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Selenium treatment affected respiratory potential in *Eruca sativa*

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ABSTRACT

The effect of selenium on *Eruca sativa* Miller was studied. The flows of electrons in the photosynthetic apparatus and in the respiratory chain were measured in control plants and in plants obtained from selenium treated seeds. The potential and effective quantum yields of photosystem II were unaffected by selenium treatment. The respiratory potential of *Eruca sativa*, measured by electron transport system (ETS) activity, significantly increased in plants, grown from selenium treated seeds.

Key words: chlorophyll *a* fluorescence, electron transport system activity, *Eruca sativa*, selenium

IZVLEČEK

VPLIV SELENA NA RESPIRATORNI POTENCIAL PRI RUKOLI (*Eruca sativa*)

V članku je opisan vpliv selena na fiziološke lastnosti rukole *Eruca sativa* Miller. Pretok elektronov v fotosinteznem aparatu in v dihalni verigi smo merili v kontrolnih rastlinah in rastlinah, ki so zrastle iz semen, tretiranih s selenom. Potencialna in dejanska fotokemična učinkovitost nista bili odvisni od tretiranja s selenom. Respiratorni potencial rukole, merjen s pomočjo aktivnosti elektronskega transportnega sistema (ETS), je bil značilno večji pri rastlinah, ki so zrastle iz semen, tretiranih s selenom.

Ključne besede: aktivnost elektronskega transportnega sistema, *Eruca sativa*, fluorescenca klorofila *a*, selen

Abbreviations: ETS, electron transport system; F, the steady state fluorescence; Fo, minimal chlorophyll *a* fluorescence yield in dark adapted samples; Fm, maximal chlorophyll *a* fluorescence yield in dark adapted samples; Fm', the maximal fluorescence of an illuminated sample; Fv, variable fluorescence; INT, iodo-nitro-tetrazolium-chloride; PPFD, photosynthetic photon flux density; SLA, specific leaf area

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1 INTRODUCTION

Selenium functions in plants as an antioxidant (Hartikainen et al., 2000). It is an essential trace element for animals, humans, and microorganisms, whereas the essentiality of selenium in higher plants is still under debate (Terry et al., 2000; Ellis and Salt, 2003). However, there are increasing indications that Se may also play a positive biological role in higher plants (Hartikainen et al., 2000; Xue et al., 2001; Seppänen et al., 2003). Plants that accumulate large amounts of selenium and even require selenium for growth are often found in selenium rich areas. It was also reported that selenium is accumulated by a number of plants in sufficient amounts to be toxic if consumed by livestock (Kampen et al., 1978; Tinggi, 2002). However, most cereal crops and fodder plants show relatively weak ability to absorb Se, even when grown on soils with higher Se content (Nowak et al., 2004). Se is taken up from the soil by plants primarily as selenate (SeO_4^{2-}) or selenite (SeO_3^{2-}) (Ellis and Salt, 2003). Se is chemically similar to sulphur, leading to non-specific replacement of S by Se in proteins and other sulphur components (Nowak et al., 2004).

Se deficiency occurs in several parts of the world, where concentrations of Se in the soil are low. These regions include some parts of China, New Zealand, Finland and Central Europe (Tinggi, 2002; Thomson, 2004). Se content of soils varies from deficit quantities of 0.01 mg kg^{-1} at the Russian Plain to heavily toxic values of 1200 mg kg^{-1} in organic soils at Meath, Ireland (Nowak et al., 2004). Se deficiency in animals and humans can lead to heart disease, hypothyroidism and a weakened immune system (Combs, 2000; Tinggi, 2002). Slovenia is a country with low amounts of selenium in the soil (Kreft et al., 2002). The selenium content in 44 vegetable samples from different regions of Slovenia was in the range $0.3\text{--}77 \text{ ng g}^{-1}$ wet weight (Smrkolj and Stibilj, 2004). The availability of selenium to plants depends on soil factors including pH, salinity and the content of CaCO_3 (Kabata Pendias, 2001). There are attempts to grow crops and vegetables with enriched content of selenium (Stibilj et al., 2004).

Eruca sativa (rucola, arugula) is an annual plant approximately 20-50 cm high, with dull-green leaves which have a distinct spicy-pungent flavour (Morales and Janick, 2002). It prefers rather rich soils, even through it can be found mixed with ruderal flora in very marginal areas. Rucola grows wild, or as a weed in Slovenia and in adjacent areas, but it is frequently cultivated, although domestication cannot be considered complete. Cultivated or wild plants are used as a salad vegetable. Morales and Janick (2002) stated that this species has been known since antiquity and is listed in the Greek herbal of Dioscorides (*Materia Medica*), written in the first century, as well as in the English herbal of John Gerard from 1597. It is an increasingly popular vegetable in Central Europe and is spreading to other areas. Rucola is easily grown and is thus suitable as an experimental plant.

