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The effect of selenium on yield and primary terminal electron transport system activity in two cultivars of bean plants *Phaseolus vulgaris*

Vpliv selena na pridelek in dihalni potencial pri fižolu *Phaseolus vulgaris*

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Abstract: The effects of soaking the seeds in solution of selenium (Se) and foliarly spraying with Se on *Phaseolus vulgaris* cv. Stanko and Topolovec were studied. The flows of electrons in the photosynthetic apparatus and in the respiratory chain were measured in control plants and in plants developed from selenium treated seeds and in once and twice foliarly treated plants. Yield of control and treated plants was measured at the end of experiment. The respiratory potential of *Phaseolus vulgaris*, measured by electron transport system (ETS) activity in cv. Stanko, significantly increased in selenium treated plants. The potential and effective photochemical efficiency of photosystem II were similar comparing treated and untreated plants. The addition of selenium induced yield in twice Se foliarly treated plants in both cultivars growing in greenhouse.

Keywords: *Phaseolus vulgaris,* selenium, yield

Izvleček: Ugotavljali smo vpliv dodanega selena na fiziološke lastnosti in pridelek pri dveh kultivarjih fižola *Phaseolus vulgaris* cv. Stanko in Topolovec. Semena smo namakali v raztopini selenata v prvem poskusu in v drugem smo rastline dvakrat listno škropili z raztopino selenata. Merili smo fotokemično učinkovitost fotosistema II in respiratorni potencial. Respiratorni potencial, merjen s pomočjo aktivnosti terminalnega elektronskega transportnega sistema, je bil višji pri mladih rastlinah. Respiratorni potencial je bil pri cv. Stanko višji pri rastlinah, obravnavanih s selenom. Fotokemična učinkovitost je bila podobna pri kontrolnih rastlinah in rastlinah, obravnavanih s selenom. Dodatek selena je povečal pridelek pri rastlinah, ki so bile dvakrat listno obravnavane s selenom pri obeh kultivarjih, gojenih v rastlinjaku.

Ključne besede: *Phaseolus vulgaris,* selen, pridelek

Introduction

Selenium (Se) is an essential nutrient for humans and animals, but also an environmental toxicant. The boundary between the two is narrow and depends on its chemical form, concentration, and other environmentally regulating variables (Fan et al. 2002, Shardendu et al. 2003).

The essentiality of Se to plants remains unclear (Terry et al. 2003). Seppänen et al. (2003) reported that according to current thinking, higher plants do not require Se. Many studies confirmed anti-oxidative role of Se in plants at low concentration (Kuznetsov et al. 2003, Germ et al. 2005, Smrkolj et al. 2006). Studies by Pennanen et al. (2002) have indicated that plant growth was promoted by Se resulting from increased starch accumulation in chloroplasts. Se can increase the tolerance of plants to UV-induced oxidative stress (Valkama et al. 2003). Applied selenium has a high impact on the activity of oxidoreductase enzymes in wheat plants (Nowak et al. 2004). Selenium concentration 0.05 mmol/kg soil positively affected antioxidant defence in wheat plants, but higher concentrations provoked stress responses. When organisms are exposed to stress and demand more energy, ATP production and O_2 consumption are increased in the mitochondria (Packard 1985, Bartoli et al. 2005). The respiratory potential of different organisms can be measured *via* the terminal electron transport system activity in mitochondria (Breznik et al. 2005).

Its wide availability and nutritional value gives the species *Phaseolus vulgaris* a strategic value as food sown in many countries (Granito et al. 2009). The amount of proteins is two to three times higher as in cereals it is also reach source of vitamins, especially group B, pholic acid and certain elements like Fe, Zn and Ca. It contains polysaharids (amids and fibres) that have positive role in digestion (Ranalli et al. 2001).

We aimed to find out the impact of applied Se on yield and the vitality of the plants of *Phaseolus vulgaris* cv. Stanko and Topolovec.

