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Inhibiting Effect of Growth Regulators on Plant Viruses

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Abstract

The effect of the growth regulator β–monomethyl ester of itaconic acid (MEIA) on two economically important viruses, namely tomato spotted wilt virus (TSWV) and tomato mosaic virus (ToMV), was studied in tomato cultivars. Tomato plants of Keti and VK1 lines were infected separately with each virus: Keti with TSWV and ToMV, and VK1 with TSWV, respectively. The tomato plants were treated with MEIA in a concentration of $5x10^{-4}$ M after inoculation with TSWV in one variant, and with ToMV in another.

An inhibiting effect was established on both viruses – TSWV and ToMV, when the tomato plants were infected and after that sprayed with MEIA solution. These initial results are a good reason to continue with the studies both for the establishment of the effect of growth regulators (MEIA in particular) on virus pathogens and the physiological indexes that prove the decrease in stress and injuries on tomato plants with virus diseases after treatment with MEIA.

Key words: growth regulator, inhibiting effect, TSWV, ToMV.

Резюме

Изследван е ефектът на растежен регулатор - β–монометилов естер на итаконовата киселина (МЕИК), върху два икономически важни за доматите вируси: на доматената бронзовост (tomato spotted wilt virus-TSWV) и на доматената мозайка (tomato mosaic virus - ToMV). Доматени растения от линиите Кети и VK1 бяха инфектирани поотделно с всеки от вирусите съответно Кети с TSWV и ToMV а VK 1 с TSWV. След инфектиране в един вариант с TSWV и в друг вариант с ToMV, доматените растения бяха третирани с МЕИК в концентрация $5x10^{-4}$ М.

Инхибиращ ефект бе установен и върху двата вируса – TSWV и ToMV при инфектиране на доматени растения и след това пръскане с разтвор на МЕИК. Тези първоначални резултати дават основание да продължат изследванията не само за установяване ефекта на растежни регулатори (в частност на МЕИК) върху вирусни патогени, но и за установяване на физиологични параметри, доказващи намаляване на стреса и повредите при вирусно болни доматени растения след третирането им с МЕИК.

Introduction

Tomato spotted wilt virus (TSWV), *Bunyaviridae* family, *Tospovirus* genus and tomato mosaic virus (ToMV), *Virgaviridae* family, *Tobamovirus* genus cause yield reduction and deteriorate the quality of field and greenhouse-grown tomatoes. (Dikova, 2014a). The inhibition (full or partial suppression of virus multiplication, transport and activity in the host plants) with chemical resources acting, simultaneously as growth regulators is a perspective approach to the control of economically important viral diseases, such as tomato spotted wilt and tomato mosaic. This is especially important for greenhouse production, where the smaller area will make the treatments economically profitable vs. field tomato growing. The inhibitors are antiviral substances that suppress or fully impede the replication of the viruses and the external manifestation of the symptoms. These substances include growth regulators and substances originating from different organisms (antibiotics, enzymes, antisera) or higher plants (Kovachevsky *et al*., 1995). There are data on the inhibiting effect of fennel and lavender essential oils on plant viruse when used at a

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dilution of 3000 ppm (Shukla *et al.*, 1989; Dikova, 2014b). Extracts of different plants, such as *Plectranthus tenuiflorus,* had antiphytoviral activity to TSWV, which can be attacked *in vitro*. When this extract was mixed with the virus inoculum for three hours, it inhibited the local lesion development in the indicator plant *Chenopodium amaranticolor* by 100 % (Othman *et al.*, 2004).

Phytohormones have been used to control some viral and phytoplasma diseases. For example, the treatment with kinetin before or right after inoculation with some plant viruses decreased viral multiplication and the intensity of symptoms in local infection and delayed plant perishing in systemic infection. For the time being, only gibberellin acid is used for the restriction of the *Sour cherry yellows virus* infection on cherry in field conditions (Stancheva, 2004).

MEIA is a growth regulator with a wide spectrum of activity, which increases the yield and improves the quality of various economically important crops. This compound with low toxicity is a derivative of naturally occurring plant metabolites. It is known that applied on cereals and leguminous, MEIA leads to a decrease in stress and injuries caused by abiotic and biotic stress factors. MEIA reduces the negative effect of some herbicides (Karanov *et al*., 2001), induces systemic acquired resistance (SAR) in tomato plants *(Lycopersicon esculentum L.*) against a tomato mosaic virus (ToMV) (Krezhova *et al.*, 2010).

The objective of the study was to establish the effect of the growth regulator MEIA on tomato plants infected with two pathogenic plant viruses typical of tomatoes.

Material and Methods

The investigations were carried out on two tomato genotypes characterized by different expression of anthocyanins in plant vegetative parts. VK1 is a non- anthocyanin line, while Keti has high anthocyanin content in all plant parts (Fig. 1).

