial disease more effectively than the strain who caused HR in pepper. The part of the virus inoculated plants developed HR after *X. vesicatoria* infection. Hence, it was assumed that the virus activates the plant unspecific defence mechanisms which restrict the development and propagation of the bacteria t. i. SAR has developed in the pepper plants.

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Original Research Paper
Оригинален научен труд

BACTERIAL AND VIRUS DISEASES SPREAD IN BULGARIA AND MACEDONIA ON FIELD AND GREENHOUSE PEPPER

РАСПРОСТРАНЕТОСТ НА БАКТЕРИСКИ И ВИРУСНИ БОЛЕСТИ НА ПИПЕРКАТА ОДГЛЕДУВАНА НА ОТВОРЕНО И ВО ЗАШТИТЕН ПРОСТОР ВО БУГАРИЈА И ВО МАКЕДОНИЈА

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Summary

In Macedonia three viruses of tobamovirus groups were distributed. Tobacco mosaic virus (TMV) and tomato mosaic virus (ToMV) isolates, causing green mosaic, were found in the field pepper where ToMV isolates predominated. Paprika mild mottle virus (PaMMV) isolates, causing mild green mosaic, were isolated from glasshouse pepper only. All CMV isolates had typical biological properties for this virus.

Bacterial spot of pepper was caused by *Xanthomonas vesicatoria* in Bulgaria and by *Xanthomonas euvesicatoria* in Macedonia. The natural Bulgarian population of *X. vesicatoria* belonged to pepper-tomato pathotypes, whereas Macedonian population of *X. euvesicatoria* referred to pepper pathotypes. The strains of *X. vesicatoria* were strongly amilolytic and weakly pectolitic, with a metabolitic profile typical for 14 DNA homologous group, containing beta band of molecular weight 27 kDa. The strain of *X. euvesicatoria* were non-amylolytic and non-pectolitic, utilize cis-aconitate and had 32 kDa protein.

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Original Research Paper
Оригинален научен труд

ToMV INDUCED SYSTEMIC RESISTANCE AGAINST SINGLE AND MIXED BACTERIAL INFECTION OF *PSEUDOMONAS SYRINGAE* PV. *TOMATO* AND *XANTHOMONAS VESICATORIA* ON TOMATO

ToMV ИНДУЦИРАН СИСТЕМ НА ОТПОРНОСТ НА ПОЕДИНЕЧНИ И МЕШАНИ БАКТЕРИСКИ ИНФЕКЦИИ ОД *PSEUDOMONAS SYRINGAE* PV. *TOMATO* И *XANTHOMONAS VESICATORIA* КАЈ ДОМАТОТ

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Summary

Tomato plants primarily inoculated with an attenuated B-5 strain of tomato mosaic virus (ToMV) and virus free tomato plants were challenge inoculated with individual and mixed infections of *Pseudomonas syringae* pv. *tomato* (Pst) and *Xanthomonas vesicatoria* (Xv). Systemic acquired resistance (SAR) was developed in the B-5 infected tomato plants and SAR suppressed the development of Pst (R0, R1) and (T1, T2, T3). In mixed-bacteria infected tomato plants concurrences by varieties and strains were observed. The virus induced SAR did not suppress the concurrence between the two bacteria and their varieties when they developed on the vaccinated plants.

Pst was more pathogenic and aggressive than Xv. XvT2 was the most virulent compared to T1 and T3 of Xv and SAR which were not as effective in these cases.

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Original Research Paper
Оригинален научен труд

INFLUENCES OF ALTERED LEAF ANATOMY ON PS1 ACTIVITY IN CMV INOCULATED LEAVES OF RESISTANT PEPPER LINES

ВЛИЈАНИЕ НА ИЗМЕНЕТАТА АНАТОМИЈА НА ЛИСТОТ НА PS1 АКИВНОСТ ВО ИНОКУЛИРАНИ ЛИСТОВИ КАЈ ПИПЕРКАТА ОД ОТПОРНАТА ЛИНИЈА

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Summary

Necrotic local lesions and chlorotic spots were developed on CMV inoculated leaves of resistant lines L113 and L57. CMV inoculated yellow-green leaves of L57 showed a rise in ΔA830 leaf absorbance that expressed changes in PS1 activity, in comparison to the L113 line. It was found that the changes in ΔA830 were a result of altered leaf anatomy and not due to enhanced PS1 activity in chlorotic leaves. The presence of the virus in leaf tissues of CMV inoculated leaves resulted in some similar changes in the leaf structure of both resistance lines studied by light microscopy. A noticeable increase in the volume of the intercellular spaces in the L57 line was observed by light microscopy. Enhanced mean volume of intercellular spaces per unit leaf area decreased light scattering properties of leaf tissues and higher values for ΔA830 leaf absorbance were established in chlorotic leaves of the L57 pepper line.

