Journal of Plant Protection Research ISSN 1427-4345

ORIGINAL ARTICLE

Effect of selenium on alleviating oxidative stress in pea leaves caused by pea aphid feeding

Sabina Łukaszewicz[1], Barbara Politycka[1]*, Beata Borowiak-Sobkowiak[2]

[1] Department of Plant Physiology, Poznań University of Life Sciences, Poznań, Poland

[2] Department of Entomology and Environmental Protection, Poznań University of Life Sciences, Poznań, Poland

Vol. 61, No. 1: 83–94, 2021

DOI: 10.24425/jppr.2021.136272

Received: October 5, 2020
Accepted: November 18, 2020

*Corresponding address:
barpolit@yahoo.pl

Abstract

The aim of this study was to evaluate the antioxidant effect of selenium in *Pisum sativum* L. plants pre-treated with sodium selenite or sodium selenate at a concentration of 10 and 20 μM, and then colonized by pea aphid *Acyrthosiphon pisum* (Harris). It has been hypothesized that selenium at low concentrations alleviates oxidative stress caused by aphid feeding on pea leaves. The study focused on the generation of reactive oxygen species (superoxide anion, hydrogen peroxide and hydroxyl radical), the activities of the antioxidant enzymes (superoxide dismutase and ascorbate peroxidase) scavenging the reactive oxygen species levels, as well as on total antioxidant activity in pea leaves. Selenium in pea leaves exposed to aphid feeding affected changes in the levels of reactive oxygen species, the activity of studied antioxidant enzymes, and the total antioxidant capacity. Effects depended on the form and concentration of selenium, as well as on the time after the colonization of pea plants by aphids. Obtained results showed beneficial effects of selenium in alleviating oxidative stress in pea leaves caused by aphid feeding.

Keywords: *Acyrthosiphon pisum*, antioxidant capacity, oxidative stress, *Pisum sativum*, selenium

Introduction

Aphids feeding on phloem sap are the main pests of plants, depriving them of assimilates, and act as viral vectors. Among various insects feeding on pulses, aphids are particularly harmful. The pea aphid [*Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae)] is a monoecious, oligophagous species infesting all plant species of the Fabaceae family (Holman 2009).

A common, fast response of plants to various stress factors is an oxidative burst, manifested by intensive overproduction of reactive oxygen species (ROS) (Dat *et al.* 2000; van Breusegem *et al.* 2001). It is a response to stress factors with high intensity which is revealed by an extreme increase of the intracellular levels of the ROS, such as: hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\bullet-}$) and hydroxyl radical ($^{\bullet}OH$). Oxidative stress involves a disturbance of the balance between the formation and scavenging of ROS which causes damage to cell components, especially proteins, lipids and nucleic acids (Apel and Hirt 2004; Lehmann *et al.* 2015; Shao *et al.* 2019). Plants have developed various complex antioxidant systems responsible for the removal of ROS in cells, including small-molecules such as glutathione, ascorbic acid, flavonoids, carotenoids, and enzymes, such as superoxide dismutases, peroxidases, catalase, glutathione reductase and others (Bartosz 2013).

Superoxide dismutases (SODs) represent the first line of defense against ROS by catalyzing the dismutation of the $O_2^{\bullet-}$ to hydrogen peroxide and oxygen. In contrast, ascorbate peroxidase (APX) catalyzes the decomposition reaction of H_2O_2 with ascorbate, as an electron donor. This enzyme has a high affinity for

H_2O_2, therefore, it may play an important role in scavenging this ROS and protecting cells from damage.

On the other hand, generation of ROS is also strongly associated with plant defense responses. Successful defense of plants against biotic invaders depends on quick plant response. Enhanced production of ROS is an early plant response against attack by phytophagous insects. The oxidative status of host plants is an important factor of plant resistance to insects (Wu and Baldwin 2010; He *et al.* 2011). The specialized nature of aphid feeding in the sieve tubes of the phloem prevents the avoidance of any damage by stress induced oxidative burst, since the sieve cells contain a complete antioxidant system (Walz *et al.* 2002). However, there is still no evidence that ROS generated in phloem can directly reduce aphid performance. Mai *et al.* (2013) reported that an early, strong generation of H_2O_2 may be related to its role as a signalling molecule in the defense mechanism of pea against *A. pisum*.

