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MEIA ACTS AS PROTECTOR AGAINST UV-C IRRADIATION IN YOUNG WHEAT PLANTS

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Abstract

Young wheat plants (*Triticum aestivum* L., cv. Sadovo 1) grown as water culture were treated with 1 mM β-monomethyl ester of itaconic acid (MEIA) and 24 h later were irradiated with 0.75 kJ m^{-2} day^{-1} of UV-C light for 5 consecutive days. Twenty hours after the cessation of the stress programme, the amount of malondialdehyde (MDA), hydrogen peroxide, free proline, free thiols and total phenols was measured in the first leaf of plants. All measured parameters were increased by UV-C irradiation as compared to relative control values. Application of MEIA prior to UV-C led to reduction in stress marker contents (MDA and free proline) accompanied with an additional increase in the amount of low-molecular thiols and total phenols (measured as part of non-enzymatic antioxidant defence system) as compared to these measured in plants treated only with UV-C. Data obtained suggest that MEIA protects young wheat plants against UV-C irradiation.

Key words: hydrogen peroxide, malondialdehyde, free proline, protector, thiols, total phenols, UV-C radiation, wheat

Abbreviations: GAE – gallic acid equivalents; H_2O_2 – hydrogen peroxide; MDA – malondialdehyde; MEIA - β-monomethyl ester of itaconic acid; ROS – reactive oxygen species; UV – ultraviolet radiation

Introduction. The three major classes of ultraviolet (UV) radiation are UV-A (315–400 nm), UV-B (280–315 nm) and UV-C (200–280 nm). Except for high mountain locations UV-C irradiation does not reach the Earth's surface due to its absorption in the atmosphere [1]. Importantly, UV-C is most detrimental

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for live organisms because of its highest energy. UV-C photons destroy chemical bonds, causing a photochemical reaction. The overproduction of different reactive oxygen species (ROS) and development of oxidative stress affect important plant processes in UV-C irradiated plants [2]. Since monocots have a vertical pattern of leaf growth, they tend to capture less direct light than dicots, whose leaves tend to grow horizontally [3]. In this regard, monocotyledons are more tolerant to UV irradiation than dicotyledons [4].

Plants possess different defence systems (mainly including various antioxidant enzymes and non-enzymatic compounds) in order to defeat harmful stress consequences. When the strength of the stressor does not exceed the endogenous defence capacity, plants are able to overcome negative stress effects. The effectiveness of the antioxidant defence systems could be enhanced by application of compounds possessing different chemical nature or physiological mode of action. Applied in low doses, these protectors could activate cell metabolism, improve plant physiological processes, and increase plant resistance to various unfavourable stress factors [5, 6]. It was previously shown that the derivatives of dicarboxylic acids (for example β-monomethyl ester of itaconic acid, MEIA) had a protective effect against the herbicide chlorsulfuron in maize [7] and biotic stress in tomatoes [8]. The aim of the current study was to evaluate the possibility of MEIA to act as a protector on wheat plants irradiated with UV-C.

Materials and methods. ***Plant material, treatment, and measurements.*** Young wheat plants (*Triticum aestivum* L., cv. Sadovo 1) were grown as water culture in growth chamber (16/8 h photoperiod; 60–70% relative air humidity; 160 µmol $m^{-2}s^{-1}$ photon flux density; 24±2 °C). Five days after germination, part of the seedlings were leaf sprayed with 1 mM MEIA solution, and 24 h later the plants were subjected to UV-C irradiation (150 s day^{-1}or 0.75 kJ m^{-2} day^{-1}) for 5 consecutive days. The analyses were performed 20 h after cessation of UV-C stress programme with fresh material collected from the first leaf of 12-day-old wheat seedlings. Hydrogen peroxide was measured spectrophotometrically according to Alexieva et al. [9]. Free proline content was determined by the method of Bates et al. [10]. Malondialdehyde (MDA) content was determined as indicator of lipid peroxidation [11]. A content of free thiol groups was determined according to Edreva and Hadjiiska [12]. Total phenols were determined using gallic acid (GA) as a standard by the method of Swain and Goldstein [13].

