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PROTECTIVE EFFECT OF PLANT GROWTH REGULATORS MEIA AND 4PU-30 AGAINST *TOMATO SPOTTED WILT VIRUS* (TSWV) ON TWO TOMATO GENOTYPES

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Abstract

The effects of β-monomethyl ester of itaconic acid (MEIA) and phenylurea cytokinin 4PU-30 on two genotypes of tomato ("Keti" and VK1) infected with *Tomato spotted wilt virus* (TSWV) were investigated. The stress markers malondialdehyde and free proline as well as the viral concentration, expressed by the extinction values of TSWV as indicators of the level of oxidative stress and rate of virus replication were measured. The β-monomethyl ester of itaconic acid (MEIA) rendered inhibiting effect on TSWV infection in both lines of tomato while the phenylurea cytokinin 4PU-30 (N^1-(2-chloro-4-pyridyl)-N^2-phenylurea) was effective only in line "Keti". It was found that line "Keti" was more susceptible to TSWV infection than line VK1.

Key words: *Tomato spotted wilt virus* (TSWV), β-monomethyl ester of itaconic acid (MEIA), phenylurea cytokinin (4PU-30), tomato, oxidative stress

Introduction. Tomato spotted wilt virus, *Bunyaviridae* family, *Tospovirus* genus is a reason for considerable yield and quality reduction of field and greenhouse grown tomatoes. The use of plant growth regulators is a promising approach to achieve control of economically important virus diseases such as tomato spotted wilt and tomato mosaic. This is valid especially for greenhouse production

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of tomatoes where the treatments will be more effective and cheaper due to the limited areas and closed space.

The virus inhibitors are antiviral substances which suppress the viral replication and also affect the disease symptoms. Such substances with an inhibiting effect against plant viral pathogens are the essential oils [1].

The oxidative stress is a common result of pathogen attack and unfavourable environmental conditions. The overproduction of reactive oxygen species disturbs key physiological processes [2].

Improvement of the plant tolerance to stresses could be achieved by treatment with plant growth regulators. The major advantage of such approach is that the grower can provide a rapid adequate reaction to the specific conditions of the environment. The phenylurea cytokinin 4PU-30 (N^1-(2-chloro-4-pyridyl)-N^2-phenylurea) was reported to mitigate the negative consequences of drought in maize seedlings [3], bean plants [4], and to alleviate the detrimental effects of the glyphosate action [5]. The application of cytokinins could increase plant resistance to some viruses [6].

MEIA (β-monomethyl ester of itaconic acid) is a plant growth regulator with a wide spectrum of activity, which increases the yield and improves the quality of various economically important crops. This compound is a derivative of naturally occurring plant metabolites. It is known that MEIA causes decrease of injuries due to abiotic and biotic stress factors. MEIA reduces the negative effect of some herbicides [7]. It was also reported to induce systemic acquired resistance (SAR) in tomato plants against tomato mosaic virus (ToMV) [8]. In our previous investigations we found that the treatment with MEIA of infected with TSWV or ToMV tomato plants resulted in a long lasting inhibitory effect on the viral infection [9].

The effects of two plant growth regulators with different modes of action were investigated on two genotypes of tomato infected with TSWV. The aim of this study was to search for inhibitory effects on the virus infection, and for physiological parameters reflecting the alleviation of the oxidative damages in TSWV infected tomato plants.

Materials and methods. The investigations were carried out with two tomato genotypes, differing in the anthocyanin levels in the vegetative parts of the plants. VK1 is a sterile non-anthocyanin line, while "Keti" is characterized by high levels of anthocyanin content.

The tomato plants were grown on soil containing pods (30/30/10 cm) under greenhouse conditions: temperature 20—25 °C; 60–70% relative air humidity (which is a precondition for development of the viral infection), and daylight of 15 h. When plants developed 3–4 leaves they were treated with 1 mM water solution of MEIA (β-monomethyl ester of itaconic acid) or 0.1 mM water solution of the phenylurea cytokinin 4PU-30 (N^1-(2-chloro-4-pyridyl)-N^2-phenylurea). Tween 80 was used as a surfactant (0.05%v/v). The control plants were treated with wa-

ter solution of Tween 80 only. Twenty-four hours later part of the plants were inoculated with TSWV inoculum. The TSWV inoculum was obtained as follows: Tobacco plants (*Nicotiana tabacum* L.) cv. Samsun NN were inoculated with pepper isolate of TSWV and two weeks after symptom development the leaves were harvested. The tobacco leaves were ground in Na-K phosphate buffer (pH 7.0) containing 0.2% ascorbic acid and 0.2% Na_2SO_3 (leaf material:buffer ratio 1:1.5). The resulted plant sap was filtered through cheese cloth and was used to infect the tomato plants. The inoculated tomato plants were incubated in darkness under high humidity conditions for one night. After 7–14 days phenological observations of the TSWV infection were made. Samples for analysis were collected 14 days after inoculation with TSWV.

