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Selenium Modifies the Effect of Short-Term Chilling Stress on Cucumber Plants

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Abstract The objective of this study was to investigate the effect of selenium (Se) supply (0, control; 2.5, 5, 10, or 20 μM) on cucumber (*Cucumis sativus* L.) cv. Polan F1 plants grown under short-term low temperature stress. About 14–16 day-old seedlings, grown at an optimal temperature (25/20°C; day/night), were exposed to short-term chilling stress with a day/night temperature of 10°C/5°C for 24 h, for a further 24 h at 20°C/15°C, and then transferred to 25/20°C (re-warming) for 7 days. Se did not affect the fresh weight (FW) of plants at a concentration of 2.5–10 μM, but in the presence of 20 μM Se, the biomass of shoots significantly decreased. The contents of chlorophylls and carotenoids witnessed no significant change after Se supplementation. Compared with the control, the Se-treated plants showed an increase of proline content in leaves, once after chilling and again after 7 days of re-warming. However, proline levels were much higher immediately after chilling than after re-warming. The malondialdehyde (MDA) content in the root of plants treated with 2.5–10 μM Se decreased directly after stress. This was in comparison with the plants grown without Se, whereas it increased in roots and leaves of plants exposed to 20 μM Se. Seven days later, the MDA level in the root of plants grown in the presence of Se was still lower than those of plants not treated with Se and generally witnessed no significant change in leaves. Although Se at concentrations of 2.5–10 μM modified the physiological response of cucumber to short-term chilling stress, causing an increase in proline content in leaves and diminishing lipid peroxidation in roots, the resistance of plants to low temperature was not clearly enhanced, as concluded on the basis of FW and photosynthetic pigments accumulation.

Keywords Assimilation pigments · Chilling stress · *Cucumis sativus* L. · Malondialdehyde · Proline · Selenium

Introduction

Low temperature stress is a major environmental stress, inducing damage of agricultural crops and limiting the distribution of both wild and agricultural crop species. Exposure of

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cold-sensitive plants to above-freezing low temperatures causes physiological disturbances due to alternations of certain metabolic processes [1]. The best known among these alterations are changes of the lipid composition of cell membranes affecting the maintenance of their fluidity, the accumulation of compatible solutes, the inhibition of chlorophyll biosynthesis, and the decrease of photosynthetic capacity and of carbohydrate metabolism. Consequently, crop yields and quality are often reduced [2–4]. In several plant species, cold damage may also result from water loss through open stomata at a time when the root's hydraulic conductance is low [5]. Furthermore, chilling increases the level of active oxygen species (AOS) in plants. AOS are highly reactive and can damage membrane lipids, proteins, and nucleic acids, thus disrupting cellular homeostasis. As chilling-sensitive species tend to have lower antioxidant capacity than do tolerant species [1, 6], a promising approach to enhance their resistance to cold stress would be to increase their antioxidant activity [7].

Selenium (Se), similar to sulfur (S) as regards its chemical properties, so far has not been classified as an essential element in proper growth and development of higher plants. However, there is evidence that Se might be an essential micronutrient for accumulator plants species such as *Astragalus* and *Stanleya* [8, 9]. Moreover, it has been shown that Se is required for an optimal growth of the unicellular green alga (*Chlamydomonas reinhardtii*), from which two Se-depended glutathione peroxidases (GSH-Px) were isolated [10]. In contrast, Se non-accumulator plants, including most species of crops, do not appear to require Se for their growth, and in general, these plants have a low tolerance to this element [8].

Although Se is toxic at high concentrations, recent studies have shown that it can have a positive influence on plants at relatively low concentrations and may play a role in plant protection from several types of abiotic stresses. Se can increase the tolerance of plants to UV-induced oxidative stress [11], delay senescence [12], and promote the growth of NaCl-stressed seedlings [13]. In addition, it has been shown that Se has the ability to regulate the water status of plants under conditions of drought [14, 15]. Recently, a protective effect of Se on plants subjected to toxic concentrations of cadmium has been reported [16, 17]. Chen and Sung [18] and Chu et al. [19] performed the investigations concerning the influence of this trace element on plants response to sub-optimal temperature. They reported that plants treated with Se and subjected to sub-optimal temperature generally grew better than plants grown without Se addition. However, to our knowledge, the role of Se in alleviating chilling-induced stress in cucumber plants has not been studied before.