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

Results and discussion. UV-C irradiation caused a significant increase (33% as compared to the control level) in MDA content (Fig. 1*A*). Application of MEIA prior to UV-C irradiation led to lower MDA concentration than the amount measured in plants treated only with UV-C. The lipid peroxidation of unsaturated fatty acids was diminished and it might be speculated that MEIA application

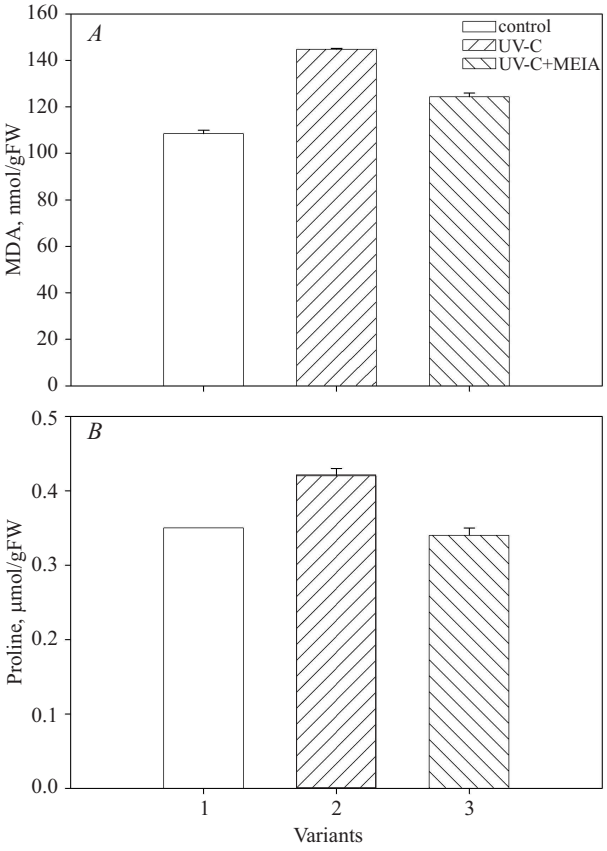

Fig. 1. Content of malondiladehyde (A) and free proline (B) in the first leaf of wheat plants preliminary treated with 1 mM β-monomethyl ester of itaconic acid (MEIA) and irradiated with 0.75 kJ m^{-2} day^{-1} UV-C light for 5 consecutive days. Data are mean values ± SE

could save cell membranes from ROS harm caused by subsequent UV-C exposure. Free proline content (Fig. 1B) was also augmented after UV-C exposure (20% in comparison with the control level) while leaf spraying of MEIA prior to irradiation reduced the accumulation of this stress marker and it reached the level in non-treated plants. Generally, the unfavourable environment (including UV radiation) causes oxidative events in stressed plants which provokes formation of ROS [14]. Usually ROS lead to increased content of MDA [11] and proline [15], which are sensitive stress markers. It was obvious that under conditions of UV-C irradiation, oxidative stress was developed since the amount of stress markers was significantly increased. At the same time, the reduced content of stress markers as a result of preliminary application of MEIA indicated that this compound mitigated the harmful effect of UV-C irradiation.

Both treatments caused an increase (up to 29% as compared to the control) in H_2O_2 content (Fig. 2). There was no significant difference in H_2O_2 amounts

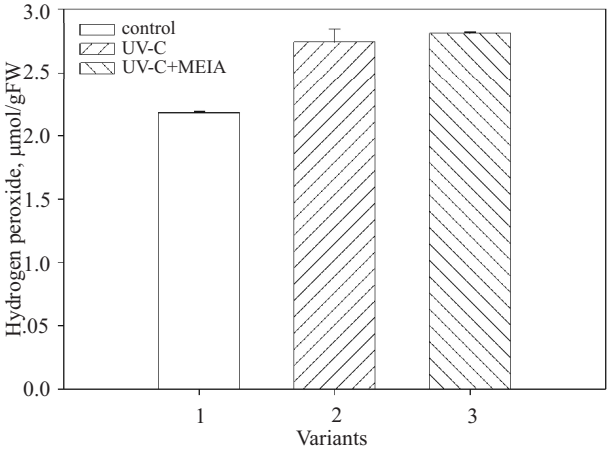

Fig. 2. Hydrogen peroxide content in the first leaf of wheat plants preliminary treated with 1 mM β-monomethyl ester of itaconic acid (MEIA) and irradiated with 0.75 kJ m^{-2} day^{-1} UV-C light for 5 consecutive days. Data are mean values ± SE