Materials and methods

Growth conditions

Se solution was used for soaking of seeds (12 h) before sowing in water with addition of Na_2SeO_4 in the concentration 2.5; 5.0 and 10.0 mg Se/L. Seeds were then dried and sown the next year in greenhouse in glinopor substrate.

Foliar treatment was performed in two different setups:

A. Bean plants were foliarly treated with Se solution Na_2SeO_4 in the concentration 2.5; 5.0 and 10.0 mg Se/L in the beginning of flowering period. After 10 days we repeated the procedure with the same concentrations, so plants were treated foliarly twice. Experiment was performed in soil in the open area. We have sown 20 plants per m².

B. The same experiment was performed in the greenhouse in Glinopor substrate to provide semi-controlled conditions. We have sown 16 plants per m².

Foliarly treatments with Se were done in three replicates and in two subsequent years. The plant yield was measured in both years. Physiological parameters of plants were measured in the second year only.

Analyses were performed on the plants with similar state of development.

Respiratory potential

Terminal electron transport system (ETS) activity was measured using the assay originally proposed by Packard (1971), and modified by Kenner and Ahmed (1975). A known fresh weight of leaves was crushed in a mortar in 4 ml final volume of ice-cold 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 an ultrasonic homogenizer (4710; Cole-Parmer, Vernon Hills, IL, USA) for 20 sec at 40 W. The homogenates were centrifuged in refrigerated ultracentrifuge for 4 min at 0 ºC at 10,000 rpm (2K15, Sigma). Within 10 min, 0.5 ml of supernatant (in triplicate) was incubated in 1.5 ml 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 0.5 ml of INT (20 mg 2-p-iodo-phenyl 3-p-nitrophenyl 5-phenyl tetrazolium chloride in 10 ml of bidistilled water), for 40 min at standard (20 °C) temperature. The formazan production was determined spectrophotometrically (Lambda 12, Perkin-Elmer) by measuring absorption at 490 nm against the blank. ETS activity was measured as the rate of tetrazolium dye reduction, and converted to equivalent oxygen as described by Kenner and Ahmed (1975).

Fluorescence measurements

Fluorescence measurements were taken on the first fully expanded leaf of randomly selected plants using the OS-500 (Opti-Sciences, Tyngsboro, MA, USA) fluorometer. Prior to measurements samples were dark adapted for 20 min. Measurements of minimal (F_0) and maximal (F_m) chlorophyll fluorescence were provided by dark-adaptation clips. Fluorescence was excited with a saturating beam of "white light" (PPFD = 8000 µmol m^{-2} s^{-1}, 0.8 s). The difference between F_m and F_0 is called the variable fluorescence (F_v). The effective quantum yield of photosystem II (PSII) was measured under saturating irradiance (1 800 µmol m^{-2} s^{-1}) at the prevailing ambient temperature by providing a saturating pulse of "white light" (PPFD = 9 000 µmol m^{-2} s^{-1}, 0.8 s) using a standard 60° angle clip. The effective quantum yield of PSII provides an estimate of the actual efficiency of energy conversion in PSII and is defined 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).

Seeds of bean plants were soaked in Se solution 10.0 mg Se/L, afterwards dried and stored, and then sown in greenhouse in glinopor next year. On the plants, grew from these seeds, respiratory potential and fluorescence measurements were performed. Respiratory potential and fluorescence measurements were done also on the young, once and twice foliarly treated bean plants cultured in the greenhouse in glinopor and in soil with concentration 10.0 mg Se/L. Both analyses were done on the same plants.

Respiratory potential and fluorescence measurements were done on the first, fully developed leaves.

Statistical Analysis

The data were evaluated by ANOVA (Statgraphics Version 4) and significance accepted at $p < 0.05$.

Results

Yield of bean plants cultivated in the soil after foliar spraying

Yield of bean plants cultivated in the soil in open area after one foliar spraying did not differ between treated and untreated plants (Table 1). However in the case of two foliar spraying in the same experimental conditions, bean yield significantly increased with increase Se treatment concentrations in both cultivars only in the first year (Table 1). When plants were treated with Se twice in the second year, there was a trend of

Table 1: Yield of bean plants cultivated in the soil in open area after one and two foliar spraying. Mean values are presented (n = 3). Columns marked with different letters are significantly different for each cv. A - cultivation in the first year, B - cultivation in the second year.