Cucumber (*Cucumis sativus* L.) is an important crop plant cultivated in many parts of the world. However, it is sensitive to temperatures below about 15°C, and considerable yield losses may occur if the temperature suddenly falls below this level during the vegetation period [20]. Thus, the main aim of present work was to determine the effect of exogenous Se (2.5, 5, 10, or 20 μM) on cucumber seedlings grown under conditions of short-term chilling stress. The Se concentrations were chosen on the basis of literature data [13, 21] and our previous experiments [22].

Material and Methods

Plant Material and Growth Conditions

The seeds of cucumber (*C. sativus* L.) cv. Polan F1 were obtained from the Seed Centre in Lublin and were sown onto the wet quartz sand. After 7–8 days, the seedlings were

transferred to 1 dm^3 glass jars (two plants per jar) containing 1.5-times concentrated Hoagland's nutrient solution, supplemented by 2% ferric citrate and micronutrients in the form of 1% Hoagland's A-Z solution [23]. The medium pH was adjusted to 5.5 using dilute NaOH. Then, the nutrient solution was differentiated to a concentration of selenium (0, control; 2.5, 5, 10, or 20 μM). Se was applied in the form of sodium selenate (Na_2SeO_4).

The plants were grown in a vegetation chamber (Sanyo MRL 350 HT) under a 14-h day length, temperature 25/20°C (day/night) at a photosynthetic photon-flux density of 270 μmol m^{-2} s^{-1}, with a relative humidity of 75%. The 2-week-old plants were exposed to short-term sub-optimal temperature (24 h 10/5°C and 24 h 20/15°C; day/night). After chilling treatment, the plants were transferred to 25/20°C for 7 days (re-warming). Immediately after chilling stress, the proline and malondialdehyde (MDA) content were determined. Seven days after stressing, the plants were examined for chlorophyll *a* and *b*, carotenoids, proline, and MDA content. Then, the plants of each treatment were harvested, divided into roots and shoots, and the fresh mass (FW) was immediately determined.

The experimental design contained five treatments and six replications per treatment. The experiment was repeated three times under the same conditions.

Plant Analysis

The assimilation pigments were extracted from fully expanded second leaves in an aliquot of 80% (*v*/*v*) acetone and estimated as described by Lichtenthaler and Welburn [24]. The contents of chlorophyll *a*, chlorophyll *b*, and carotenoids were determined by equations using the absorbance at 663, 646, and 470 nm and calculated as milligrams per gram FW.

The level of lipid peroxidation was evaluated by MDA measurement [25]. In order to determine the MDA content, tissues (0.5 g) were homogenized in 4.5 cm^3 0.1% trichloroacetic acid (TCA) solution. The extract was centrifuged (10,000 rpm for 5 min) and 4 cm^3 of 20% TCA containing 0.5% of thiobarbituric acid were added into 1 cm^3 of achieved supernatant. The mixture was then heated to 95°C for 30 min., quickly cooled, and re-centrifuged. Finally, the specific absorbance of the product was recorded at 532 nm, with the value for nonspecific, background absorbance at 600 nm subtracted from 532 nm. The MDA content was calculated using its extinction coefficient (155 mM cm^{-1}) and expressed as nanomole MDA per gram FW.

The proline content was determined using a colorimetric method [26]. The leaf samples (0.5 g) were homogenized in 10 cm^3 of aqueous solution of 3% sulphosalicylic acid. Then, the solution was filtered, and 2 cm^3 of the extract was reacted with 2 cm^3 of acid ninhydrin and 2 cm^3 of glacial acetic acid for 1 h at 100°C. Finally, the reaction was terminated in an ice bath. Thereafter, the reaction mixture was extracted with 4 cm^3 of toluene and mixed vigorously by vortexing for 15–20 s. After this, the chromophore-containing toluene phase was sucked using a pipette and kept at room temperature to stabilize. Proline content was measured by a spectrophotometer at 520 nm using toluene as a blank and calculated as micrograms per gram FW against standard proline.

