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BIOCHEMICAL RESPONSES OF TWO TOMATO GENOTYPES DIFFERING IN GENE *ANTHOCYANINLESS OF HOFFMANN* (*AH*), TREATED WITH UV-B IRRADIATION AND *β*-MONOMETHYL ESTER OF ITACONIC ACID (MEIA)

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Abstract

Tomato (*Solanum lycopersicum*) – cv. Ailsa Craig (ACr, wild type) and its isogenic/near isogenic line [IL/NIL] *ah* (*anthocyaninless of Hoffmann*) were grown as a soil culture. Four-week-old plants were treated with 1mM *β*-monomethyl ester of itaconic acid (MEIA) and 24 h later were irradiated with 12.8 $kJm^{-2}d^{-1}$ UV-B. Hydrogen peroxide (H_2O_2), free proline, free thiols and total phenols were measured in the fourth leaf of plants at 0, 24 and 48 h after cessation of UV-B irradiation. At the end of experiment all irradiated plants showed desiccation and curling of some leaf nodes. These negative effects were less expressed by application of MEIA prior to UV-B especially for ACr cv., containing anthocyanins. Concentration of H_2O_2 rise in UV-B treated plants but preliminary application of MEIA lessen this stress marker in ACr cv. whereas in anthocyaninless mutant it was permanently enhanced. Combined treatment provoked permanently augmented proline levels in both lines, with exception of data for anthocyaninless mutant at 24 h after irradiation. Preliminary application of MEIA also led to lower accumulation of free thiols and total phenolics as compared to irradiated only plants especially in ACr cv. Anthocyaninless mutant is more sensitive to UV-B stress than the wild type and possesses less total phenolic compounds, compensated by higher concentrations of free thiols measured at 24 and 48 h in combined variant. Comparative data analyses of phenotypic effects and non-enzymatic antioxidant's amount

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suggest that MEIA has protective effect against UV-B irradiation through activation of different defence mechanisms related to particular characteristics of both tomato genotypes, and this effect was more pronounced for anthocyanins containing genotype.

Key words: antioxidants, hydrogen peroxide, protector, tomato, UV-B radiation

Abbreviations: ACr – Ailsa Craig, wild type, *ah – anthocyaninless of Hoffmann* mutant line, GAE – gallic acid equivalents, H_2O_2 – hydrogen peroxide, MEIA – β-monomethyl ester of itaconic acid, ROS – reactive oxygen species, UV – ultraviolet radiation

Introduction. During the past few decades a decrease of ozone layer as a result from human activities has been observed. The reduction of the ozone layer could lead to a significant increase of UV-B irradiation (290–320 nm). UV-B irradiation has a range of negative effects on plant organisms: it reduces growth and alters morphology, disrupts important macromolecules, modifies biosynthesis of secondary metabolites, provokes oxidative stress via overproduction of reactive oxygen species, disturbs the normal physiological processes and may even cause death [1, 2]. To reduce the noxious effects of UV-B radiation, plants have developed a variety of detoxification mechanisms, such as enhancement of the antioxidant system, activation of photolyases and accumulation of UV-absorbing compounds [1–3]. Plants are able to overcome the harmful stress effects by themselves when the strength of the stressor does not exceed the endogenous defence capacity. Application of compounds possessing different chemical nature or physiological mode of action could enhance the effectiveness of the antioxidant defence systems when the strength of the stressor exceeds the plant protection capacity. When applied in low doses, the substances activate cell metabolism, improve plant physiological processes, and increase plant resistance to various unfavorable stress factors [4–6]. It was previously shown that the β-monomethyl ester of itaconic acid, MEIA (derivative of naturally occurring dicarboxylic acid) had defensive effect against the herbicide chlorsulfuron in maize [7], UV-C radiation in wheat [8] and biotic stress in tomatoes [9]. Contemporary researches show that anthocyanins play a certain role in tolerance to stressors as diverse as drought, UV-B, and heavy metals, as well as resistance to herbivores and pathogens [3]. Nine mutations that result in the complete absence of anthocyanin in all plant organs during the whole vegetation period are known in tomato [10]. One of them, mutation *ah (Hoffmann's anthocyaninless)* was characterised by co-ordinate reduction in the activities of dihydroflavonol 4-reductase, chalcone synthase and flavone 3 hydroxylase – the key enzymes involved in phenolic secondary metabolites [11]. The plants have ability to synthesise flavones and/or flavonols, but not anthocyanins.