Selenium (Se) has a dual effect on living organisms – at lower concentrations it is a beneficial element, however, at higher concentrations it is toxic (Dat *et al.* 2000). Although there are many studies which investigate the antioxidant effects of Se during stress caused by abiotic factors, there are very few which are focused on biotic factors, especially phytophagous insects (Hartikainen *et al.* 2000; Gupta and Gupta 2017). Selenium can affect plant-herbivore interactions. It was found that Se accumulation in plants protected them against aphids, caterpillars, moths, grasshoppers and crickets, but the protective mechanism of Se has not been fully elucidated (Mechora and Ugrinović 2015). Selenium is not an essential element for plants. However, recent research shows that it can counteract the negative effects of stress factors through changes in the activity of the antioxidant system, the process of generating and scavenging ROS, and the performance of the antioxidative function. Selenium can regulate the ROS levels in stressed plants through three pathways: (i) by stimulating the spontaneous dismutation of $O_2^{\bullet -}$ into H_2O_2, (ii) by direct reaction between Se-containing compounds and ROS, and (iii) by the regulation of antioxidant enzymes (Feng *et al.* 2013).

In the natural environment, plants are most often exposed to several stress factors that work simultaneously or in succession. The combined effect of two or more stress factors can modify plant response to their action. Studies on the influence of unfavorable factors on plants have shown that there is a so-called cross-tolerance phenomenon involving increased resistance or susceptibility to a stress factor, as a result of simultaneous or an earlier action of another factor (Foyer *et al.* 2016; Saxena *et al.* 2016). It has also been shown that the mobilization of elements of the antioxidant system, due to the occurrence of one stress factor, increases the resistance to the next stress factor. Numerous studies have shown that Se, by affecting the activity of the antioxidant system, can counteract the negative effects of various stress factors on plants (Sieprawska *et al.* 2015). Łukaszewicz *et al.* (2018) determined the range of Se concentrations in the nutrient solution and in pea plants which caused low intensity stress.

The aim of our study was to assess the effect of Se on metabolic changes accompanying oxidative stress in pea plants pre-treated with Se and subjected to aphid feeding. These changes relate to the generation of ROS ($O_2^{\bullet -}$, H_2O_2 and $^{\bullet}OH$) and the activities of antioxidant enzymes (SOD and APX), as well as to the total antioxidant capacity of pea leaves.

Materials and Methods

Plants

Leaves of pea (*Pisum sativum* L.) 'Akord' were the object of the research. Pea seeds, sprouted for 4 days, and the seedlings were transferred to containers with 4 l of Hoagland nutrient solution No. 1, where they grew under growth chamber conditions for the next 7 days. The experiment was carried out in the controlled environment of a growth chamber with the following conditions: under luminescent light with photon flux density 135 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Philips lamps), a 14/10 day/night photoperiod, at 27/23°C day/night temperatures and relative humidity of about 60%. On day 7, Se was added to the nutrient solution as sodium selenite ($Na_2SeO_3 \cdot 5H_2O$) or sodium selenate (Na_2SeO_4) at concentrations of 10 and 20 µM. Seedlings not treated with Se were the control. After 4 days of pea growth on the nutrient solution with Se, the plants were transferred to nutrient solution without Se and 20 apterous female aphids were placed on one seedling. For comparison, half of the pea seedlings were uncolonized by aphids. The leaves, after carefully removing the aphids, were collected at 24, 48 and 72 h after the aphids had been placed on the plants, and samples were taken for the determination of ROS ($O_2^{\bullet -}$, H_2O_2, $^{\bullet}OH$), antioxidant enzymes (SOD, APX) and total antioxidant capacity (TAC). Reactive oxygen species determinations were performed in fresh plant material, while material frozen in liquid nitrogen was used for SOD and APX activity and TAC determinations. The experiment was repeated 3 times in 3 replications, each replication involving one container with 35 pea seedlings.