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Original Research Paper
Оригинален научен труд

DISTRIBUTION OF (AAT)n MICROSATELLITE DNA REPEAT SEQUENCE IN PEPPER LINES RESISTANT TO ECONOMICALLY IMPORTANT DISEASES COMPARED TO OTHER PLANT FAMILIES

ДИСТРИБУЦИЈА НА (AAT)n МИКРОСАТЕЛИТ ДНА ПОВТОРЕНА СЕКВЕНЦА КАЈ ОТПОРНИТЕ ЛИНИИ ПИПЕРКА НА ЕКОНОМСКИ ЗНАЧАЈНИ БОЛЕСТИ И КОМПАРАЦИЈА СО НЕКОИ ДРУГИ РАСТИТЕЛНИ ФАМИЛИИ

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Summary

Microsatellites, also called sequence tagged microsatellite sites (STMSs), have become important markers for genome analysis but are currently not sufficiently understood in plants. Characterization of the type of (AAT)n distribution is of substantial importance due to its use as a genetic marker in a broad spectrum of gene screening approaches. By the application of molecular genetic approaches, we studied the distribution of (AAT)n simple repeat sequence in several pepper lines resistant to economically important diseases and compared the results to other plant families. We found that (AAT)n repeat is well distributed in some plant genomes, including the genus *Capsicum*, in big clusters in DNA regions lacking sites for restriction endonucleases.

Key words: DNA markers, sequence tagged microsatellite site, *Solanaceae*, complex disease resistance

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Original Research Paper
Оригинален научен труд

CHARACTERISATION OF RESISTANCE AND VALUABLE AGRICULTURAL QUALITIES OF PEPPER LINES

КАРАКТЕРИСТИКИ НА ОТПОРНИ И СТАНДАРДНИ ЗЕМЈОДЕЛСКИ ЛИНИИ ПИПЕРКА

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Summary

Newly created sweet pepper lines were pyramided with disease resistant genes and genes for taste, quantity of β-carotene (provitamin A), vitamin C, reducing sugars, lycopene, dry matter content and the anthocyaninless trait contributing to improved nutritional quality of the pepper.

Three sweet pepper lines resistant to CMV, TMV, ToMV and *Ph. capsici*, anthocyaninless and two lobed kapiya type fruits were developed. The fruits were of different colour at technical maturity: L14 - dark green, L16 and OKalR– light green. At botanical maturity the fruits of OKalR were orange while those of L14 and L16 were red. The quantity of vitamin C and lycopene in L16 and reducing sugars and lycopene in L14 was higher than that of the standard cultivars. The values of remaining traits were found to be approximately equal. The content of β-carotene in the orange fruits of OKalR and OKal was nearly three times higher than in the red fruits of the standard cultivars.

All lines were found to be suitable for early and middle early field production and may be efficiently used for pepper breeding programs.

Development and Characteristics of the First Bulgarian F_1 Transgenic Tomato Hybrids

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Keywords: *Lycopersicon esculentum*, *Tomato spotted wilt virus* (TSWV), heterosis

Abstract

Data on the development of the first Bulgarian F_1 transgenic tomato hybrids are presented. The hybrids analyzed were developed by crossing the tomato line R480, obtained at Agrobioinstitute and possessing a transgene (virus gene coding the nucleoprotein of the virus of *Tomato spotted wild virus* - TSWV) with tomato lines, developed at the Institute of Genetics and possessing a complex of valuable economic traits. Complete immunity to TSWV of the tomato hybrids was established by double testing because of the dominant inheritance of the resistance to TSWV. The transgenic hybrids along with their high resistance to TSWV showed high vigorous habitus, photosynthetic activity, earliness and yield. The inheritance of earliness and total productivity is overdominant with a high coefficient of inheritance and of mean fruit weight, from intermediate to slight overdominant. The first promising Bulgarian transgenic tomato hybrids were evaluated on the basis of their main economic traits. F_1 hybrids (R480 × L.751) and (R480 × L.753) performed best, and showed resistance to TSWV, earliness, high productivity, large fruits and good quality. These hybrids are suitable for middle-early production in the open field.