We used the growth regulator β–monomethyl ester of itaconic acid (MEIA) in the trial for TSWV and ToMV inhibition. The stimulating effect of MEIA, which is a derivative of carboxylic acids, was proved on healthy tomato plants parallel to the trials for the establishment of the inhibiting effect on viruses. MEIA was applied after infecting the plants with TSWV in one variant, and with ToMV in the other. The plants were treated at the 3rdto 5th real leaf stage with MEIA solution in a concentration of 5.10^{-4} M with an addition of Tween 80 0.05 % v/v tap water solution. Control plants were treated with Tween 80 0.05 % v/v tap water solution only.

a) b)

Fig. 1. a) line VK 1 ; b) line Keti

TSWV infection was done by artificial mechanic inoculation with infectious material of pepper isolate of the same virus, replicated in tobacco plants – *Nicotiana tabacum* cv. Samsun NN. The trial was carried out with tomato seedlings, naturally infected with ToMV, probably by transmission of the virus with seeds of the Keti line. We used virus-carrier seedlings without mechanical inoculation with ToMV and after that treated with MEIA the tomato plants which manifested symptoms. The tests for establishing the inhibiting effect of MEIA on TSWV and ToMV were carried out by the serological method DAS-ELISA according to Clark and Adams (1977). Tomato plants of lines VK1 and Keti were analyzed in the following variants: 1. Healthy plants (negative controls for TSWV and ToMV); 2. Plants infected with TSWV and ToMV (positive controls for TSWV and ToMV); 3. Plants treated with MEIA; 4. Plants infected with TSWV or ToMV and subsequent treatment with MEIA. All trials for artificial inoculation of the tomato plants and the positive controls for both viruses were carried out according to Noordam (1973).

Results

The damages caused by TSWV and ToMV affected the shelf appeal of the fruits picked from the infected plants (Fig. 2a and 2b).

The growth regulator MEIA had a stimulating effect when applied on healthy non-infected tomato plants (Fig. 3, right). The leaves of the treated plants, compared to healthy non-infected and not treated with MEIA controls were larger and of darker green color (Fig. 3).

a) b)

Fig. 2. Damages, caused by plant viruses on tomato fruits
a) - chlorotic concentric spots, caused by TSWV
b) - necrotic figures, caused by mixed TSWV and ToMV infection

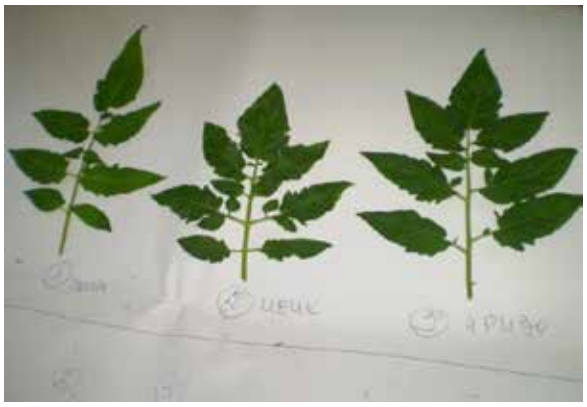

Fig. 3. Effect of the growth regulator MEIA on tomato line VK1
Left - leaf of a tomato plant not infected with TSWV or ToMV and not treated with MEIA
Right - leaf of a tomato plant not infected with TSWV or ToMV but treated with MEIA

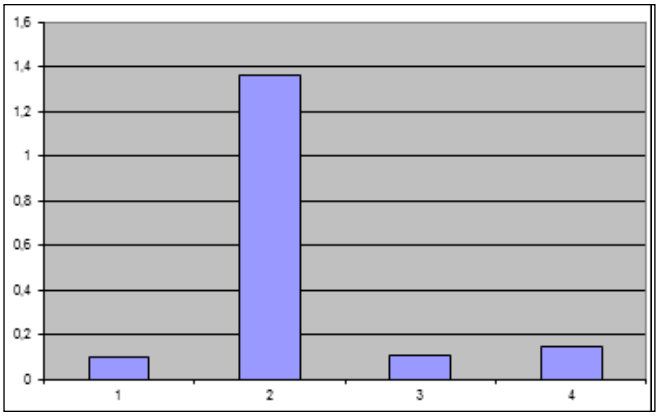

Fig. 4. Effect of TSWV infection and MEIA treatment on VK1 tomato line
On ordinate – Optical density (average extinction values for TSWV of tomato plants of the separate variants)
On abscissa – variants: 1. Healthy plants; 2. TSWV infected plants; 3. Plants treated with MEIA; 4. TSWV infected + MEIA treated plants

We established the inhibiting effect of MEIA on TSWV (Fig. 4 and 5) and ToMV (Fig. 6) by comparing the optical density values (the extinction values of samples from all tested tomato plants).