Tabela 1: Pridelek fižola, gojenega na poskusnem polju, ki je bil enkrat in dvakrat listno škropljen. Predstavljene so povprečne vrednosti (n = 3). Stolpci, označeni z različnimi črkami, se med seboj statistično značilno razlikujejo znotraj cv. A - gojene rastline v prvem letu, B- gojene rastline v drugem letu.

Cultivar	Conc. of Se (mg Se/L)	One foliar treatment				Two foliar treatments			
		A		B		A		B	
		g/m²	g/plant	g/m²	g/plant	g/m²	g/plant	g/m²	g/plant
Stanko	0	147 a	7.3	137 a	6.9	150 a	7.5	145 a	7.3
	2.5	148 a	7.4	136 a	6.8	149 a	7.5	145 a	7.3
	5.0	148 a	7.4	139 a	7.0	161 b	8.1	150 a	7.5
	10.0	148 a	7.4	139 a	7.0	160 b	8.0	150 a	7.5
Topolovec	0	146 a	7.3	137 a	6.9	148 a	7.4	134 a	6.7
	2.5	144 a	7.2	136 a	6.8	153 ab	7.7	138 a	6.9
	5.0	147 a	7.3	135 a	6.8	158 b	7.9	132 a	6.6
	10.0	148 a	7.4	138 a	6.9	167 c	8.4	135 a	6.8

increasing mean bean yield with increasing Se concentrations in cv. Stanko. Bean yield was lower in the second year in both treatment (one and two foliarly spraying) because of the heat stress in the period of plant growth.

Yield of bean plants cultivated in the glinopor after foliar spraying

There was a trend of increasing yield in bean plants, cultivated in glinopor in greenhouse in cv. Topolovec once foliarly treated with Se (10.0 mg Se/L) (Table 2) in the first year. Additionally, plants twice foliarly treated with selenium (10.0 mg Se/L) have statistically significant higher yield ($p<0.05$) in cv. Topolovec in both years. Yield was similar in control and treated plants in cv. Stanko regarding year and number of foliar treatments. The exception was higher bean yield in twice foliarly treated plants in the second year. In this case yield was the highest in plants, foliarly treated with 5 mg Se/L.

Respiratory potential

ETS activity of plants cv. Stanko, grown from the seeds, soaked in Se was higher in comparison to the control (Fig. 1). ETS activity enhanced with Se application also in young bean plants in the same cultivar grown in the greenhouse in glinopor, once foliarly treated with Se (Fig. 2a). ETS activity in cv. Stanko was unaffected by Se in mature plants, twice foliarly treated with Se grown in glinopor in greenhouse and soil in open area (Fig. 2b). ETS activity in the cv. Topolovec was similar in plants grown from seeds soaked in the solution of Se comparing to control. In addition ETS activity in the same cultivar was similar in once and twice foliarly treated plants grown in the glinopor in greenhouse. ETS activity in the cv. Topolovec decreased in the once and twice foliarly treated plants grown in the soil in open area. ETS activity of both cultivars was higher in young plants comparing to mature plants (Fig. 2a, 2b).

Table 2: Yield of bean plants cultivated in the glinopor in the greenhouse after one and two foliar spraying. Mean values are presented (n = 3). Columns marked with different letters are significantly different for each cv. A - cultivation in the first year, B - cultivation in the second year

Tabela 2: Pridelek fižola, gojenega v rastlinjaku , ki je bil enkrat in dvakrat listno škropljen. Predstavljene so povprečne vrednosti (n = 3). Stolpci, označeni z različnimi črkami, se med seboj statistično značilno razlikujejo znotraj cv. A - gojene rastline v prvem letu, B- gojene rastline v drugem letu