Aphids

Pea aphids (*A. pisum*) were obtained from aphid stock culture provided by the Department of Entomology and Environmental Protection, Poznań University of

Life Sciences. Aphids lived on the seedlings of their host, *P. sativum,* under growth chamber conditions. In order to maintain the vitality of the colony, aphids were transferred to uncolonized pea seedlings every week. Adult apterous females were used for further research. They were placed on pea seedlings (20 insects per plant), pre-treated for 4 days with sodium selenite or sodium selenate. Control consisted of aphids colonizing plants untreated with Se compounds. New born larvae were removed every 12 h.

Superoxide anion ($O_2^{\bullet-}$)

Determination of $O_2^{\bullet-}$ was based on its ability to reduce nitroblue tetrazolium (NBT) (Doke 1983). Pea leaves (0.5 g) were immersed in 3 ml of 0.01 M potassium phosphate buffer (pH 7.8) containing 0.05% NBT and 10 mM sodium azide and then incubated at room temperature for 1 h and shaken every 15 min. Yellow colored NBT was reduced by $O_2^{\bullet-}$ to deep blue formazan. Then 2 ml of extract was incubated at 80°C for 15 min and cooled. The absorbance was measured spectrophotometrically at a wavelength of 580 nm. $O_2^{\bullet-}$ content was expressed in absorbance units per 1 g of fresh weight of leaves.

Hydrogen peroxide (H_2O_2)

H_2O_2 was determined by using the colorimetric method (Messner and Boll 1994). This method involves the measurement of the content of green colored product 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) in conjugation with H_2O_2 catalyzed by peroxidase. Pea leaves (0.5 g) were homogenized in a cooled mortar with pestle with 0.1 M potassium phosphate buffer (pH 7.0) and centrifuged at 1500 × *g* for 30 min. The level of H_2O_2 was determined in the reaction mixture consisting of supernatant (1.5 ml), extraction buffer (0.1 ml) and a solution of horse-radish peroxidase (0.1 ml, 150 units per 1 ml). The reaction was initiated by adding 0.05 ml of 0.05 M ABTS. Absorption was measured spectrophotometrically at a wavelength of 415 nm, 3 min after the addition of ABTS. The H_2O_2 content was expressed in moles per 1 g of fresh weight of leaves.

Hydroxyl radical ($^{\bullet}OH$)

The level of $^{\bullet}OH$ was determined according to von Tiedemann (1997). Pea leaves (0.6 g) were placed in 2.4 ml of 1 mM 2-deoxy-D-ribose and incubated in the dark at room temperature for 45 min. Then 0.5 ml solution was added to a mixture of 2.8% trichloroacetic acid (0.5 ml) and 1% 2-thiobarbituric acid (0.5 ml) and heated in a boiling water bath for 10 min. Next, the samples were cooled on ice for 10 min. Absorbance was measured spectrophotometrically at a wavelength of 540 nm. The $^{\bullet}OH$ content was expressed in absorbance units per 1 g of fresh weight.

Superoxide dismutase (SOD) assay

Samples of leaves (200 mg) were homogenized in cooled mortars with 4 ml of 0.05 mM sodium phosphate buffer at pH 7.0, with 1% PVP, 1 mM EDTA-Na and 0.5% NaCl. The homogenate was centrifuged for 30 min at 15,000 × *g* at 4°C. The obtained supernatant was used to determine the activity of SOD and protein content. Activity of SOD (EC 1.15.1.1) was determined according to the method of Beauchamp and Fridovich (1971), using the ability of this enzyme to inhibit the photochemical reduction of NBT. The reaction mixture (3 ml) contained 0.05 mM phosphate buffer at pH 7.8, 97 mM methionine, 2 mM NBT, enzymatic extract and 120 µM riboflavin. The reaction was initiated by adding riboflavin. Cuvettes with the reaction mixture were exposed to UV light for 15 min. At the same time a reference sample (without enzyme extract) was incubated. The measurement was carried out at a wavelength of 560 nm. The result of enzymatic activity was converted into the number of enzymatic units (U) per mg of protein, with 1 U being the amount of SOD that inhibited the NBT reduction by about 50%. Superoxide dismutase activity was expressed in $U \cdot mg^{-1}$ protein.