Statistical Analysis

The data were tested using variance analysis (ANOVA), and the significance of the differences between the mean values was separated using a Tukey's test. Differences were considered significant at $P<0.05$. The data shown are mean values±standard errors.

Results

Biomass and Photosynthetic Pigments Accumulation

The influence of Se supplementation range of 2.5–20 μM on short-time cold-stressed cucumber plants biomass and photosynthetic pigments content was studied 7 days after chilling stress (Figs. 1 and 2). There was no significant effect of Se treatment at a concentration range of 2.5–10 μM on the FW of roots and shoots, as compared with control plants. However, a significant decrease (28%) in shoot FW was noted in the presence of 20 μM Se. Exposure to the Se at 5 μM concentration resulted in a 13% increase (but insignificant) in roots FW (Fig. 1).

It was found that the level of Se fertilization had no significant effect on the content of both chlorophyll forms (*a* and *b*) as well as carotenoids. Although, in Se-treated plants, the content of chloroplast pigments was 3–8% lower than that in control plants, but these differences were not statistically significant (Fig. 2).

Free Proline Content

Immediately after low temperature exposure, the proline contents in both the control and the Se-treated plants were considerably higher than that after 7 days of re-warming (Fig. 3). Data recorded after a 7-day recovery period were approximately 4–9-fold lower than that directly after chilling. The cold-stressed cucumber plants grown in the Se presence showed an increase in the level of free proline directly after stress, as well as 7 days later as compared with the control plants. Immediately after cold stress in all Se-treated plants, a significant increase of proline level was recorded. The proline content reached a maximum value of 332 μg g^{-1} FW at treatment with 10 μM Se and was almost two-fold higher than that noted in the control plants. After re-warming, the proline content exceeded the control value by 100%, 286%, and 88% in the presence of 5, 10, and 20 μM Se, respectively.

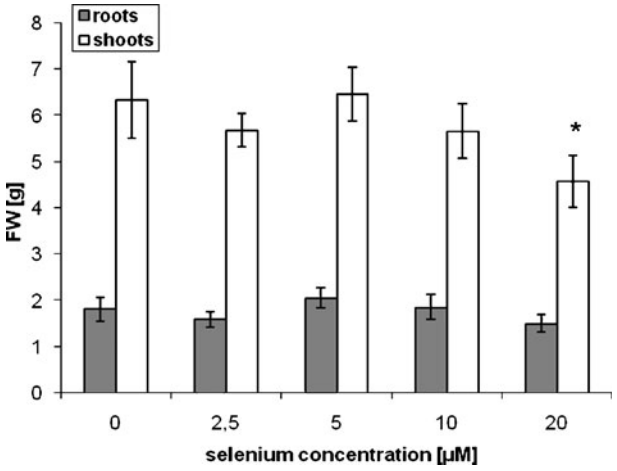

Fig. 1 The effect of selenium on biomass of cucumber seedlings exposed to short-term chilling stress. The level of significance is represented by (*asterisk*) $P<0.05$ as compared with value in control plants

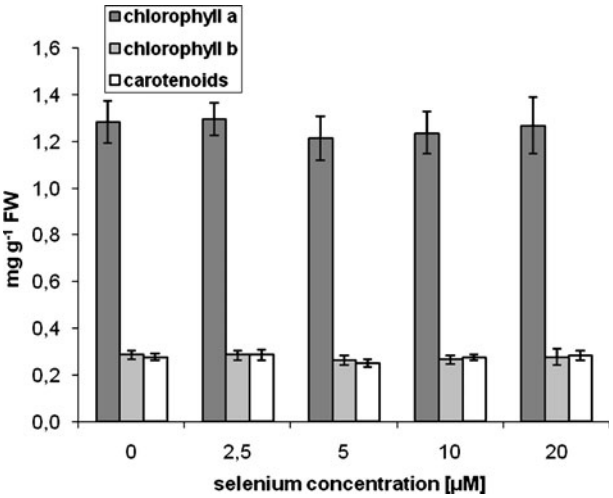

Fig. 2 The effect of selenium on photosynthetic pigment content in cucumber seedlings exposed to short-term chilling stress