Cultivar	Concentration of Se (mg Se/L)	One foliar treatment				Two foliar treatments			
		A		B		A		B	
		g/m²	g/plant	g/m²	g/plant	g/m²	g/plant	g/m²	g/plant
Stanko	0	99 a	6.2	98 a	6.1	100 a	6.3	95 a	5.9
	2.5	99 a	6.2	100 ab	6.3	103 a	6.4	98 a	6.1
	5.0	97 a	6.1	104 b	6.5	101 a	6.3	99 a	6.2
	10.0	99 a	6.2	100 a	6.3	105 a	6.6	98 a	6.1
Topolovec	0	96 a	6.0	102 a	6.4	97 a	6.1	92 a	5.8
	2.5	96 a	6.0	102 a	6.4	99 a	6.2	94 ab	5.9
	5.0	96 a	6.0	106 a	6.6	97 a	6.1	96 b	6.0
	10.0	100 a	6.3	104 a	6.5	105 b	6.6	97 b	6.1

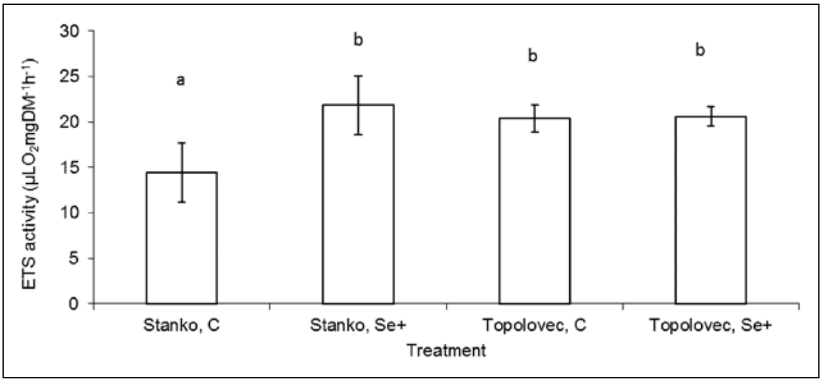

Figure 1: ETS activity in cv. Stanko and Toplovec. Mean values±SD are presented (n = 3). Columns marked with different letters are significantly different. C - control, Se+ - selenium treated plants

Slika 1: Aktivnost ETS pri kultivarjih Stanko and Toplovec. Predstavljene so povprečne vrednosti ±SD (n = 3). Stolpci, označeni z različnimi črkami, se med seboj statistično značilno razlikujejo. C - kontrola, Se+ - s selenom obravnavane rastline

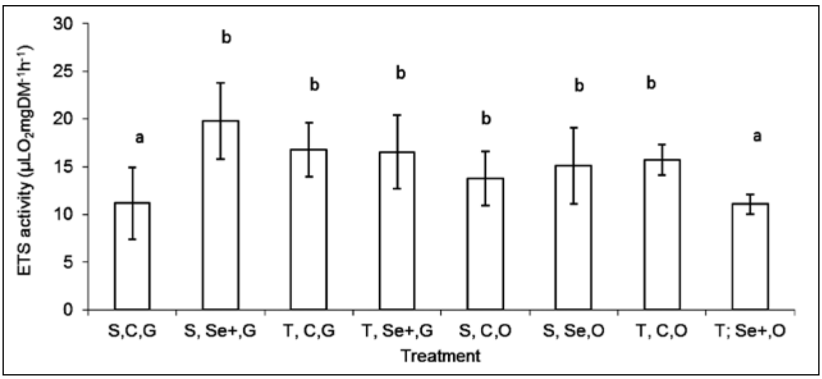

Figure 2a: ETS activity in cv. Stanko and Toplovec once foliarly treated with solution of selenate. Mean values ±SD are presented (n = 3). Columns marked with different letters are significantly different. S - cv. Stanko, T - cv. Topolovec, C - control, Se+ - selenium treated plants, G - greenhouse, O - open area