Ascorbate peroxidase (APX) assay

Samples of leaves (0.5 g) were homogenized in cooled mortars with 4.0 ml of 0.1 mM potassium phosphate buffer at pH 7.0 (with 30 mg of Polyclar AT). The extract was centrifuged for 30 min at 15,000 × *g* at 4°C. The obtained supernatant was used to determine the activity of APX and protein content. Activity of APX (EC 1.11.1.11) was determined according to the Nakano and Assada (1981) method. The reaction mixture contained 0.1 mM potassium phosphate buffer at pH 7.0 (2.3 ml), to which extract (0.2 ml) and 5 mM ascorbic acid (0.2 ml) were added. The reaction mixture was placed in a quartz cuvette and 0.3 ml of H_2O_2 was added. The decrease in the absorbance of the sample caused by the oxidation of ascorbate was measured for 2 min at a wavelength of 290 nm. Ascorbate peroxidase activity was calculated on the basis of the molar absorption coefficient for L-ascorbate, which is 2.8 $mM^{-1} \cdot cm^{-1}$ (Doke 1983), and expressed in $nkat \cdot mg^{-1}$ protein.

Protein concentration

The protein concentration was measured spectrophotometrically, according to the Bradford method (1976). Up to 25 µl of extract was added to 1.975 ml

Coomassie Brilliant Blue. Absorbance was measured after 10 min at a wavelength of 595 nm. The protein concentration was read from the standard curve of bovine albumin and used to express the activities of antioxidant enzymes.

Total antioxidant capacity (TAC)

TAC was measured using the ability of antioxidants present in the extract to reduce the cation $ABTS^{\bullet+}$ according to the method described by Re *et al.* (1999) and modified by Bartosz (2013). The starting $ABTS^{\bullet+}$ solution was prepared by dissolving 279 mg of ABTS in 100 ml of 0.1 M potassium phosphate buffer (pH 7.4), and 47 mg of potassium persulfate. After mixing for 2 h the solution was left at room temperature in the dark for 12–16 h. Just prior to the determination, the starting $ABTS^{\bullet+}$ solution was diluted with 0.1 M potassium phosphate buffer, pH 7.4, so that absorbance at a wavelength of 414 nm was 1.0. To determine TAC, 0.5 g samples of pea leaves were homogenized in 3 ml of 5% trichloroacetic acid. The samples were then centrifuged at 15 000 × *g* for 30 min at 4°C. The absorbance (A_0) of 1.9 ml of diluted $ABTS^{\bullet+}$ was measured at a wavelength of 414 nm. Then 100 µl of extract from pea leaves was added and the absorbance was measured again after 10 s (A_1). TAC was calculated according to formula $\Delta A = A_0 - A_1$. Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) was used as an antioxidant standard. The standard curve was prepared by adding 5–25 µl portions of 1 mM Trolox to the diluted $ABTS^{\bullet+}$ and the decrease in absorbance was measured. The results of TAC were expressed as micromoles of Trolox per gram of fresh weight.

Statistical analyses

All determinations were performed in three replicates from three independent experiments. Three-way analysis of variance (ANOVA) was used to verify the significant differences. Means were compared using Duncan test at $\alpha \leq 0.05$ level of significance.

