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Acclimation of *Lactuca sativa* to increased UV irradiation at various selenium levels

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Introduction

Selenium (Se) is an essential component of glutathione peroxidase (GSH-Px) enzyme, an important scavenger of lipid hydroperoxides. Through its antioxidative function, Se can ameliorate UV-B radiation damage in human beings (La Ruche and Cesarini 1991, Leccia et al. 1993). On the other hand, plants are generally considered not to require Se and to have a low tolerance to it. They may respond to increased UV-B radiation via multiple morphological or metabolic mechanisms, e.g. by changes in epidermal layer, epicuticular waxes, leaf thickness, canopy morphology and architecture, or by synthesizing UV-absorbing compounds, e.g. flavonoids (Koostra 1994, Krizek et al. 1998, Laakso et al. 2000). UV-B radiation exerts harmful effects on photosynthetic reactions (Allen et al. 1997), photosystem II being the most sensitive part (Vass et al. 1996). However, recently Se has been shown to enhance the antioxidative capacity of plants (Hartikainen et al. 1997), to alleviate UV-induced oxidation damage and even to promote growth of plants subjected to short UV-B episodes (Hartikainen and Xue 1999). The protective role of Se is found to be related to enhanced GSH-Px activity. This experiment was undertaken to study the effect of Se addition and pretreatment with short UV episodes on the functional and structural responses of the photosynthesis apparatus in Lactuca sativa subjected to increased UV stress.

Material and Methods

Lettuce (*Lactuca sativa*) was cultivated in a greenhouse without or with Se. After sprouting, half of the plants in both treatments were pre-acclimated for 9 days by subjecting them daily to 1-min UV irradiation (280-400 nm) of 0.15 kJ m^{-2}. The non-acclimated plants were grown under a photosynthetically active photon flux density of 390 μmol m^{-2} s^{-1}. The natural daylength was approximately 19 hours, and the daylight was supplemented with Osram Vialox Nav-T 400W lamps. After 9 days, the pre-acclimated and non-acclimated plants were subjected daily to a 60-min UV treatment at an intensity of 9 kJ m^{-2}. The control plants were cultivated with and without Se and UV. During the experiment the temperature in the greenhouse varied between 15C° and 33C°.

Soil in the experimental pots was prepared as follows: a coarse-textured subsoil was supplied with nutrients (200 mg N, 200 mg K and 159 mg P kg^{-1} soil). Half of the pots were treated with Se (as H_2SeO_4, Merck) at the rate of 0.01 mg kg^{-1} soil, and the other

half was left without Se addition. Twelve seeds of lettuce (*Lactuca sativa* L. Australischer gelber) were sown onto the upper layer of the soil and covered with 100 g of soil.

For enzyme analyses, fresh plant material was stored in liquid N_2 immediately after harvesting. The activity of gluthatione peroxidase (GSH-Px, EC 1.11.1.9) was analyzed by a modified method of Flohe and Gunzler (1984) using H_2O_2 as substrate. Proteins were determined by Bradford method (BioRad). Pigments were analyzed according Scott et al. 1996.

Fluorescence

Fluorescence measurements were carried out with a Bio Monitor PSMII meter. After 30-min incubation in the dark, fluorescence emission of leaf tissue was measured at a light level of 400 umol/m^2/s. Beginning with the onset of the increased UV treatment, fluorescence was monitored for 40 days in all treatments.

Ultrastructure

For ultrastructural studies, leaf samples were taken from the non-acclimated and pre-acclimated plants cultivated without or with added Se. The sampling took place before noon. The leaf samples were fixed in 2.9% (v/v) glutaraldehyde in 0.1 M Na-phosphate buffer (PB, pH 7.2) for 3 hours at room temperature. For thin sections, postfixation was done with 1% osmium tetroxide in PB for 2 hours at room temperature. Dehydration of the samples was carried out in graded ethanol series and propylenoxide, followed by embedding and staining in Epon 812. The grids were examined and photographed with a Jeol 1200 EX transmission electron microscope.