Slika 2a: Aktivnost ETS pri kultivarjih Stanko in Topolovec, ki sta bila enkrat listno škropljena z raztopino selenata. Predstavljene so povprečne vrednosti ±SD (n = 3). Stolpci, označeni z različnimi črkami, se med seboj statistično značilno razlikujejo. S - cv. Stanko, T - cv. Topolovec, C - kontrola, Se+ - s Se obravnavane rastline, G - rastlinjak, O - poskusno polje

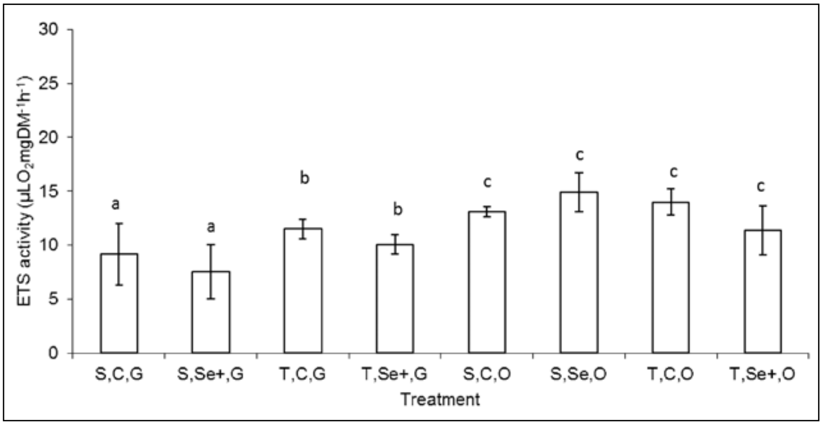

Figure 2b: ETS activity in cv. Stanko and Toplovec twice foliarly treated with solution of selenate. Mean values ±SD are presented (n = 3). Columns marked with different letters are significantly different. S - cv. Stanko, T - cv. Topolovec, C - control, Se+ - selenium treated plants, G - greenhouse, O - open area

Slika 2b: Aktivnost ETS pri kultivarjih Stanko in Topolovec, ki sta bila dvakrat listno škropljena z raztopino selenata. Predstavljene so povprečne vrednosti ±SD (n = 3). Stolpci, označeni z različnimi črkami, se med seboj statistično značilno razlikujejo. S - cv. Stanko, T - cv. Topolovec, C - kontrola, Se+ - s Se obravnavane rastline, G - rastlinjak, O - poskusno polje

Chlorophyll fluorescence

Se did not influence potential - F_v/F_m and effective - $\Delta F/F_m$' photochemical efficiencyof PSII in any treatment (Table 3).

Discussion

Growth and development

Se induced higher yield in twice foliarly sprayed plants in both cultivars grown in greenhouse and in cv. Topolovec grown in the soil in open area (Tables 1, 2). Present results of increased bean yield, cultivated with application of Se are in line with Marschner (2002) who claimed that Se induce luxuriant growth and higher yield of plants. Xue et al. (2001) reported about stimulating effect of added Se on the growth on lettuce in lower concentration of Se (0.1 mg/kg).

Se treatment significantly enhanced pumpkins (Germ et al. 2005) and buckwheat yield (Tadina et al. 2007). Hartikainen et al. *(*2000) also observed the growth promoting effect of Se in ryegrass that was partly a consequence of anti-oxidative effects, which can counteract the senescence processes. Xue et al. (2001) reported about stimulatory effect of foliar application of Se on growth of lettuce. Se also enhanced growth of potato (Turakainen et al. 2004), and leaves of green tea (Hu et al. 2003).

Respiratory potential and photochemical efficiency of PS II

Young plants had higher respiratory potential comparing to mature plants. Plants demand more energy during intensive growth and development, in order to build structural components (Smrkolj et al. 2006). Higher respiratory potential in young plants was reported for an aquatic plant *Potamogeton crispus* (Mazej and Gaberščik 1999), for *Fagopyrum esculentum* and *F. tataricum* (Breznik et al. 2005) and for *Pisum sativum* (Smrkolj et al. 2006). ETS activity of plants, grew from the seeds, soaked in Se was higher comparing to the control in cv. Stanko (Fig. 1). In addition, ETS activity was highest in leaves of young bean plants once foliarly treated with Se in cv. Stanko grown in the greenhouse comparing to control (Fig. 2a). The results are in line with the results from Smrkolj et al. (2006) obtained from the study on *Pisum*

