



Research article

Melatonin and resveratrol reverse the toxic effect of high boron (B) and modulate biochemical parameters in pepper plants (*Capsicum annuum* L.)



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ABSTRACT

The objectives of this research were to test a possible involvement of melatonin (MEL) and resveratrol (RES) in restoring growth and to control boron (B) toxicity in peppers. The plants were subjected to four different nutrient solution treatments as following: 1) half-strength Hoagland's nutrient solution (Control), 2) half-strength Hoagland's nutrient solution+100 μ M B (100 μ MB), 3) half-strength Hoagland's nutrient solution+100 μ M boron+100 μ Mresveratrol (100 μ MRES), and 4) half-strength Hoagland's nutrient solution+100 μ M B+1 μ Mmelatonin (1 μ M MEL). Pepper plants subjected to B excess (100 μ M) for 68 days (d) exhibited visible B toxicity symptoms, reduced rate of photosynthesis (P_n) and reduced dry weight (DW), while their leaf and fruit had the greatest increase of B concentration. The reduction of photosynthesis was restored, the reduction of DW was prevented, while the B leaf and fruit accumulation was moderated with the application of both 100 μ Mresveratrol (RES) and 1 μ Mmelatonin (MEL). Moreover, plants exposed to MEL and/or RES displayed no visible B toxicity symptoms. The present study revealed a novel role of MEL and/or RES in the adaptation of pepper plants to B excess based on plant growth, physiological and biochemical criteria.

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1. Introduction

Boron is an essential micronutrient for normal plant growth. However, at high concentrations in the growing medium it is toxic to plants. Pepper (*Capsicum annuum* L.) plants are moderately sensitive to B (1–2 mg L⁻¹) (Gupta et al., 1985; Keren and Bingham, 1985). The physiological effects of B excess include a reduction in: root cell division (Liu et al., 2000; Reid, 2007), cell wall expansion, shoot and root growth, and photosynthetic rate (Nable et al., 1997).

One of the targets of plant physiology is to develop tools for improving plant growth under unfavorable growing conditions. Growing media with a B excess is one of the most serious problems. High concentrations of B may occur naturally in the soil or be added to the growing medium through irrigation water and fertilizers (Nable et al., 1997). This toxic effect, resulting from a high oxidative state may be alleviated by several antioxidative systems to which

indole compounds (indole-3-acetic acid, indole-3-butyric acid) and melatonin belong (Arnao, 2014; Kladna et al., 2003).

Melatonin (N-acetyl-5-methoxytryptamine, MEL) has been detected in many plants in the last two decades, and its role as an antioxidant or growth promoter (Paredes et al., 2009) as well as a retardant of senescence processes (Arnao and Hernandez - Ruiz, 2015) has been reported. MEL is synthesized in plants when they are exposed to both biological and non-biological stresses such as fungal infection and extremes of temperature, toxins, increased soil salinity, drought, etc (Hardeland, 2015; Reiter et al., 2015). Furthermore, MEL has been reported to provide salt adaptation in sour orange (Kostopoulou et al., 2015). In other studies, MEL has been found to be a strong antioxidant in reducing the oxidative damage of important molecules, such as nucleic acids, proteins and lipids (Manda et al., 2007; Zhang and Zhang, 2014; Manchester et al., 2015).

On the other hand, the ability of resveratrol (3,5,4'-trans-trihydroxystilbene, RES) to reduce oxidative stress compared to melatonin has been discussed by Ramis et al. (2015). Resveratrol as a

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phenolic compound contributes to the antioxidant potential of red wine (Fang et al., 2002; Fremont, 2000; Leonard et al., 2003), participates in inducible defense mechanisms (Chong et al., 2009), and accumulates endogenously in response to UV-C irradiation (Tang et al., 2010). Furthermore, RES mediates salt adaptation in citrus plants (Kostopoulou et al., 2014).

Both MEL and RES participate in many plant functions and have a strong antioxidant capacity, however, their participation in controlling B toxicity is still unknown. Thus, the aim of the present study was to investigate whether the treatment of the growing medium with MEL or RES may increase pepper plant tolerance to B excess.

2. Materials and methods

2.1. Plant material and B treatments

A greenhouse experiment was conducted at the farm of the Aristotle University of Thessaloniki, Greece from April to June, 2014. Pepper (*Capsicum annuum* L.) seedlings (cv. Balga) provided by Agris Advancing Horticulture S.A, Greece were transplanted at the fourth true leaf stage of growth in plastic pots (2 plants per pot) containing 2 L of a 1/1 mixture of sand/perlite (v/v). The conditions in the greenhouse were RH 60–70%, temperature 20–25 °C and a PPFD of 900 $\mu\text{mol m}^{-2} \text{s}^{-1}$ measured at the top of the plants with a quantum sensor.