The tomato plants were divided into the following variants:

1. Control; 2. Inoculated with TSWV; 3. Treated with MEIA (1mM); 4. Treated with 4PU-30 (0.1mM); 5. Treated with MEIA and inoculated with TSWV; 6. Treated with 4PU-30 and inoculated with TSWV.

All procedures of artificial inoculation of tomato plants were preformed according to Noordam [10].

Each sample derived from individual plant was analyzed by DAS-ELISA [11] with antiserum for TSWV. The TSWV antiserum was purchased from LOEWE Biochemica (Germany). The optical density (OD) was determined by measurements of the extinction values for TSWV on a SUMAL PE spectrophotometer, Karl Zeiss Jena (Germany).

The biochemical analyses were carried out with fresh plant material homogenized with 0.1% (w/v) trichloracetic acid and assayed according to the appropriate methods listed below. The lipid peroxidation was assessed by the content of malondialdehyde (MDA) which is a side product of the biomembrane unsaturated fatty acids peroxidation. The concentration of MDA was determined after derivatization for 45 min with thiobarbituric acid at 100 °C, and was calculated by using of extinction coefficient $\varepsilon = 155$ $mM^{-1}cm^{-1}$ [12]. The content of free proline was measured according to the method of Bates et al. [13]. The absorbance was measured at 520 nm after incubation for 1 h in acid ninhydrin at 100 °C.

The experiments were performed three times in three replicates. Data presented are mean values with standard errors (±SE).

Results and discussion. The data of DAS-ELISA demonstrated that line "Keti" was about 31% more susceptible to TSWV infection than line VK1. The preliminary treatment with MEIA restricted the virus development in both genotypes of tomato – 48% of the control in line "Keti" and 73% of the control in line VK1. The treatment with 4PU-30 showed inhibitory effect on virus distribution only in line "Keti" – 34% as compared to the Control (Fig. 1). Similarly to DAS-ELISA, the concentrations of the stress markers MDA and free proline were altered in a similar trend.

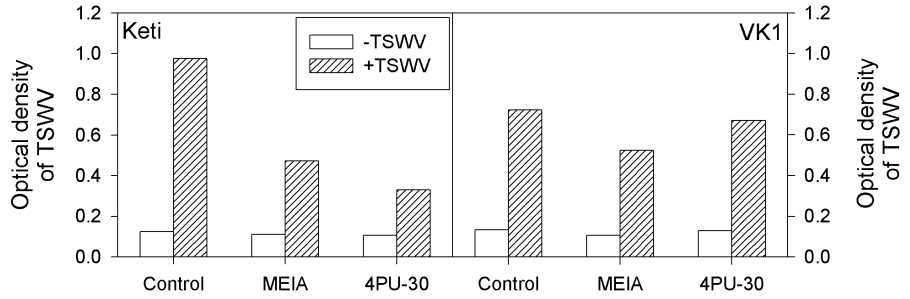

Fig. 1. Effects of MEIA and 4PU-30 on the optical density (OD) of TSWV, represented by the average extinction values for TSWV in non-infected (–TSWV) and infected (+TSWV) with TSWV tomato lines "Keti" and VK1

The changes in the concentrations of MDA and free proline are widely used criteria to assess the injuries occurring in plants due to various stress factors. The reactive oxygen species generated during the stress, attack the unsaturated fatty acids of biomembranes and initiate chain reactions of lipid peroxidation with final products malondialdehyde and ethane. The consequences of this, result in disintegration of the biomembranes, destruction and disorder of the normal membrane permeability [14]. Another result of a number of stresses like drought, salinity, etc., is that plants accumulate the imino acid proline. This increase of proline content is of dual nature – it could be a protective response or it could be only a symptom of stress. In all cases however, the increased content of free proline is an evidence of disturbance in the physiological status of plants [13, 15, 16]. Proline accumulation also occurs in response to pathogen attack [17].

It was found that the inoculation of line "Keti" with TSWV resulted in a significant increase of the concentrations of MDA (295%) and free proline (206%), while in line VK1 the content of MDA was slightly increased (125%) and that of proline was even slightly decreased (83%) as compared to the respective non-treated Controls (Fig. 1, 2). These data clearly demonstrate that line "Keti" is more susceptible to TSWV than line VK1. This observation is in accordance with the data obtained by the DAS-ELISA analyses.