Table 3: Potential (Fv/Fm) and effective ($\Delta F/F_m$') photochemical efficiency of PS II in control and Se treated plants. Mean values ±SD are presented (n = 5)

Tabela 3: Potencialna (Fv/Fm) in dejanska ($\Delta F/F_m$') fotokemična učinkovitost FS II pri kontrolnih in obravnavanih rastlinah. Predstavljene so povprečne vrednosti ±SD (n = 5).

Treatment	Cultivar	Substrate		F_v/F_m	$\Delta F/F_m$'
Soaked	Cv. Stanko		Control	0.80 (0.01)	0.67 (0.06)
			Treated	0.82 (0.01)	0.72 (0.02)
	Cv. Topolovec		Control	0.80 (0.01)	0.72 (0.06)
			Treated	0.81 (0.01)	0.70 (0.04)
One foliarly treatment	Cv. Stanko	Glinopor	Control	0.78 (0.02)	0.20 (0.09)
			Treated	0.81 (0.01)	0.32 (0.08)
		Soil	Control	0.75 (0.04)	0.32 (0.10)
			Treated	0.77(0.02)	0.26 (0.06)
	Cv. Topolovec	Glinopor	Control	0.76 (0.02)	0.21 (0.07)
			Treated	0.81 (0.02)	0.22 (0.05)
		Soil	Control	0.77 (0.02)	0.26 (0.09)
			Treated	0.77 (0.04)	0.32 (0.10)
Two foliarly treatments	Cv. Stanko	Glinopor	Control	0.79 (0.02)	0.21 (0.05)
			Treated	0.78 (0.04)	0.22 (0.01)
		Soil	Control	0.76 (0.03)	0.22 (0.05)
			Treated	0.75 (0.04)	0.34 (0.08)
	Cv. Topolovec	Glinopor	Control	0.75 (0.05)	0.22 (0.07)
			Treated	0.78 (0.03)	0.21 (0.08)
		Soil	Control	0.79 (0.04)	0.30 (0.08)
			Treated	0.76 (0.04)	0.31 (0.09)

Legend: Soaked - cultivation on glinopor in a greenhouse with the technique of soaking seeds prior to sowing in a solution of selenate; once and twice foliar spraying with solution of selenate. Glinopor - glinopor in the greenhouse, Soil - in the soil in the open area

Legenda: Namakanje - semena so bila namakana v raztopino selenata, rastline iz teh semen so bile gojenje na glinoporju v rastlinjaku; enkrat oz. dvakrat foliarno škropljene rastline z raztopino selenata. Glinopor - glinopor v rastlinjaku, Soil – tla na poskusnem polju

