

MELATONIN APPLICATION EFFECTS ON TWO BULGARIAN WHEAT CULTIVARS

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Abstract

Wheat is a staple crop for humankind and it is important to have environmentally safe tools to cope with the most important stress factors for agriculture. Melatonin is reported as universal molecule, possessing plant growth regulating properties and various important functions, including its usage as biostimulant against stress factors in plants. We aimed to investigate if melatonin application could cause unexpected adverse effects in two Bulgarian wheat cultivars (Fermer and Gines). The assessment of the content of selected stress markers such as malondialdehyde, electrolyte leakage and free proline, together with phenotype characteristics distinctly showed that 75 μ M melatonin supplementation via roots does not cause deterioration of plant physiology responses in tested Bulgarian wheat cultivars.

Key words: plant growth regulator, stress markers, *Triticum aestivum* L.

Abbreviations: MDA – malondialdehyde, EL – electrolyte leakage, ROS – reactive oxygen species

Introduction. Melatonin (N-acetyl-5-methoxytryptamine) is an endogenous molecule in plants shown to possess plant growth regulating and biostimulating properties toward various environmental stress factors [1, 2]. It is nontoxic biomolecule and its usage could be strategic in improving plants' stress tolerance

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[3]. In general, stress factors boost massive generation of the reactive oxidative species (ROS), which harm plants by cell membrane deterioration [2]. Standard method for assessment of stress damages to plant cells is the determination the content of stress biomarkers such as malondialdehyde (MDA) and electrolyte leakage (EL), along with free proline. Melatonin was shown to have positive impact on the metabolism and physiology of plants subjected to stress, which is manifested by diminished content of the stress markers and decreased EL level [3]. It is reported to be ROS scavenger, to possess stress-signalling function and to act together with phytohormones, but also to regulate diverse biochemical reactions throughout the plant life [4].

The literature survey showed that melatonin application does not provoke significant alterations of stress-related biomarkers in plants grown under non-stress conditions [5–9], including foreign wheat cultivars [10–12]. Recently, phylogenetic analyses of 117 modern bread wheat cultivars have revealed that 78 Bulgarian varieties except one differed from other 38 bread wheat cultivars originated from the Central and Western European regions [13]. Until now the effects of melatonin application were not studied in Bulgarian wheat cultivars. The aim of the current study is to perform comparative analyses of the physiological reactions to melatonin treatment in two Bulgarian wheat cultivars possessing different tolerance to drought, i.e. cv. Gines and cv. Farmer. The cultivar choice was done based on the previous publications concerning studies of the drought tolerance of the aforementioned cultivars [14,15]. The monitoring of well-known stress markers MDA, EL and free proline was performed to test whether melatonin application could not cause negative effect on Bulgarian wheat cultivars and to explore further its capability to cope with the drought stress in the selected varieties.

Material and methods. Plant material and treatments. Bulgarian wheat cultivars (*Triticum aestivum* L., cv. Fermer and Gines) were soil-grown in growth chamber with the following parameters: 14/10 h (day/night) photoperiod, providing 200 $\mu\text{mol}/\text{m}^2/\text{s}$ photon flux density, 21 °C/19 °C (day/night) temperatures, and 60% relative air humidity. Water solution of 75 μM melatonin were root supplemented to 17-day-old seedlings (with fully developed second leaf) or to 24-day-old plants, while the control plants were irrigated with tap water. Based on the preliminary experiments the melatonin concentration was selected as promising in attenuating drought injuries in Bulgarian wheat cultivar Sadovo-1, which is officially certified as a standard for bread wheat. The plant samples were collected from above-ground parts and used for analyses of the selected stress marker contents and EL.

Stress markers content determination. Approximately 250 mg of fresh leaf material was ground in 4 mL of 1% (w/v) cold trichloroacetic acid (TCA) and then centrifuged for 30 min (15000 $\times$ g, 4 °C). Appropriate aliquots of the resultant supernatants were used for stress markers' free proline and MDA content. Malondialdehyde and free proline were determined according to the methods of

KRAMER et al. [16] and BATES et al. [17], respectively, with some modifications. Relative leakage of electrolytes was determined according to the method of CUI et al. [18] by using FiveEasy Plus Mettler Toledo conductivity meter.