Results and discussion

Ultrastructural investigations revealed that both the non-acclimated and pre-acclimated plants supplied with Se were developing more rapidly than those without added Se: grana and starch grains in the chloroplasts were larger, and plastoglobuli appeared earlier. According earlier studies (Gonzales et al. 1998, Grammatikopoulos et al. 1998), UV treatment resulted in a slower developmental rate and affected the ultrastructure. In our study, a thicker outer epidermis began to form in response to the pre-acclimation UV treatment, this reaction pattern being more pronounced in the plants supplied with Se. The younger leaves of the pre-acclimated plants adapted to UV stress (60 min daily) within a few days. Prolonged UV stress (more than 14 days) caused structural changes in cell components both in pre- and non-acclimated plants.

During the pre-acclimation UV treatment the Fv/Fm values remained rather stable, demonstrating that PS II electron flow was not affected. On the other hand, the Fv/Fm parameter revealed that at the beginning of the daily UV stress, PS II was relatively sensitive irrespective of whether the leaves were pre-acclimated or not (Fig. 1). The repair mechanism of PSII normalized the Fv/Fm values within 5 to 7 days. When the UV stress was prolonged and the leaves became older, the beneficial role of selenium in stabilizing the maximum quantum yield became apparent, and the Fv/Fm values remained more constant. This positive effect of Se was also reflected in Fo values. An increase in the Fo parameter displays photoinhibitory damage (Maxwell & Johnson 2000). In this experiment prolonged UV stress raised the Fo values but the added Se diminished the increase. During plant development (40-day period) Fo increased in all treatments, but the protective role of selenium against photoinhibitory damage became more pronounced.

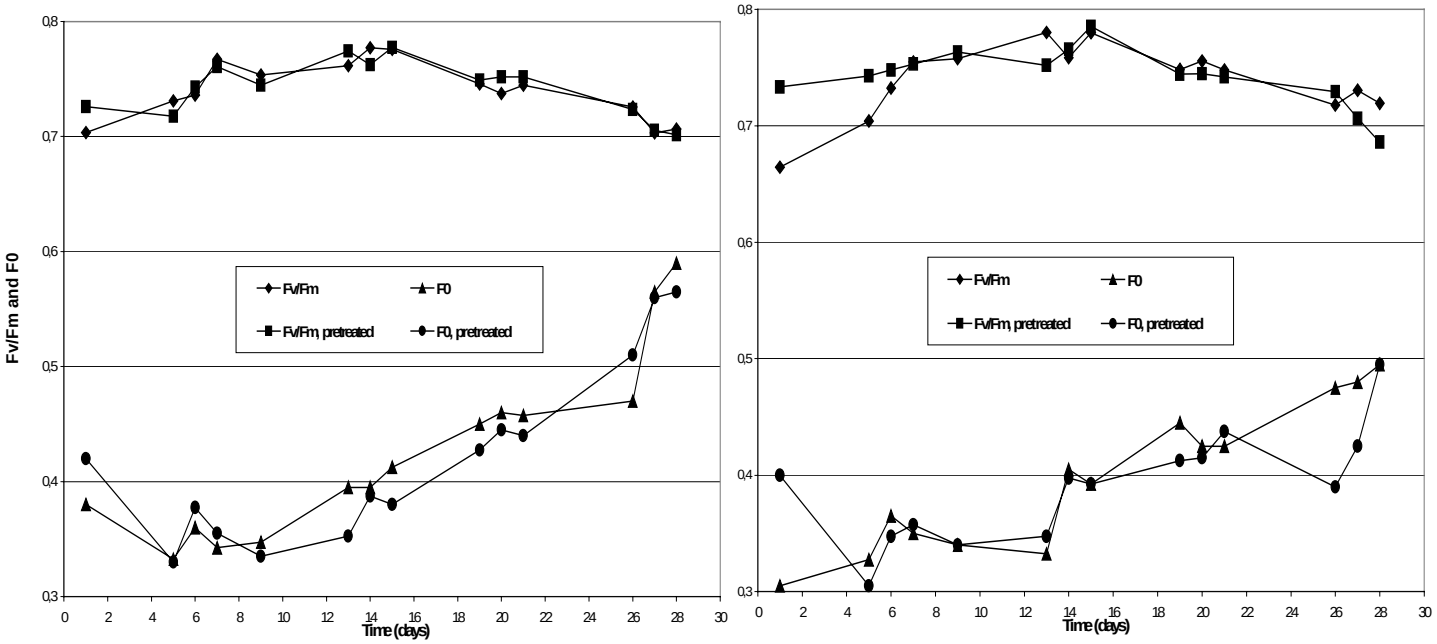

Figure 1. Effects of UV irradiation (9 KJ m-2) on Fv/Fm and Fo values in not pretreated and UV pretreated (0.15 KJ m-2 9days) plants without Selenium (Left, A) and with Selenium (Right, B).

