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Combined impact of solar UV-B radiation and selenium treatment on respiratory potential in pumpkins (*Cucurbita pepo* L.)

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ABSTRACT

The effects of ambient and filtered solar UV-B radiation and of selenium treatment on respiratory potential measured by electron transport system (ETS) activity in pumpkins, *Cucurbita pepo* L. were studied. Measurements were conducted three times in the growth period. Solar UV-B radiation decreased ETS activity in plants, regardless selenium treatment. The results suggested that the solar UV-B radiation impaired flow of electrons in the respiratory chain. Selenium decreased ETS activity in plants exposed to solar UV-B radiation in the end of the vegetation period.

Key words: Electron transport system, Selenium, Ultraviolet radiation, pumpkins

Abbreviations: ETS, electron transport system; INT, iodo-nitro-tetrazolium-chloride; UV, ultraviolet; UV1Se0, ambient radiation; UV1Se1, ambient radiation and selenium spray; UV0Se0, UV-B excluded ambient radiation; UV0Se1, UV-B excluded ambient radiation and selenium spray.

IZVLEČEK

VPLIV SONČNEGA UV-B SEVANJA IN SELENA NA DIHALNI POTENCIAL PRI NAVADNI BUČI (*Cucurbita pepo* L.)

V raziskavi smo preučevali vpliv UV-B sevanja in selena na dihalni potencial ocenjen s pomočjo meritev elektronekega transportnega sistema (ETS) pri navadni buči *Cucurbita pepo* L.. Rastline smo izpostavili naravnemu sevanju in sevanju, kjer smo s pomočjo ustreznih filtrov onemogočili prehajanje UV-B žarkom. Ob istem času so bile rastline listno gnojene s selenom. Meritve dihalnega potenciala so bile opravljene trikrat v rastni sezoni. Sončno UV-B sevanje je povzročilo znižanje aktivnosti ETS pri rastlinah, ne glede na dodatek selena. Rezultati kažejo, da je UV-B sevanje povzročilo motnje pri pretoku elektronov v dihalni verigi. Rastline, ki smo jim dodali selen, so imele nižjo aktivnost ETS v primeru, ko so bile istočasno izpostavljene naravnemu sevanju ob koncu vegetacijske sezone.

Ključne besede: elektronski transportni sistem, selen, buče, UV-B radiation

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1 INTRODUCTION

A depletion of the stratospheric ozone layer in the atmosphere has occurred over the last two decades that result in an increase in ultraviolet-B radiation (UV-B) at the earth's surface (Madronich et al, 1998; Searles et al., 2001). Regarding the presumed continuous increase of the ambient UV-B level due to climate changes (Taalas et al., 2000) it is reasonable to have information about the sensitivity of crop plants to UV-B radiation. The direct dependence of primary producers on solar energy, that includes also harmful UV-B rays, results in damage to biomolecules (Björn et al., 1999; Britt, 1999), alteration in transpiration and photosynthesis (Gaberščik et al., 2002a), respiration potential (Förschler et al., 2003), and growth, development and morphology (Flint et al., 2003). Changes in plant morphology that result from UV-B exposure may alter competitive relationships among weeds and associated crop species (Furness and Upadhyaya, 2002). The UV-B damage depends on species and even cultivars and balance of potential damage and induction of protective mechanisms in the plants (Stephanou and Manetas, 1998; Gaberščik et al., 2001). Damage caused by UV-B radiation can generate free radicals and superoxides in the plants (Rozema et al., 1997). Plants need to accumulate molecules which can quench the free radicals. Seppänen et al. (2003) and Xue et al. (2000) reported about positive role of selenium that protects plants against UV-induced oxidative stress.

The majority of studies of the effect of UV-B radiation on plants have been carried out under laboratory or glasshouse conditions (Zhenk et al., 2003) and little is known about its effects on plants growing under field conditions. The aim of the present study was to determine the effect of ambient UV-B radiation on respiratory potential (measured as ETS activity) in pumpkin. Possible combine effect of Se substances and UV-B radiation was studied as well.