Despite reports that selenium may have an antioxidative effect in plants, it is not known what the basic effects of selenium are. We studied its possible role in photochemical efficiency of photosystem II, and in terminal electron transport system (ETS) activity of mitochondria. Information about ETS activity enables the general metabolic activity of individual organisms to be estimated (Packard, 1985). The ETS is ubiquitous from bacteria to man and is responsible for 90% of the biological O_2 consumption in the biosphere (Packard, 1985; Tóth et al., 1994). The aim of the present research was to

investigate the possible involvement of selenium in the energy household of plants and to find out whether selenium influences processes in photosystem II and in the respiratory chain.

2 MATERIAL AND METHODS

Plant material

Eruca sativa Miller, cv. Coltivata seeds were obtained from a seed company, Magnani sementi, Milano, Italy. Seeds were soaked for 4 hours in a solution of Na selenate (10 mg Se/L). Control seeds were soaked in distilled water. Seeds were sown in a glasshouse in peat substrate on 5th of March 2004 and watered regularly, with water containing no detectable amount of selenium.

Measurements of chlorophyll a fluorescence and ETS activity were performed on the 4th and 5th of May, respectively, 2004, when plants had 6 to 7 developed leaves.

Measurements

Chlorophyll fluorescence was measured *in situ* using a portable fluorometer OS-500 (Opti-Sciences, Tyngsboro, MA, USA). The first fully developed leaf, lacking any visible injury symptoms, was kept in the cuvette for 15 min prior to measurement of minimal (F_o) and maximal (F_m) chlorophyll a fluorescence. Fluorescence was excited with a saturating beam of "white light" (PPFD = 8 000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 0.8 s). The yield of variable fluorescence (F_v) was calculated as $F_v = F_m - F_o$. The potential quantum yield was evaluated in terms of the ratio F_v/F_m . The effective quantum yield of PSII is an estimate of the actual efficiency of energy conversion in PSII. It was calculated as $(F_m' - F)/F_m' = \Delta F/F_m'$. F_m' is the maximal fluorescence of an illuminated sample and F is the steady state fluorescence (Schreiber et al., 1995). The effective quantum yield of PSII was determined by a saturating pulse of white light (PPFD = 9000, $\mu\text{mol m}^{-2} \text{s}^{-1}$, 0.8 s), using a standard 60° angle clip under saturating irradiance ($1500 \mu\text{mol m}^{-2} \text{s}^{-1}$) at the prevailing ambient temperature.

Respiratory potential of mitochondria was measured *via* the potential electron transport system (ETS) activity, as described by Packard (1971). The ETS acts as a bridge between the oxidizing organic matter and O_2 . Determination of ETS activity is based on reduction of the artificial electron acceptor iodo-nitro-tetrazolium-chloride (INT), with the spectrophotometric measurement of the rate of formazan production, which is directly related to the oxygen consumption of the tissue. Leaves with known fresh weight were crushed 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 in a mortar and with an ultrasound homogeniser, and centrifuged at 8500 x g, 0°C, for 4 min in a refrigerated centrifuge (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. After stopping the reaction with stopping solution (formaldehyde and phosphoric acid, 1 : 1), the formazan absorption at 490 nm was determined. ETS activity was measured as the rate of INT reduction, which was converted to the amount of oxygen (Kenner and Ahmed, 1975) utilised per weight of dry mass (DM) of leaves per hour.

Leaves of known surface area were weighed and then dried to constant weight. Specific leaf area was calculated by dividing leaf area by dry mass.

Statistical analysis

The measurements were carried out on 5 (F_v/F_m and $\Delta F/F_m'$) and 8 (ETS) parallel samples. The significance of differences between control and Se treated plants was tested by Student

t-test where appropriate. Differences at the 5% level were accepted as significant, marked with different letters in the figures.