measured in both treated variants and it seemed that MEIA did not influence H_2O_2 content. Most likely the enhancement of its level was due to UV-C irradiation. Recent articles have demonstrated that when concentration of H_2O_2 is not extremely elevated this compound might serve as a signal molecule for activation of endogenous plant defence system under stress conditions and trigger acclimation of plants [14, 16–18]. As a part of non-enzymatic endogenous defence system [19, 20], we have measured the content of free thiol groups (Fig. 3*A*) and total phenols (Fig. 3*B*). UV-C treatment increased the free thiols content up to 19% as compared to the control. Preliminary spraying with MEIA augmented additionally its content (30% in comparison with the control level). Similarly, total phenols were increased by UV-C treatment (21% as compared to the control) and pretreatment with MEIA led to additional rise in its concentration (41% in comparison with the control). In plants the low-molecular thiol compounds include mainly glutathione and cysteine. Glutathione is the most abundant thiol compound in living cells. Generally, the enhancement of glutathione and total phenols under stress conditions is assumed to be a beneficial response, whereas its diminution leads to negative consequences for plants [14, 21]. Augmentation of low-molecular thiols and total phenols (Fig. 3 A, B) indicates activation of the non-enzymatic defence system in wheat plants, much more pronounced after combined administration of MEIA and UV-C than after UV-C treatment alone. Possibly the increase in concentration of low-molecular thiols and total phenols by UV-C was adaptation response of plants. Since application of MEIA prior to UV-C irradiation led to much higher concentration of low-molecular thiols and total phenols in wheat plants, we assumed that MEIA acted as a protector which

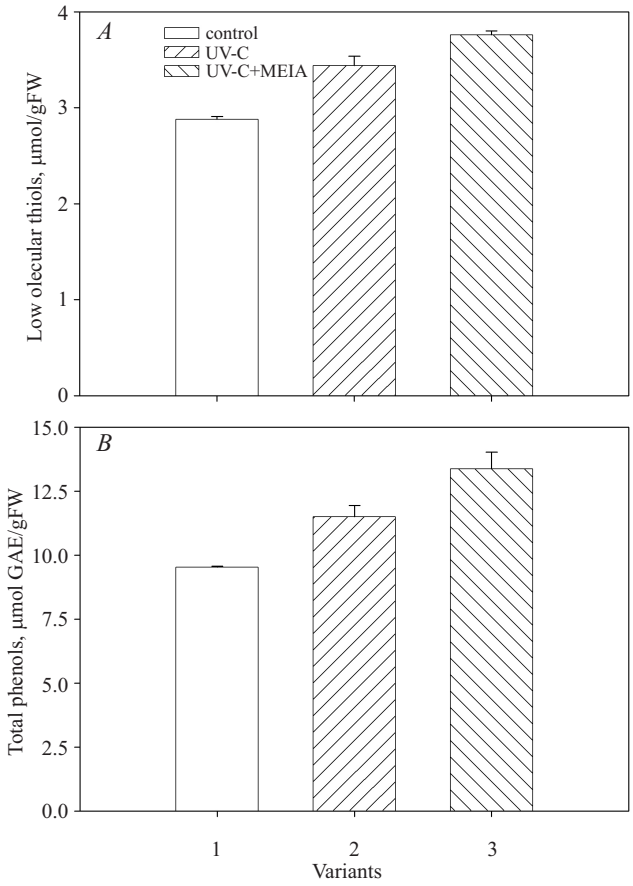

Fig. 3. Content of low-molecular thiols (A) and total phenols (B) in the first leaves of wheat plants preliminary treated with 1 mM β-monomethyl ester of itaconic acid (MEIA) and irradiated with 0.75 kJ m^{-2} day^{-1} UV-C light for 5 consecutive days. Data are mean values ± SE

strengthens the antioxidative defence systems. This suggestion is in line with the generally accepted idea that free thiols and total phenols which possess antioxidative properties are involved in the non-enzymatic defence system of plants [19, 20] and their increase is a beneficial response under adverse environment [14, 21].

In conclusion, all measured parameters were increased by UV-C treatment. Application of MEIA prior to UV-C irradiation caused a significant reduction in the amount of stress markers (MDA and free proline) as compared to UV-C variant, accompanied with an additional augmentation of free low-molecular thiols and total phenols (as a part of non-enzymatic antioxidant defence system), which suggested a positive adaptation response of wheat plant treated with MEIA and UV-C. On the basis of the results presented here, we suppose that MEIA could act as a protector of young wheat plants against UV-C irradiation. However,

additional investigations on other non-enzymatic antioxidants and activities of defence enzymes are needed to confirm the protective role of this plant growth regulator against UV-C irradiation of wheat plants.

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