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Оригинален научен труд
Original Research Paper

ДОЛГОТРАЈНА ПРЕЗЕРВАЦИЈА НА ВИРУСОТ НА МОЗАИКОТ НА ТУТУН СО МЕТОДОТ СМРЗНУВАЊЕ-СУШЕЊЕ

LONG-TERM PRESERVATION OF CUCUMBER MOSAIC VIRUS BY FREEZE-DRYING

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Summary

Freeze-drying is the most suitable method for long term preservation of plant viruses. The longevity of lyophilized cucumber mosaic virus CMV-O was examined for a period of 12 years. The strain survived better in cryoprotected purified preparation than in plant sap but this variant is not convenient for wide use because of expensive procedure of CMV purification. An investigation of the survival of six CMV strains from the collection of NBIMCC after 10-13 years was performed. Two lyophilic forms for CMV preservation were used – freeze-dried leaves and protected with sorbito/dextran plant sap. The leaf age before the virus conservation was of great importance. The CMV infectivity was better preserved when the leaves lyophilized immediately after the symptom appearance (10-14 days) than after waiting 20 days for greater biomass formation. Part of the studied strains was kept similarly in freeze-dried leaves and protected plant sap. The weak preservation in lyophilized sap expressed the two strains causing mild green mosaic. The application of both lyophilic forms is appropriate for long-term conservation of CMV depending on the aims of the next investigations.

INOCULATED LEAVES OF PEPPER LINES RESISTANT TO THE V

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Summary

The virus presence in the blades and petioles of CMV inoculated leaves of two resistant pepper lines was followed in parallel with the provoked changes in the leaves and petioles morphology. The results emphatically proved the virus presence in CMV inoculated leaves (blades and petioles) of L57 and L113 while the other plant parts were virus free. CMV spreading through the petiole is slow and its quantity there reached a small value. This supports the thesis that CMV moves from cell-to-cell but not *via* the petiole phloem flow. The process of virus accumulation is restricted mainly into the leaf blades of both virus resistant lines which lead to visible and histology symptoms of faster leaf and petiole-induced senescence. The resulting premature leaf abscission in turn prevents the virus systemic spread. Some of the leaf histology changes testify to a development of compensatory mechanisms enabling a higher photosynthetic activity of the mesophyll cells located in proximity to the virus-damaged leaf areas.

Key words: cucumber mosaic virus, resistance, pepper

UDC 632.3:635.64

Оригинален научен труд
Original Research Paper

СТЕКНАТА СИСТЕМИЧНА ОТПОРНОСТ ИНДУЦИРАНА ОД САЛИЦИЛНА КИСЕЛИНА И ВИРУСОТ НА МОЗАИКОТ НА ДОМАТОТ ПРОТИВ БАКТЕРИСКАТА БОЛЕСТ НА ДОМАТОТ

SYSTEMIC ACQUIRED RESISTANCE INDUCED BY SALICYLIC ACID AND TOMATO MOSAIC VIRUS AGAINST BACTERIAL SPOT AND SPECK DISEASES IN TOMATO

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Summary

The tomato plants cv. Moperou possess in the gene Tm-2 for resistance against tomato mosaic virus (ToMV) were two fold treated with salicylic acid (SA), pre-inoculated with ToMV J and B-5 strains and challenge infected with trud*Pseudomonas syringae* pv. *tomato* (R0 and R1) and *Xanthomonas vesicatoria* (T1, T2 and T3). ToMV J stain infected systemically tomato plants and yellow mosaic was developed on the young leaves but B-5 did not cause any symptoms and it can not infect systemic tomato with gene Tm-2. The percentage of protection of ToMV pathotype P0 strain was between 93.4-98.6% and this of ToMV pathotype P2 strain - 82.5-91.7%. SAR induced by SA was no effective against bacterial spot and speck diseases. Both types of virus strains - virulent and avirulent - induce SAR which protects plants from challenge pathogen infection.