Level of Lipid Peroxidation

The content of MDA, a lipid peroxidation marker, was determined to investigate the oxidative effect induced by Se supplementation in chill-treated cucumber. MDA concentration was higher immediately after low temperature stress than 7 days later, especially in roots, regardless of the presence of Se (Fig. 4a, b). However, when plants were exposed simultaneously to chilling and Se at concentrations of 2.5–10 µM, it was found that immediately after stress, the MDA level notably decreased (33–44%) in roots and remained almost unmodified in leaves, as compared with the control. In the plants treated with 20 µM Se, the level of MDA was 25% and 33% higher in roots and leaves respectively, as compared with plants grown without Se addition (Fig. 4a). After 7 days of re-warming, the MDA contents in roots were several-fold lower than directly after cold

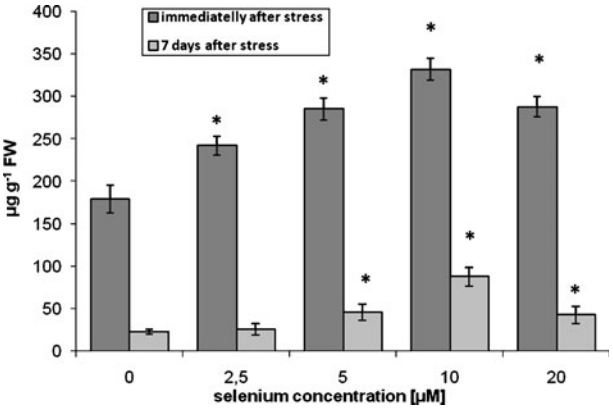

Fig. 3 The effect of selenium supplementation on free proline content in cucumber seedlings exposed to short-term chilling stress

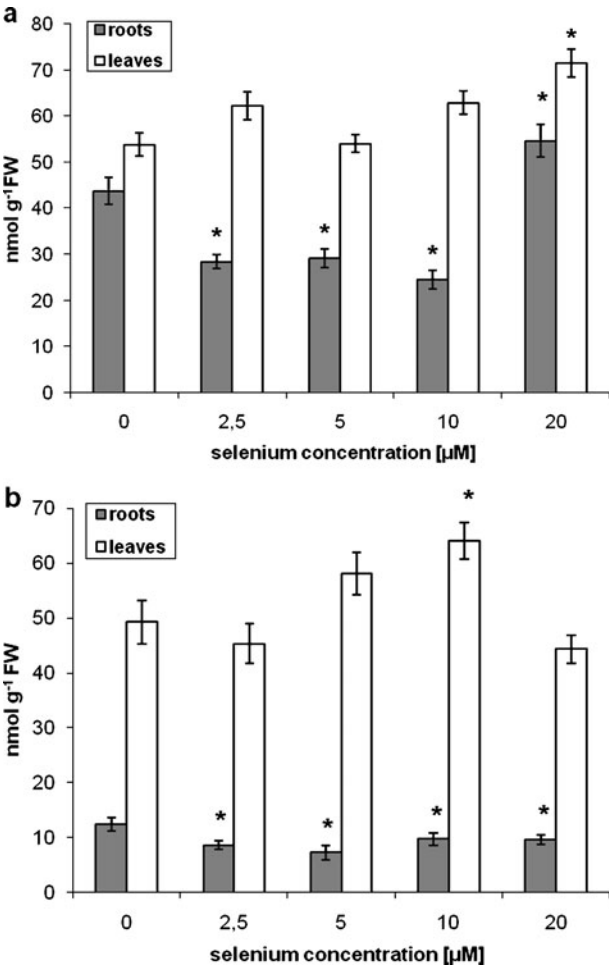

Fig. 4 The effect of selenium supplementation on MDA content in cucumber seedlings immediately after chilling stress (**a**) and 7 days after re-warming (**b**)

stress treatment, but at all experimental Se concentrations, a significant decrease (22–31%) of MDA concentration was still observed. On the other hand, in the presence of 10 μM Se, about 30% increase of MDA level was noticed in leaves (Fig. 4b).