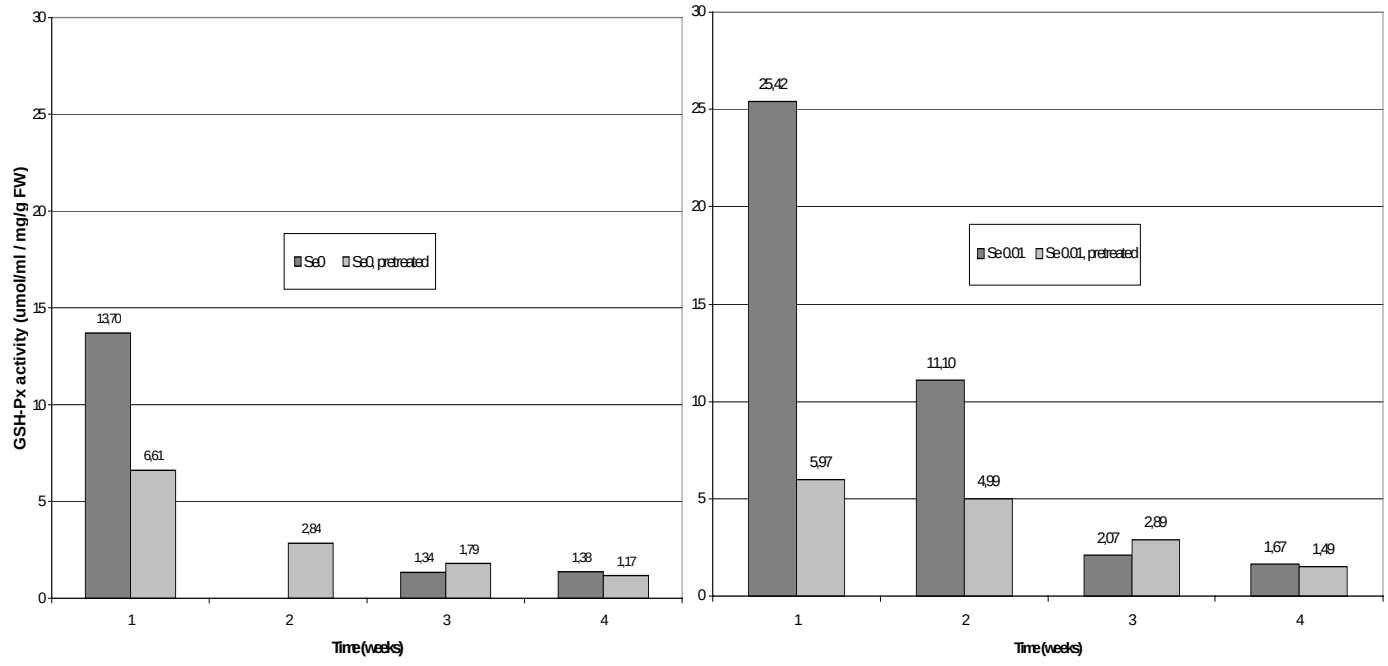

Figure 2. Effects of UV irradiation (9 KJ m-2) on GSH-Px activity in not pretreated and UV pretreated plants (0.15 KJ m-2) without Selenium (left, A) and with Selenium (right, B).

The intensity and duration of UV irradiation affected the acclimation process. The pre-acclimation treatment reduced the GSH-Px activity irrespective of Se treatment, and the pre-acclimated plants also showed a lower GSH-Px activity when subjected to UV stress (Fig.2). However, the antioxidative capacity seemed to be affected more by the aging of the leaves than by environmental factors: GSH-Px was low in all 30-day old plants irrespective of UV stress.

The acclimation to increased UV stress was dependent on the developmental stage. The younger leaves were able to build up protective systems quickly. In the aging leaves the protective effect of Se became apparent. In fact, added Se had a dual function: it accelerated development but, on the other hand, retarded the aging process through its antioxidative function. Furthermore, without Se addition UV irradiation increased lutein and β-carotene concentrations. It is noteworthy that in the Se-supplied plants UV stress did not affect the concentration of these carotenoids. This suggests that the added Se may exert a protecting function, diminishing the synthesis of lutein and β-carotene.

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