The aim of the current study is to evaluate whether the protective effect of MEIA against UV-B irradiation varies within tomato genotype which contains

Fig. 1. Phenotypic changes of ACr tomato plants preliminary treated with 1 mM β-monomethyl ester of itaconic acid (MEIA) and irradiated with 12.8 kJ m^{-2} day^{-1} UV-B light at the 48th hour after irradiation

Fig. 2. Phenotypic changes of *ah* tomato plants preliminary treated with 1 mM β-monomethyl ester of itaconic acid (MEIA) and irradiated with 12.8 kJ m^{-2} day^{-1} UV-B light at the 48th hour after irradiation

anthocyanins (as it is already known that anthocyanins possess defensive role against unfavorable environments [3]) and mutant tomato genotype which does not contain leaf anthocyanins (the wild type ACr and its isogenic/near isogenic line [IL/NIL] *ah*, respectively).

Materials and methods. ***Plant material, treatment, and measurements.*** Young tomato plants (*Solanum lycopersicum* L.) the wild type (cv. Ailsa Craig, ACr) and its isogenic/near isogenic line [IL/NIL] *ah* (*anthocyaninless of Hoffmannline*) constructed and described by Maxon-Smith and Ritchie [12] were grown as soil culture in a growth chamber (16/8 h photoperiod; 60–70% relative air humidity, 160 µmol $m^{-2}s^{-1}$ photon flux density; 25 ± 2 °C). Twenty-eight days after germination, part of the seedlings were leaf sprayed with 1 mM MEIA solution, and 24 h later half of them were subjected to UV-B irradiation (12.8 kJ m^{-2} day^{-1}). UV-B dose was chosen since it was published that in the middle attitudes it reached 6–13 kJ m^{-2} day^{-1} [13]. The analyses were performed at 0, 24 and 48 h after cessation of UV-B stress program with fresh material collected from the 4th true leaf of seedlings. Hydrogen peroxide was measured spectrophotometrically according to Alexieva et al. [2]. Free proline content was determined by the method of Bates et al. [14]. Content of free thiol groups were determined according to Edreva and Hadjiiska [15]. Total phenols were determined using gallic acid (GA) as a standard by the method of Swain and Goldstein [16].

Replication and statistics. All experiments were repeated three times with three to six replications. The results reported in the figures are means of the values with standard error (SE).

Results and discussion. The typical for UV-B-treated plants morphogenic responses leaf curling and desiccation [17] were observed in irradiated ACr tomato cv. and its *ah*-IL and these effects were more pronounced in anthocyaninless mutant (Figs 1 and 2). Preliminary application of MEIA diminished these negative UV-B consequences; this positive effect of MEIA was obvious mostly for ACr genotype.

High concentrations of active oxygen species lead to oxidative stress events but in low amounts they act as a signal molecule triggering appropriate defence response [18, 19]. All the treatments (except combined treatment in *ah*-line at first measurement point) caused an increase of H_2O_2 content as compared to the respective controls (Fig. 3), but it was most augmented in UV-B irradiated plants of both cultivars. Hydrogen peroxide tended to increase and rise up to 146% (3rd measurement point) when MEIA was preliminary applied to UV-B treated anthocyaninless *ah*-mutant plants. However, the application of MEIA prior to UV-B irradiation maintained hydrogen peroxide levels lower as compared to UV-B irradiated only ACr plants. The data correspond well with the phenotypic response of tomato plants to both UV-B and combined treatment and lead us to presume that application of MEIA before irradiation protects the plants (especially ACr