sativum. Enhanced ETS activity due to added Se, as presented here, has been known from chicory, foliarly treated with selenium twice with an aqueous solution containing 1 mg Se L^{-1} in the form of sodium selenate (to give 2 µg Se per plant) (Germ et al. 2007) and for *Eruca sativa,* where seeds were soaked for 4 hours in a solution of Na selenate (10 mg Se/L) (Germ and Osvald 2005). On the contrary, when *Glycine max* plants were foliary sprayed with an aqueous solution containing 10 mg Se/L in the forum of Na selenate, ETS activity was lower in treated compared to untreated plants (Mechora and Germ 2010). Sreekala et al. (1999) reported that when Se was applied to *Trigonella foenum-graecum* seedlings, mitochondrial oxygen uptake increased with the enhanced mitochondrial SOD activity. In pumpkin plants and common buckwheat (Germ et al. 2005, Breznik et al. 2005) foliar treatment with Se had no effect on the ETS activity. Higher respiratory potential presented here may reflect increased GSH-Px activity in mitochondria. Xue and coworkers reported that Se exposure increased GSH-Px activity in ryegrass and lettuce (Xue and Hartikainen 2000, Hartikainen et al. 2000, Xue et al. 2001). Higher respiratory potential in Se treated plants can be also a respond to higher demand of energy needed to mitigate toxic effect of Se. This is in line with the fact that Se can mimic sulphur, forming Se analogues of S compounds like replacing S in methionine and cysteine. The conformation of proteins containing selenoaminoacids could be perturbed leading to disturbing of their catalytic activity (Brown and Shrift 1982). However, visual symptoms of Se toxicity did not appear on bean plants. In addition the high potential photochemical efficiency of PSII in the plants, grew from the seeds, soaked in Se and Se foliarly sprayed plants evidenced that Se did not induce damage to photosynthetic apparatus (Table 3). Similar results were obtained in the study on pumpkins (Germ et al. 2005) and chicory (Germ et al. 2007). Potential photochemical efficiency of PSII were close to the theoretical maximum of unstressed plants (0.8- 0.83), that indicated an undamaged antenna complex (Bischof et al. 1998, Schreiber et al. 1995). In research on common buckwheat results shown that Se did not affect potential photochemical efficiency of PSII (Breznik et al. 2005). However, Se treatment induced the increase of the effective photochemical efficiency of PSII in strawberry as well as in common buckwheat (Valkama et al. 2003, Breznik et al. 2005). Se exerted a positive role on the photochemistry of PSII in these species.

Conclusions

Se increased the yield in twice foliarly sprayed plants in both cultivars of bean plants. Present results indicated positive effect of Se on crop. Young plants had higher respiratory potential than mature plants. ETS activity was higher in Se treated plants in cv. Stanko. Photochemical efficiency was unaffected by addition of Se.

Povzetek

Selen je široko razširjen po zemeljski obli in na razpolago rastlinam vsaj v majhnih količinah. Gojenje rastlin, obogatenih s selenom, je učinkovit način dodajanja selena ljudem in izboljšanju zdravja. V znanstvenem svetu poteka debata, ali je selen potreben za rastline. Obstajajo pa dokazi, da selen pri rastlinah pospešuje antioksidacijsko aktivnost, zavira procese, povezane s staranjem in omili stres zaradi visoke svetlobe in tudi suše. Ugotavljali smo vpliv dodange selena na fiziološke lastnosti in pridelek pri dveh kultivarjih fižola *Phaseolus vulgaris* cv. Stanko in Topolovec.

Semena smo namakali v raztopini selenata, jih posušili in naslednje leto posejali v rastlinjak. Na rastlinah, zrastlih iz obravnavanih in neobravnavanih rastlin, smo v drugem letu izvedli fiziološke meritve. V dveh zaporednih letih smo rastline listno škorpili z raztopino selenata in ugotavljali, kakšen je bil pridelek rastlin. Na kontrolnih, enkrat in dvakrat obravnavanih rastlinah smo izvedli meritve. Respiratorni potencial, merjen s pomočjo aktivnosti terminalnega elektronskega sistema, je bil višji pri mladih rastlinah. Mlade rastline rabijo energijo za izgradnjo svoje biomase. Respiratorni potencial je bil pri cv. Stanko višji pri rastlinah, obravnavanih s selenom, kar je lahko posledica višje aktivnost GSH-Px v mitohondrijih. Lahko pa je višji respiratorni potencial odraz povečane potrebe po energiji, ki jo rastlina rabi, da popravi škodo, ki jo je povzročil dodatek selena. Fotokemična učinkovitost je bila podobna pri kontrolnih rastlinah in rastlinah, obravnavanih s selenom, kar kaže na odsotnost stresa zaradi dodanega Se. Dodatek selena je povečal pridelek pri rastlinah, ki so bile dvakrat listno škropljene s selenom pri obeh kultivarjih, ki sta bila gojena v rastlinjaku. Z raziskavo smo želeli ugotoviti, ali selen stimulativno vpliva na pridelek fižola, ki je široko razširjena kmetijska rastlina. Raziskava je zanimiva tudi zato, ker je znano, da je v Sloveniji v tleh malo selena.

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