The inhibiting effect on TSWV was manifested in VK1 and Keti line, and on ToMV – in Keti, when the infected plants were treated with MEIA in a concentration of $5x10^{-4}$M. The average extinction values of TSWV and ToMV infected tomato plants treated later with MEIA in a concentration of $5x10^{-4}$M did not exceed the values of the healthy plants more than 2.5 times (threshold value), therefore, they were not virus carriers. This phenomenon could be due to the effect of the growth regulators (Figures 4, 5 and 6). The values of the virus infected plants were manyfold higher than those infected and afterwards treated with MEIA. The viral concentration of TSWV and ToMV during the vegeta-

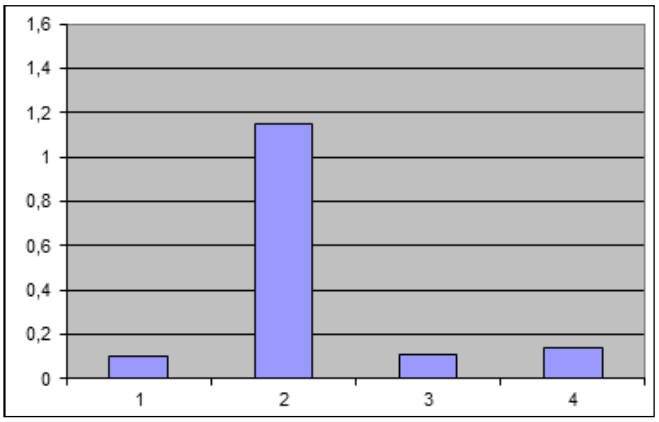

Fig. 5. Effect of TSWV infection and MEIA treatment on tomatoes cv. Keti
On ordinate – Optical density (average extinction values of TSWV infected tomato plants for the separate variants)
On abscissa – Variants: 1. Healthy plants; 2. TSWV infected plants; 3. Plants treated with MEIA; 4. TSWV infected + MEIA treated plants

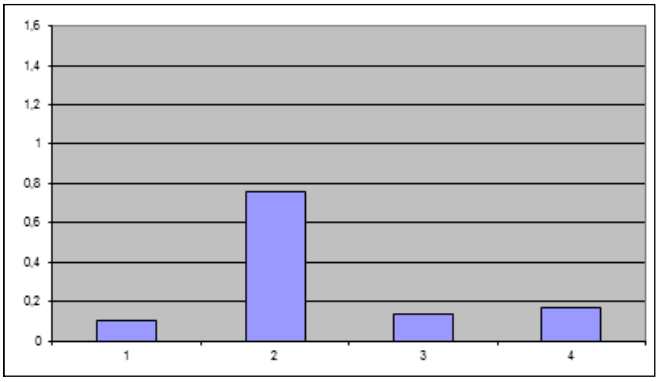

Fig. 6. Effect of ToMV infection and MEIA treatment on tomato line Keti
On ordinate – Optical density (average extinction values of ToMV infected tomato plants for the separate variants)
On abscissa – Variants: 1. Healthy plants; 2. ToMV infected test plants from the species *Nicotiana tabacum* cv. Samsun; 3. ToMV infected + MEIA treated plants from 03.09.2015; 4. ToMV infected + MEIA treated plants from 17.09.2015

tive period could be reduced with MEIA treatment. The results were encouraging to continue the investigations in view of decreasing the damages caused by TSWV and ToMV on the vegetative parts of tomato plants and their fruit, and guaranteeing a higher quality produce.

Discussion

It was found that anthocyanins had a protective effect with regard to abiotic and biotic stress (Krezhova *et al.,* 2010). There were no statistically significant differences in terms of virus inhibition both in the anthocyanin line and the one without anthocyanin. It is known that MEIA, applied together with herbicides with a different mechanism of action, can decrease the herbicide rate by half and still maintain its effect. Thus MEIA has a protective effect on mono-and dicotyledonous cultivated plants (Karanov *et al.,* 2010). The initial results gave a good reason to continue with the studies in order to establish the physiological parameters that prove the reduction of stress in virus infected tomato plants, following the treatment with MEIA.

Conclusion

β–Monomethyl ester of itaconic acid (MEIA) used as a growth regulator had an inhibiting effect on two mostcommon viral pathogens on tomato: tomato spotted wilt virus (TSWV) and tomato mosaic virus (ToMV). It is necessary to conduct a future detailed study on the mechanisms of the protection effect. The use of MEIA growth regulator as a plant virus inhibiting tool accounts for efficient control aiming at regulating biotic stress in plants. Combined with the reduction of the harmful effect of herbicides, it may become very important for the overall protection of plants and the environment.

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