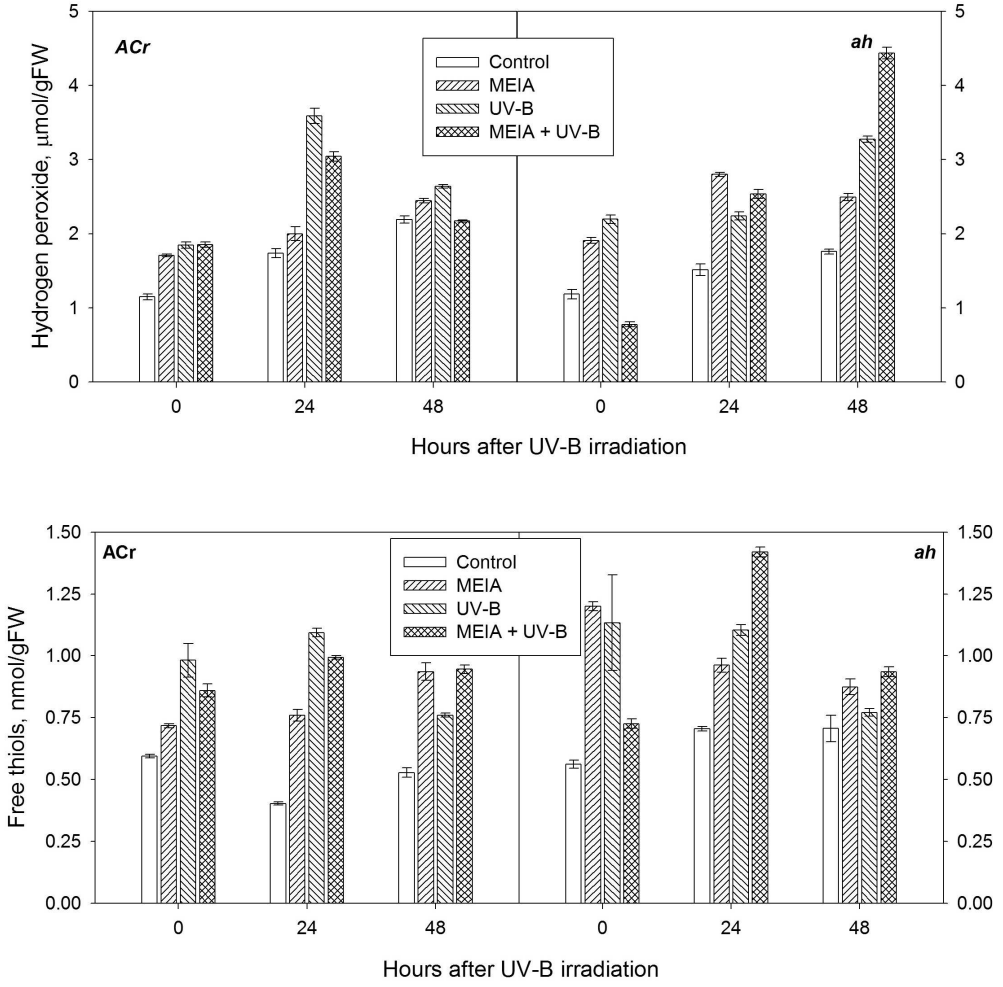

Fig. 3. Content of hydrogen peroxide and low-molecular thiol compounds in 4th leaf of tomato plants preliminary treated with 1 mM β-monomethyl ester of itaconic acid (MEIA) and irradiated with 12.8 kJ m^{-2} day^{-1} UV-B light. Data are mean values ± SE

cv.) against the detrimental effect of irradiation. Absence of anthocyanins makes anthocyaninless mutant much more sensitive to UV-B than wild type line. It is expected because like other phenolic compounds, leaf anthocyanins may quench the active oxygen species, act as solar screen absorbing UV-B and reducing photooxidative damage thus saving the UV-sensitive target macromolecules [3]. That is why our results support the importance of anthocyanins in responses of UV-B stressed plants. Additionally, the data obtained for hydrogen peroxide amount showed that preliminary application of MEIA could alleviate the oxidative stress caused by UV-B irradiation predominantly in ACr line.