Statistical analysis. The results are from three independent experiments performed in triplicate. The data are mean values $\pm$ standard error (SE). The Duncan's multiple range test was used to evaluate the significant differences at a level of 0.05.

Results and discussion. We chose two time-points of melatonin treatment: (1) on the 17-day-old and (2) on the 24-day-old seedlings, which aimed plant to be in different leaf stage. To assess if there are damages of the cellular biomembranes caused by lipid peroxidation or affecting the cellular integrity, the amounts of MDA (Fig. 1) and electrolyte leakage (Fig. 2) were determined on the 3rd, 4th and 7th day after each time-point of melatonin treatment. The amounts of MDA increased with time, which could be related with normal physiological plant ageing processes, which are well known to induce higher ROS production and the corresponding increase in lipid peroxidation of membranes [19]. Melatonin supplement via roots does not increase the content of MDA in the wheat cv. Fermer, indicating for lack of additional lipid peroxidation damages in cellular membrane.

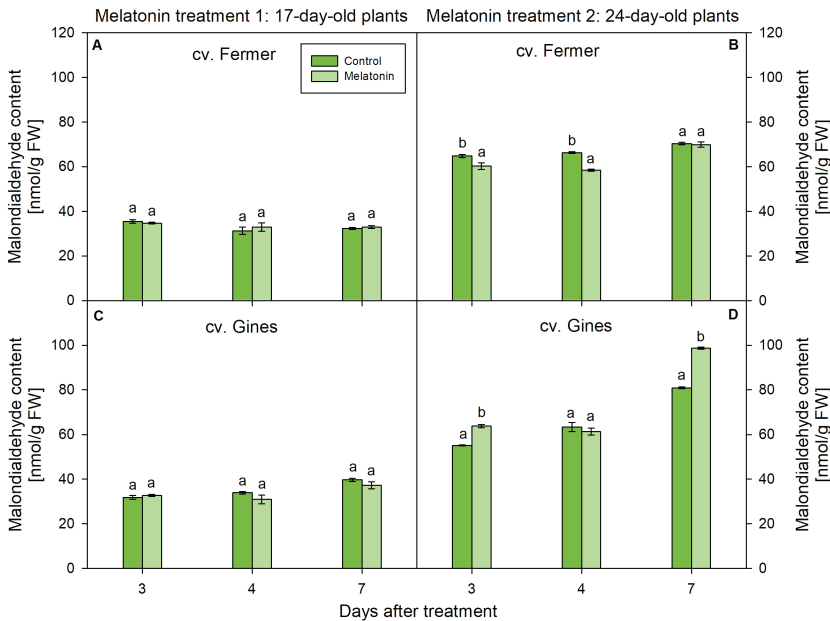


Fig. 1. Content of malondialdehyde in wheat cv. Fermer (A and B) and cv. Gines (C and D) measured on the 3rd, 4th, and 7th day after melatonin treatment 1 (A and C) and melatonin treatment 2 (B and D) presented as mean values with standard error. Different small letters indicate statistically significant differences between the treatments at $p < 0.05$