Then, the plants were subjected to four different nutrient solution treatments with a pH 5.5–6.0. These were 1) half-strength Hoagland's nutrient solution (Hoagland and Arnon, 1938) (Control); 2) half-strength Hoagland's nutrient solution+100 μM B (100 μMB); 3) half-strength Hoagland's nutrient solution+100 μM B+100 μM RES (100 μMRES); and 4) half-strength Hoagland's nutrient solution+100 μM B+1 μM MEL (1 μMMEL). The choice of MEL and RES concentrations was based on our preliminary experiments as well as on our previous experience with salt tolerance (Kostopoulou et al., 2014, 2015). Each treatment consisted of five pots containing 10 plants (2 plants per pot). Plants were irrigated three times a week with 250 ml solution per pot. The RES was diluted in a small amount of ethanol and therefore, the same amount of ethanol was added to all treatments. To avoid accumulation of B at the end of each week, each pot was leached out with 300 ml of distilled water.

At 37 days (d) after experiment initiation, the leaf water potential and photosynthetic parameters were measured in one plant per pot. Then the plants were removed and the fully expanded leaves of each plant were used to determine chlorophylls, carotenoids, total flavonoids, carbohydrates and antioxidant capacity.

The experiment lasted for 68 days when visible B toxicity symptoms appeared in the plants exposed to 100 μMB treatment. On that day, after the measurements for leaf water potential, photosynthetic parameters and plant height (cm) had been taken, the plants were removed and separated into leaves, stems, fruits and roots in order to determine their fresh and dry weights. The fully expanded leaves of each plant were used to determine chlorophylls, carotenoids, total phenols, total flavonoids, carbohydrates, electrolyte leakage, antioxidant capacity, and micro- and macronutrients. Also, the fruits (green and red) of each plant were used for the determination of carotenoids, total phenols, total flavonoids, antioxidant capacity and micro- and macronutrients.

2.2. Photosynthetic rate (P_n)

Photosynthetic rate was determined on fully expanded leaves (3rd leaf from apex) with a portable LC pro + system (ADC

Bioscientific Ltd, UK). Measurements were performed between 10:00 and 12:00 at a light intensity $>900 \mu\text{mol m}^{-2} \text{s}^{-1}$ while leaf temperature varied between 28 and 32 °C.

2.3. Leaf water potential (Ψ)

The leaf water potential was measured between 09:00 and 10.00 a.m. on the first two fully expanded leaves of each plant using a pressure chamber according to Scholander et al. (1965).

2.4. Chlorophylls

For chlorophyll concentration, 0.1 g of frozen leaves was placed in 25 ml glass test tubes and 15 ml of 96% ethanol was added. The tubes were incubated in a water bath at a temperature of 80 °C until complete discoloration of samples, after about 3–4 h. The absorbance of the extract was measured at 665 and 649 nm and the calculation of the chlorophylls was done according to Winternans and de Mots (1965).

2.5. Carotenoids

Carotenoid concentration was determined on a VIS spectrophotometer (Camspec M 106, Leeds, U.K.) as described by Lichtenthaler (1987), Porra et al. (1989) and modified by Yang et al. (1998). For the extraction, 0.5 g of frozen material was placed in a solution containing a mixture of ethanol, acetone and hexane at a ratio of 1.5:1.5:3. This was then transferred to a refrigerator until complete discoloration of the tissue took place after about 24 h. Following thorough homogenization of the extract, the mixture was allowed to stand for 15 min and then the 3 ml of the supernatant hexane was taken in order to determine the carotenoid substances. The absorbance of the extract was measured at 450 nm and the carotenoid concentration was calculated using the extinction coefficient (ϵ) of 1% = 2592 $\text{M}^{-1} \text{cm}^{-1}$ (Rodriguez – Amaya, 1999).

2.6. Total phenols

Total phenols were extracted from 0.1 and 0.3 g of frozen material (leaves and fruit pericarp, respectively) in 80% methanol. They were assayed using the Folin-Ciocalteu reagent following the method of Scalbert et al. (1989), and expressed as mg gallic acid equivalents (GAE) $\text{g}^{-1} \cdot \text{FW}$.

2.7. Total flavonoids

Total flavonoids were extracted from 0.1 and 0.3 g of frozen material (leaves and fruit pericarp, respectively) in 80% methanol and determined colorimetrically as described by Zhishen et al. (1999). Rutin was used as the standard compound for quantification of total flavonoids. Values were expressed as mg of rutin $\text{g}^{-1} \cdot \text{FW}$.

2.8. Carbohydrates

For carbohydrate extraction, 0.1 g of frozen material was placed in 25 ml glass test tubes and 15 ml of 80% ethanol was added. The tubes were incubated in a 60 °C water bath for 30 min. The extract was filtered with Whatman No 1 filter paper and carbohydrates were measured with anthrone reagent using a standard curve of 0–0.2 mM according to Fales (1951).

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