2 MATERIAL AND METHODS

Plant material

Seeds of *Cucurbita pepo* L. were germinated in the laboratory under sterile conditions and the 10 day old seedlings were transferred to the experimental field of the Biotechnical Faculty, University of Ljubljana, (320 m above see level, 46°35´N, 14°55´E). They were planted in 1.5 m x 1.5 m plots (2 plants per plot, four plots per treatment in a split plot design).

Growth conditions

The treatments included ambient radiation (UV1Se0), ambient radiation and selenium spray (UV1Se1), UV-B excluded ambient radiation (UV0Se0), UV-B excluded ambient radiation with selenium spray (UV0Se1). Wavelengths below 320 nm were excluded by covering the plots with 0.15 mm thick Mylar foil (Gehrke et al., 1996). Controls, which received ambient solar radiation, were covered with 0.15 mm thick polyethylene foil, which transmitted UV-B radiation and only minimally attenuated other wavelengths. UV-B was excluded and selenium sprayed on the 49th day after sowing. This coincided with the initiation of flowering. Selenium was applied as a foliar spray of sodium selenate at a concentration of 1.5 mg/L. Control plants were sprayed with distilled water containing no detectable amounts of selenium.

Measurements

Electron transport system (ETS) activity was measured in June, on the 49th day after sowing and before treatments, then in July, 14 days after treatments and in August, 48 days after treatments.

Respiratory potential was measured as ETS activity of mitochondria, as described by Packard (1971). Fresh leaves were weighted and crushed in a mortar in chilled 0.1 M sodium phosphate buffer (pH = 8.4) containing 0.15% (w/v) polyvinyl pyrrolidone, 75 µM $MgSO_4$, and 0.2% (v/v) Triton-X-100, followed by ultrasound homogenization (40W, 4710; Cole-Parmer, USA). The extract was centrifuged (8500 x g, 4 min, 0^{o}C) in a top refrigerated ultracentrifuge (2K15, Sigma, Osterode, Germany). An aliquot of the supernatant was added to the substrate solution (0.1 M sodium phosphate buffer (pH = 8.4), 1.7 mM NADH, 0.25 mM NADPH, 0.2% (v/v) Triton-X-100), and 20 mg 2-p-iodo-phenyl 3-p-nitrophenyl 5-phenyl tetrazolium chloride (INT) in 10 ml of bidistilled water. The mixture was incubated at 20 °C for 40 min. Absorbance of the resulting formazan was measured at 490 nm. ETS activity was calculated as the rate of INT reduction, which was converted to the amount of oxygen utilised per mg of leaf dry mass (DM) per time unit (Kenner and Ahmed, 1975).

Statistical analysis

All measurements were carried out on 8 parallel samples. The data were submitted to multifactor ANOVA (Statgraphics Version 4) and the significance calculated at p = 0.05. Measurement in July and August were tested separately.

2 RESULTS AND DISCUSSION

ETS activity was lower in plants grew under solar UV-B radiation comparing plants, which grew in conditions, where solar UV-B was filtered out. The difference was stronger in the last measurements in August; namely, ETS activity was more than once higher in the plants, from UV-B excluded experiment (Fig. 1). Ambient UV-B radiation disturbed the flow of electrons in respiratory chain and possibly proceeded senescence processes in pumpkins. The inhibitory effect of UV-B radiation on ETS activity observed in the present study on pumpkins is supported by similar results on common and Tartary buckwheat (Breznik et al., in press). Contrary to our results UV-B radiation caused enhanced ETS activity in *Ceratophyllum demersum* (Gaberščik et al, 2002b), while no influence of excluding solar UV-B radiation on ETS activity was observed for *Batrachium trichopyllum* and *Potamogeton alpinus* (Germ et al., 2002b). High ETS activity in UV-B treated primary producers was known from the research on *Scenedesmus quadricauda* and *S. capricornutum* (Germ et al., 2002a, 2004) and on natural phytoplankton populations (Ferreyra et al., 1997). When the plants are exposed to UV-B damage, the respiration needed for repair and protection is also higher (Gwynn-Jones, 2001; Bredahl et al., 2004). But when the damage, caused by UV-B radiation is too strong, plants can not overcome the stress and respiratory potential of plants is lowered, that was likely the case in the present study. Unfavourable conditions for growth lowered ETS activity in *Phragmites australi*s (Cav.) Trin. ex Steud. (Urbanc-Berčič and Gaberščik, 2001). It was also reported by Vodnik et al. (1999) that lead induced lower respiratory potential in the roots of *Picea abies* (L.) Karst.. Foliar treatment with Se had, however, no effect on the ETS activity in pumpkin plants in July, while it lowered ETS activity in August in solar UV-B exposed plants (Fig. 1). That might mean that Se treatment presented an additional stress for the plants, exposed to solar UV-B radiation.