3 RESULTS AND DISCUSSION

Optimal and effective quantum yields of photosystem II were unaffected by selenium treatment (Fig. 1). F_v/F_m is reported to be 0.80-0.83 for a variety of dark-adapted plants (Björkman and Demmig-Adams, 1995). Any deviation from the optimal value shows that the plant is exposed to stress (Schreiber et al., 1995). F_v/F_m in control and treated plants was more than 0.8, indicating that plants were in good physiological state and that the flow of electrons was undisturbed. Even though the effective was lower than the potential quantum yield of PSII, the closeness of the potential photochemical efficiency to the theoretical maximum indicated reversible inactivation rather than damage to the reaction centre (Bischof et al., 1998).

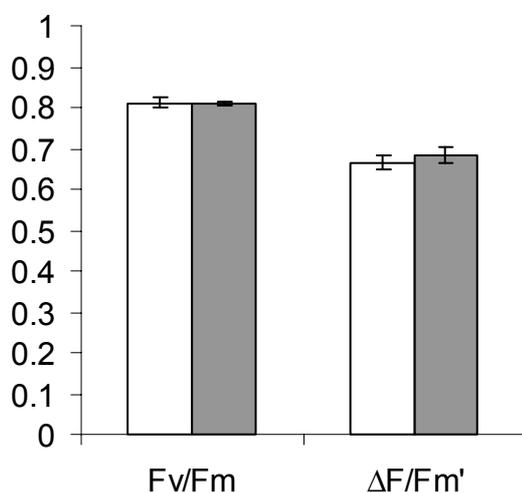


Figure 1: Potential (F_v/F_m) and effective ($\Delta F/F_m'$) quantum yield of photosystem II in *Eruca sativa* exposed to different Se treatments. Bars indicate SE. White columns stand for control plants and dark columns for selenium treated plants.

ETS activity was higher in plants, grown from the seeds, treated with selenium (Fig. 2). Possible explanations are as follows: (1) Some of respiratory enzymes might need Se for their activity; the ETS consists of a complex chain of cytochromes, flavoproteins and metallic ions that transports electrons from catabolized foodstuffs to the natural electron acceptor O_2 . (2) Se may intensify plant metabolism, (3) Plants needed energy to repair the damage caused by Se. It is known that when organisms are stressed and demand more energy, mitochondria increase ATP production and O_2 consumption (Amthor, 1995). (4) Plants needed energy to neutralise Se substances/compounds from the environment.

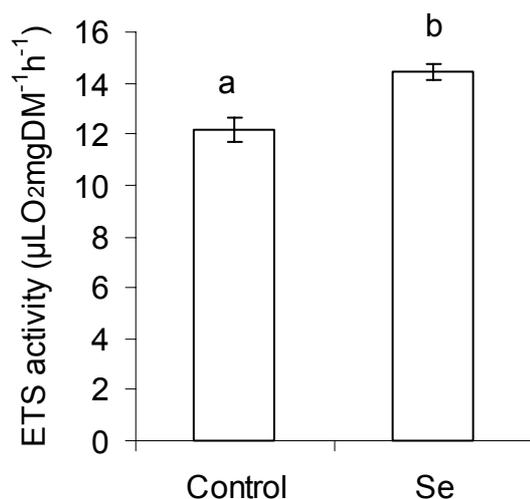


Figure 2: Terminal electron transport system (ETS) activity in *Eruca sativa* grown from Se-treated seeds. Bars indicate SE.

Specific leaf area (SLA, leaf area/leaf dry weight) relates dry matter production to leaf area expansion and, consequently, to light interception and photosynthesis (Gary et al., 1993). Specific leaf area and the ratio of fresh to dry weight were in the same range in control and plants grown from seeds, treated with Se (Table 1).

Table 1: Specific leaf area (cm²g⁻¹) and ratio of fresh to dry weight of the leaves. Mean values and the standard error of mean (SE).

| Parameter/treatment | Control | Selenium |
|------------------------|----------------|----------------|
| Specific leaf area | 332.10 (37.50) | 329.22 (11.10) |
| Fresh/dry weight ratio | 6.98 (0.70) | 6.90 (0.30) |

Results of the present study indicated that Se had probably no harmful effect on photosynthetic processes via changes of activity or biosynthesis of enzymes, or alteration of PSII. In contrast, it has been reported that high Se dosage (1 mgkg⁻¹soil) was toxic to young lettuce, while in senescent seedlings Se reduced dry, but not fresh weight (Xue et al., 2001) and reduced runner biomass (Valkama et al., 2003).

4 CONCLUSIONS

Preliminary results showed, that the addition of selenium did not disturb the flow of electrons in photosystem II, while increased the respiratory potential of *Eruca sativa*. The mechanisms of impact of Se in the respiratory chain are still not clear and deserve continued research.

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