To cope with oxidative stress triggered by UV-B irradiation the treated plants

switch on various antioxidative mechanisms. The major low-molecular thiol compound in plants is glutathione which has multiple roles, including participation in ascorbate-glutathione cycle and antioxidative properties [18, 19]. Generally the rise in low molecular thiol compounds is considered a positive adaptation reaction [20]. Initially, MEIA application led to accumulation of free thiol compounds, which was more pronounced in *ah*-mutant than in the wild type genotype ACr and probably was a compensatory mechanism for the lack of anthocyanins (Fig. 3). However, we found a different tendency of free thiol alterations in MEIA treated plants: low-molecular thiols increased with time in ACr line (21, 88 and 77% for the 1st, 2nd and 3rd measurement points, respectively) and gradually decreased with time (113, 36 and 24% for the 1st, 2nd and 3rd measurement points, respectively) in the *ah*-mutant. Low-molecular thiol compounds increased in UV-B treated plants; however their concentration rose durably during the experimental period in ACr line but decreased gradually with time in *ah*-mutant reaching the control levels at the final measurement point. The percentage increase of free thiols was found to be higher for ACr, indicating its ability better to avoid the oxidative events. On the other hand, the plants subjected to combined treatment initially have lower free thiol values than the respective irradiated variants. However, the levels rose in time and reached values higher than respective irradiated only plants. This result suggests positive adaptation reaction due to application of MEIA prior to UV-B especially in mutant tomato line. It also supports our presumption that *ah*-line compensates the lack of anthocyanins by activation of synthesis of low-molecular thiols (for example glutathione) to cope with oxidative stress provoked by UV-B irradiation.

The role of phenolic compounds was already pointed out to be part of the non-enzymatic defence system possessing antioxidative and solar screen properties. ACr cultivar showed higher constitutive levels of total phenolic compounds than its anthocyaninless-mutant (Fig. 4), which is expected as anthocyanins are part of total phenolic pool of plants. Therefore *ah*-mutant has lower capacity for UV-B screen than the ACr line, mainly due to the lack of anthocyanins. Both plants responded to UV-B radiation with gradual rise of phenolic compounds in time which was much more pronounced in ACr than in *ah*-mutant (97% in *ah*-mutant and 274% in ACr at the 3rd measurement point). The results showed that the unspecific non-enzymatic compounds free thiols and total phenolics were activated, and UV-B acclimatisation response was much better pronounced in ACr than in its *ah*-mutant.

Proline has multiple roles in plants serving as non-enzymatic antioxidant or compatible solute [21], but sometimes it could be considered as a stress marker [22]. Slight decrease of free proline is found in both MEIA treated plants (Fig. 4). ACr line maintains unaltered level of free proline in UV-B treated plants during the experimental period. Its *ah*-IL/NIL responded to UV-B stress with initial enhancement of free proline up to 55%, as compared to the respective