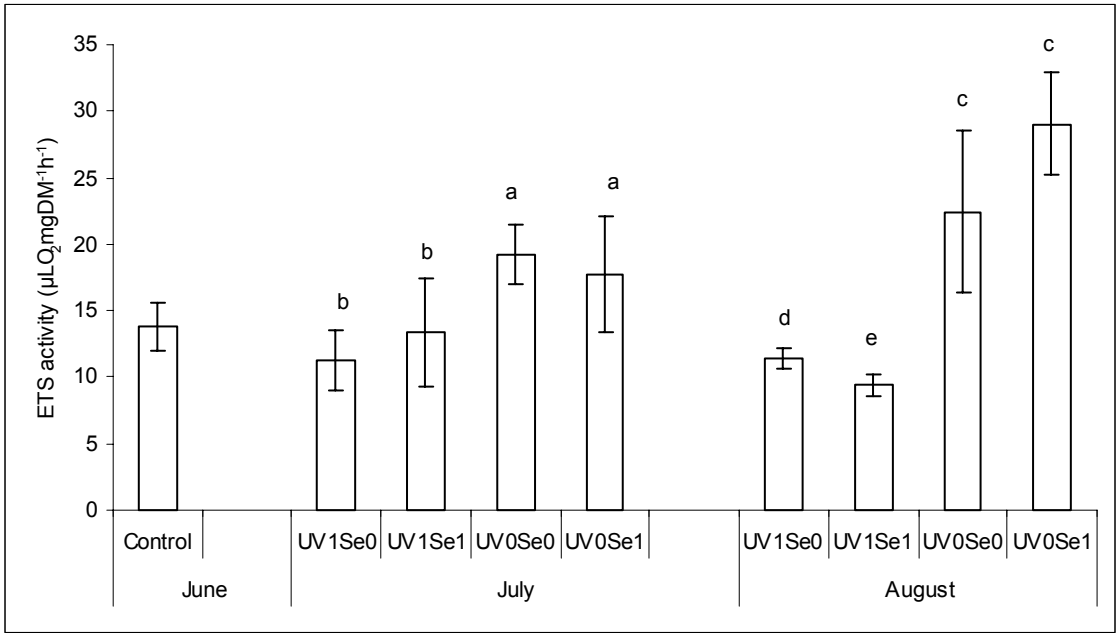

Fig. 1. Terminal electron transport system (ETS) activity in pumpkins grown under different UV-B and Se treatments. Error bars show 95% confidence intervals. Columns marked with different letters are significantly different (95% probability level). Ambient radiation (UV1Se0), ambient radiation and selenium spray (UV1Se1), UV-B excluded ambient radiation (UV0Se0), UV-B excluded ambient radiation and selenium spray (UV0Se1).

4 CONCLUSIONS

Respiratory potential, measured by ETS activity, was lower in pumpkin plants grown under ambient radiation conditions. Results showed that UV-B radiation affected the flow of electrons in the terminal electron transport system. These observations indicated the sensitivity of respiratory potential of pumpkins to current levels of UV-B radiation in our regions. Selenium did not exhibit clear role in the measured parameter.

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