Discussion

Reduction of plant growth and decrease of photosynthetic pigments content are common symptoms for plants grown under stressful conditions. In a present study, the FW of roots and shoots of cucumber plants was not affected by Se at concentration of 2.5–10 μM, but in the presence of 20 μM Se the shoots FW decreased significantly, as compared with the control plants. This confirms results of our previous study indicating that Se negatively affected the biomass of the aboveground parts of NaCl-stressed cucumber plants at 20 μM concentration [27]. Hartikainen et al. [28] suggest a dual role of Se in plants: at low

concentrations, it can act as an antioxidant and promote plant growth, whereas at high concentrations, it is a pro-oxidant causing metabolic disturbances and drastic yield losses. In Se-sensitive plants, the Se toxicity seems to be strictly related to the replacement of S atoms by Se in *S*-containing amino acids, cysteine and methionine. The differences in size and ionic properties of S and Se can cause changes in the structure and activity of Se-substituted proteins and thus having negative effect on the plants growth [8].

In presented study, Se supplementation had no significant effect on the content of both chlorophylls and carotenoids in the leaves of cucumber 7 days after chilling stress. However, some studies have indicated that relatively low Se concentrations can increase chlorophyll content by altering its biosynthetic pathway [29]. On the other hand, the excess of Se may induce chlorosis, possibly through a negative effect on the production of porphobilinogen synthetase, an enzyme required for chlorophyll biosynthesis [30].

Many plants accumulate high levels of free proline in response to a wide range of biotic and abiotic stresses, and proline is considered as a signal/regulatory compound able to activate multiple physiological or molecular mechanisms. The role of endogenous proline under oxidative stress includes stabilization of protein complexes, regulation of cytosolic pH, regulation of NAD/NADH ratio, or act as a scavenger of oxygen free radicals [31]. There are conflicting reports concerning the function of proline in the chilling resistance of plants. The higher level of free proline in cold-stressed plants has been suggested as a factor conferring chilling tolerance [6, 32]. In contrast, proline accumulation has also been considered as a symptom of injury rather than an indicator of low temperature tolerance [33]. It is clear from the presented results that Se supplementation effectively enhanced the proline accumulation under chilling stress conditions. Djanaguiraman et al. [29] have also observed an increase of proline accumulation in the Se-supplied soybean plants. On the other hand, Kuznetsov et al. [14] reported that Se caused a significant decrease in proline content in spring wheat under drought stress conditions. However, the reasons for proline increase or decrease under Se treatment is not known until date.

Our study demonstrates that the level of lipid peroxidation is much higher immediately after chilling than after re-warming. This response provides the evidence that short-term chilling develops oxidative stress in cucumber plants. Furthermore, these findings indicate that the root tissues appear to be more sensitive than leaf tissues to oxidative damage. The anti-oxidative action of Se was more pronounced in the roots, probably because the first contact of plants with Se occurs through the roots that act as a barrier. Several studies have shown that an antioxidant effect of Se in higher plants has been often associated with improved GSH-Px activity and a decreased lipid peroxidation [7, 11, 12, 28]. Moreover, the plants treated with Se showed an increase in the activity of enzymes that detoxify H_2O_2 (ascorbate peroxidase, catalase), as well as an increase in the foliar concentration of low-molecular weight antioxidants such as ascorbate and glutathione [21]. Chen and Sung [18] reported that priming bitter gourd seeds with Se solution may partially protect them against sub-optimal temperature induced oxidative injury by coupling the changes in the reduced and oxidized forms of antioxidants. However, the specific physiological and molecular mechanisms that underlie the beneficial effects of Se in plants under stress conditions have not yet been fully clarified, and further experiments are required. Nonetheless, investigations are in progress to enhance the understanding of the effect of Se treatment on plant resistance towards different abiotic stresses.

Finally, our results indicate that, although Se at concentrations of 2.5–10 μM generally caused an increase in proline content and decrease of lipid peroxidation, the resistance of

cucumber plants to short-term chilling stress was not clearly enhanced, as concluded on the basis of biomass yield and photosynthetic pigments accumulation.

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