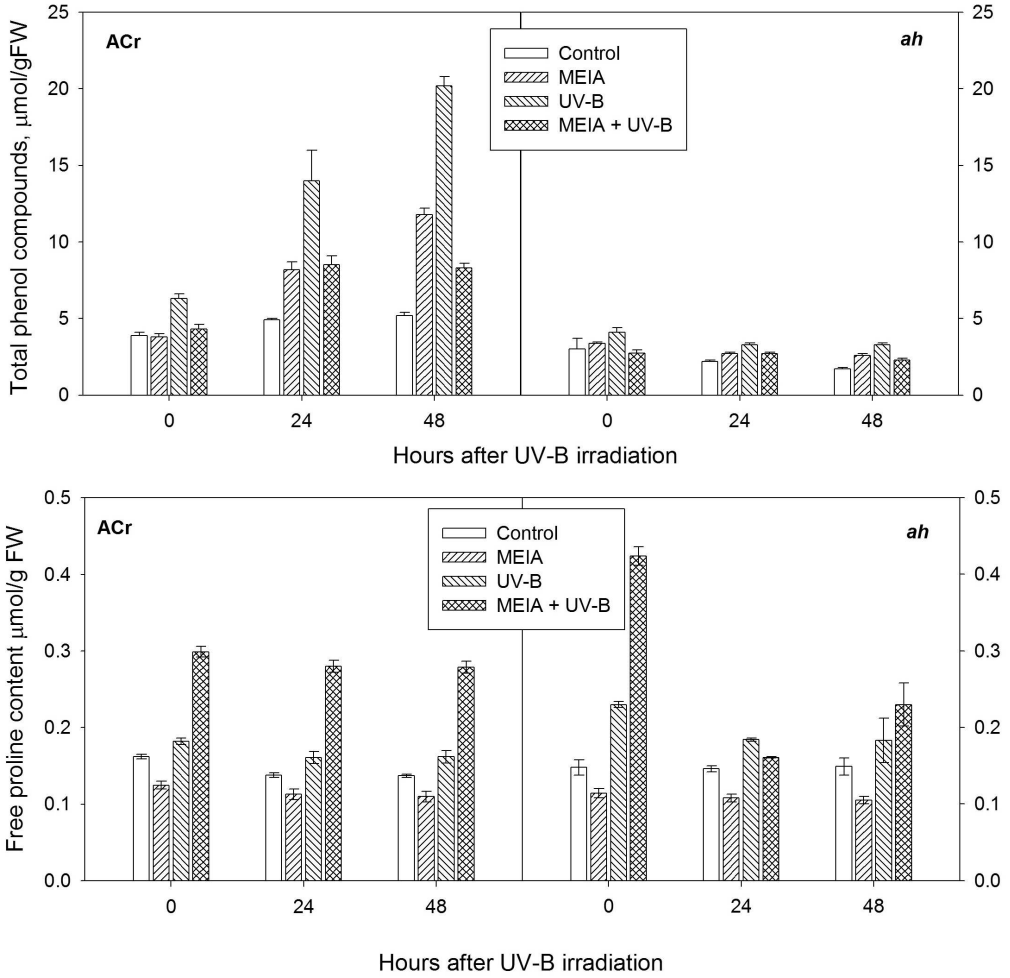

Fig. 4. Content of total phenols and proline in 4th leaf of tomato plants preliminary treated with 1 mM β-monomethyl ester of itaconic acid (MEIA) and irradiated with 12.8 kJ m^{-2} day^{-1} UV-B light. Data are mean values ± SE

control then tended to decrease. Application of MEIA prior to UV-B maintained enhanced free proline concentration (about 100%, as compared to the respective control) during the whole experimental period in ACr genotype. Initially proline rose sharply (186%) in MEIA+UV-B treated *ah*-mutants but this alteration was not stable during the experimental period. We assume that under UV-B stress conditions MEIA could be metabolised to itaconic (methylenesuccinic) acid. Thus MEIA might be forced to be involved in tricarboxylic acid cycle and to enhance proline synthesis in order to assist plant acclimatisation response to UV-B induced oxidative stress.

In general, our results support the significance of anthocyanins in defence

responses of UV-B irradiated plants. Further on the basis of comparative data analyses of phenotypic effects and non-enzymatic antioxidants' quantity it could be suggested that MEIA has protecting effect against UV-B irradiation through launch on different defence mechanisms related to specific characteristics of both tomato genotypes. The application of MEIA prior to UV-B irradiation protected better this genotype which contained anthocyanins and probably strengthened the defensive role of this natural endogenous protector. Further investigations related to determination of endogenous levels of anthocyanins of tomato plants treated with MEIA and UV-B irradiation will give additional information about the possible mechanisms of interaction between anthocyanin content and itaconic acid derivatives and how it reflects on the productivity of the tomato.

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