Line "Keti" is characterized by its high levels of anthocyanin content. It is well known that under certain unfavourable environmental conditions plants synthesize anthocyanins, which allows them to develop a resistance to a number of stresses. Some anthocyanins possess antifungal, antibacterial or antiviral properties and have the potential to protect plants from pathogen attack. Nevertheless anthocyanins are not as effective protectors as some flavonoids and hydroxycinnamic acids [18]. In our previous investigations we did not find a direct correlation between the high anthocyanin content in line "Keti" and its tolerance/susceptibility to TSWV [19]. The data indicate that the different levels of

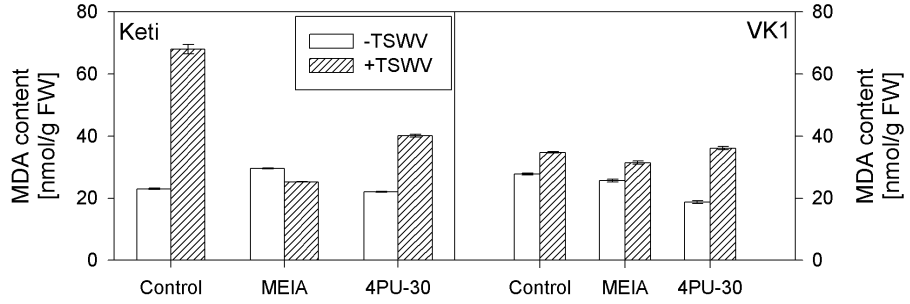

Fig. 2. Effects of MEIA and 4PU-30 on the concentration of MDA in non-infected (–TSWV) and infected (+TSWV) with TSWV tomato lines "Keti" and VK1

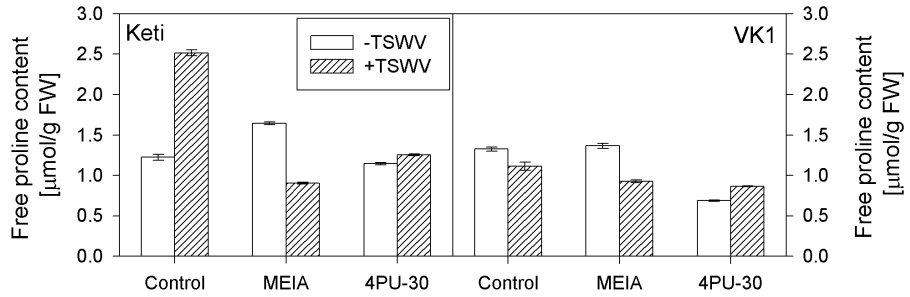

Fig. 3. Effects of MEIA and 4PU-30 on the concentration of free proline in non-infected (–TSWV) and infected (+TSWV) with TSWV tomato lines "Keti" and VK1

tolerance/susceptibility to TSWV of both lines of tomato are not related to the anthocyanin content. Regardless of the antiviral and antioxidant properties of the anthocyanins, their higher concentration in line "Keti" appears not to be a key factor for an increased tolerance to TSWV.

When applied alone, MEIA did not change the MDA content in line VK1 and slightly increased MDA (129%) and proline (135%) content in line "Keti" (Fig. 2, 3). The treated with MEIA and infected with TSWV line "Keti" contained considerably lower amount of MDA (37%) and proline (36%) than the respective infected Controls (Fig. 2, 3). Similar trend, but expressed to a lesser extent was obtained in line VK1.

The treatment with 4PU-30 only, did not cause considerable alterations in the content of MDA and free proline in line "Keti", while in line VK1 these values were lower than the non-treated Control (Fig. 2, 3). Similarly to MEIA, the concentrations of stress markers in line "Keti" when treated with 4PU-30 and infected with TSWV were significantly lower than those in the infected Controls – MDA 59% and proline 48%.

The data about the virus concentration and the content of stress markers

proline and MDA in line "Keti" showed similar trends. This could be because the growth regulators MEIA and 4PU-30 acted as inhibitors of TSWV reproduction, or they stimulated the defense systems and thus rendered protection against the oxidative damages in the treated plants. Line VK1 less susceptibility to TSWV than line "Keti" and the effects of the application of MEIA and 4PU-30 were also weaker. In this respect line VK1 could be a suitable model system for studies on the mechanisms of plant tolerance/resistance to viral infections. The role of anthocyanins in the susceptibility of both lines of tomato to TSWV is not definite.

The precise mechanisms of the protective action of MEIA and 4PU-30 against TSWV are not known yet. However, the observed encouraging effects of MEIA and 4PU-30 on tomato plants infected with TSWV give ground for further studies on the possibilities for application of plant growth regulators as tools to neutralize pathogen attacks.

Conclusions. The pre-treatment with MEIA and 4PU-30 limited the development of subsequent infection of TSWV in two lines of tomato plants and lessened the negative consequences of the oxidative stress.

Line "Keti" is more susceptible to TSWV than line VK1.

The protective effects of the investigated plant growth regulators was better expressed in the more susceptible to TSWV line "Keti".

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