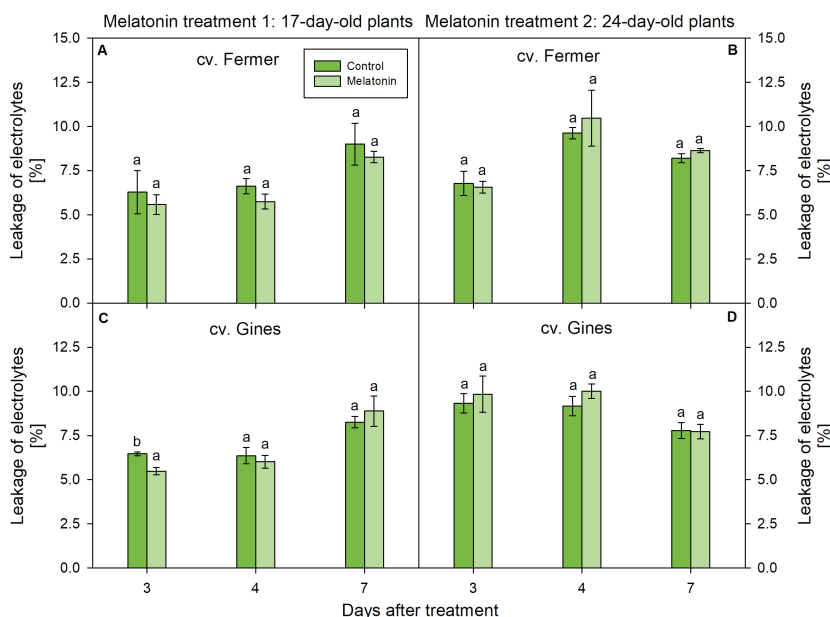


Fig. 2. Relative leakage of electrolytes in wheat cv. Fermer (A and B) and cv. Gines (C and D) measured on the 3rd, 4th, and 7th day after melatonin treatment 1 (A and C) and melatonin treatment 2 (B and D) presented as mean values with standard error. Different small letters indicate statistically significant differences between the treatments at $p < 0.05$

Moreover, when melatonin was supplemented to 24-day-old plants, there was slight but significant decrease of MDA amount compared to the control (Fig. 1B). The level of membrane lipid peroxidation in cv. Gines remained almost unaltered; however, some slight increase was observed when melatonin was supplemented to 24-day-old plants (Fig. 1D). That minor generation of MDA could be related with the still unexplored specific biochemical characteristics of the particular cultivar, which might have relatively higher endogenous melatonin concentration. It was reported that in wheat melatonin has positive outcomes in specific concentration range, and if the exogenous concentration is higher, negative consequences appear [1]. In addition to the fact that the observed increase of MDA content due to exogenous melatonin application in cv. Gines is minor, electrolyte leakage detected (Fig. 2D) at the same time point does not differ after melatonin application compared with the control showing that cellular integrity was not affected. The observed lack of significant differences between varieties with and without melatonin supplementation clearly proved that the applied plant growth regulator does not affect severely biomembrane integrity of the investigated Bulgarian wheat cultivars.

In general, proline has dual role in plant stress – as protector (carbon source,

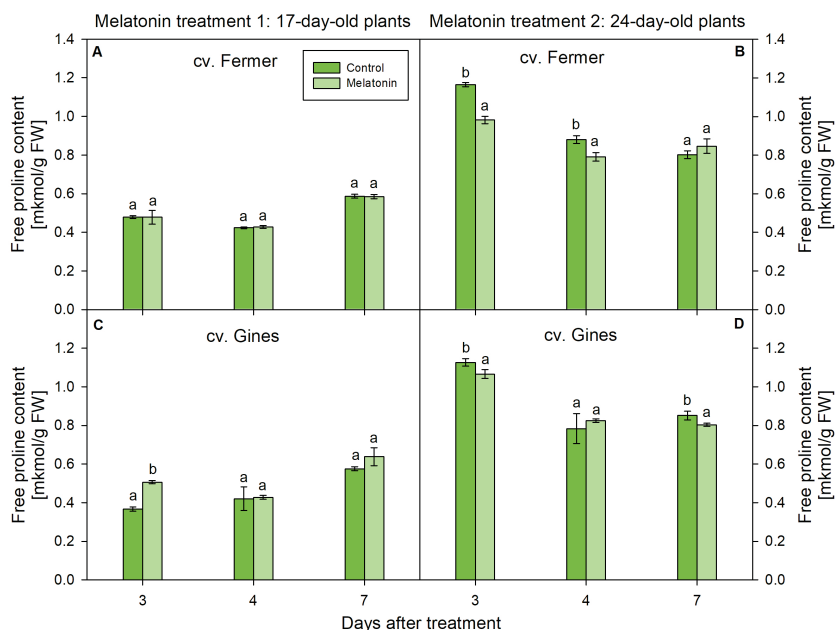


Fig. 3. Content of free proline in wheat cv. Fermer (**A** and **B**) and cv. Gines (**C** and **D**) measured on the 3rd, 4th, and 7th day after melatonin treatment **1** (**A** and **C**) and melatonin treatment **2** (**B** and **D**) presented as mean values with standard error. Different small letters indicate statistically significant differences between the treatments at $p < 0.05$

osmolyte, which is sustaining leaf turgor) or as stress marker molecule [20]. Both examined Bulgarian wheat cultivars, Fermer and Gines showed slight increase in basal free proline concentration (Fig. 3) in older plants as compared to the younger, which could be due to biochemical alterations related to growth progress changes (3rd leaf development, leaf transition from 2nd to 3rd leaf). However, melatonin supplementation does not cause severe alterations in proline levels compared with the respective controls. The result does not eliminate the possibility that melatonin could induce other biochemical alterations in proline biosynthesis level or at molecular level, but if so, the outcome for the proline amount is stable.

In addition to the stress markers, we monitored the phenotype in both wheat varieties at the end of each time-points of melatonin supplementation (Fig. 4). Although there were some phenotype differences in the wheat cultivars explored (Fermer and Gines), there were no obvious alterations noticed due to melatonin treatment.

In conclusion, the absence of phenotype changes together with the lack of stress marker boost due to 75 μM root supplementation of melatonin clearly proved that its usage did not lead to unfavourable plant physiology effects in the studied Bulgarian wheat cultivars Fermer and Gines.

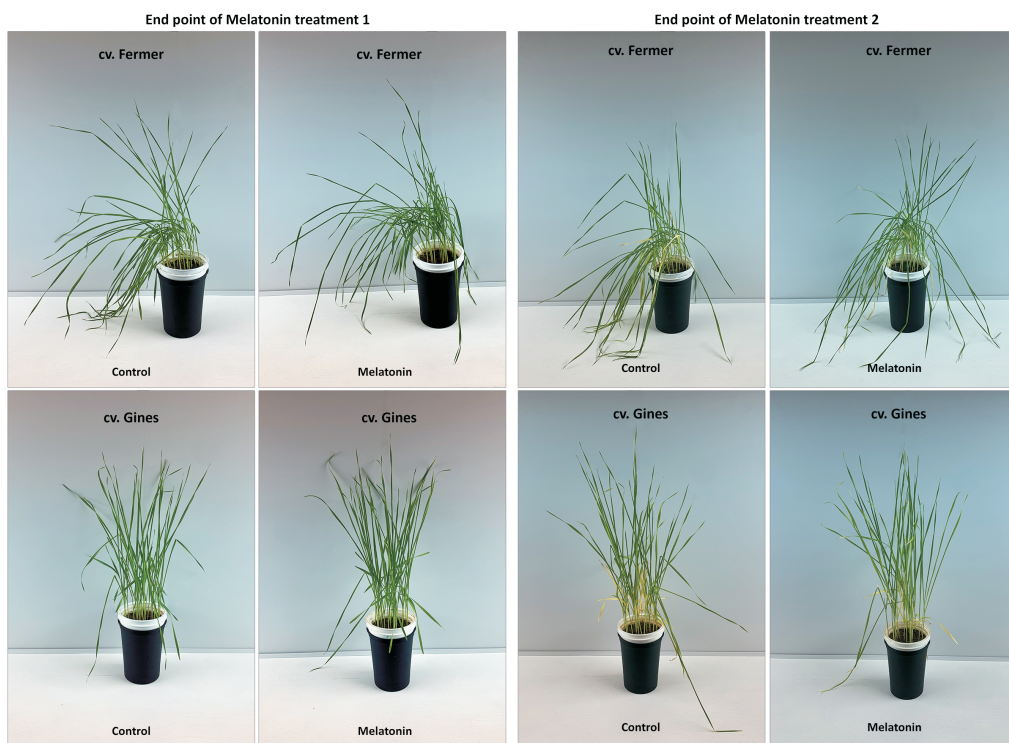


Fig. 4. Phenotype of wheat cv. Fermer and cv. Gines on the 7th day after melatonin treatment 1, and melatonin treatment 2

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