Results

No significant changes in the level of $O_2^{\bullet-}$ were observed after 24 h under the influence of pre-treatment with Se and colonization by pea aphid (Table 1), with the exception of 20 mM selenate, which in the plants uncolonized by aphids resulted in an increase of the studied radical by 41% compared to the control without Se. As a result of the infestation by aphids, a significant increase (75%) in the $O_2^{\bullet-}$ level was observed at 48 h in the plants not treated with Se, compared to the uncolonized plants. At this time, there was also a rapid increase in the studied radical observed in plants treated with 20 µM selenite. It was on average 68% higher than in the control without Se, and it did not differ significantly in the plants uncolonized and colonized by aphids. At the same time, no significant effect of selenate on the level of the studied radical was found. After 72 h, the selenite-treated plants, both uninfested and infested by aphids, had a mean $O_2^{\bullet-}$ level 89% higher than in Se untreated plants. On the other hand, in plants treated with 10 µM selenate, uncolonized and colonized by aphids, and those treated with 20 µM selenate uncolonized by aphids, the level of the studied radical was reduced

Table 1. Superoxide radical ($O_2^{\bullet-}$) level [$A_{580} \cdot g^{-1}$ fresh weight] in leaves of pea pre-treated with selenium (Se) in the form of selenite or selenate and colonized by aphids

Colonization by aphids	Time from aphid colonization of pea								
	24 h			48 h			72 h		
	Selenite pre-treatment [µM Se]								
	0	10	20	0	10	20	0	10	20
Uncolonized	0.47 b ±0.07	0.45 b ±0.06	0.41 b ±0.05	0.45 b ±0.05	0.70 c ±0.10	1.08 de ±0.11	0.55 b ±0.09	1.03 de ±0.15	0.96 de ±0.14
Colonized	0.51 b ±0.08	0.49 b ±0.08	0.48 b ±0.06	0.79 c ±0.12	0.73 c ±0.10	1.00 de ±0.17	0.51 b ±0.08	1.29 e ±0.19	0.68 c ±0.09
	Selenate pre-treatment [µM Se]								
	0	10	20	0	10	20	0	10	20
Uncolonized	0.47 b ±0.07	0.45 b ±0.06	0.61 bc ±0.09	0.45 b ±0.05	0.58 bc ±0.08	0.64 bc ±0.09	0.55 b ±0.09	0.32 ab ±0.06	0.24 a ±0.04
Colonized	0.51 b ±0.08	0.51 b ±0.09	0.72 c ±0.11	0.79 cd ±0.12	0.69 c ±0.10	0.83 cd ±0.13	0.51 b ±0.08	0.24 a ±0.04	0.47 b ±0.07

Statistical analyses – three-way ANOVA, Duncan's test, $n = 3$. Results marked with the same letters do not differ significantly at $\alpha \leq 0.05$

by an average of 50% in 72 h compared to the control without Se.

Se-pretreatment of plants caused changes in the level of H_2O_2 in pea leaves (Table 2). The highest level of H_2O_2 was observed in plants not treated with Se and infested by aphids after 24 h. It was two-fold higher than in uninfested plants. Selenite in plants uncolonized by aphids caused a decrease in the level of H_2O_2 within 24 h. It was 28% after treatment with 10 μM selenite and 63% after treatment with 20 μM selenite, compared to the plants untreated with Se. Selenate caused a decrease in the level of H_2O_2 in 24 h in plants uncolonized by aphids by an average of 25%, and by 36% in those infested by aphids and treated with 20 μM selenate compared to the control without Se. At 48 h an increase in the level of H_2O_2 was observed in plants uncolonized by aphids and treated with selenite, by an average of 68%, and those infested and treated with 10 μM selenite by 37%, compared to plants not treated with Se. In contrast, in plants treated with selenate, the highest level of H_2O_2 at 48 h was observed at the concentration of 20 μM in plants infested by aphids. It was 30% higher than in plants uncolonized by aphids. Within 72 h, no significant differences were found in the level of H_2O_2 in plants, neither under the influence of Se, nor as a result of the colonization by aphids.

Generally, no effect of plants pretreatment with Se and colonization by aphids on the $^{\bullet}OH$ level in pea leaves was observed (Table 3). Compared to the value at 24 h, in all combinations at 48 h and 72 h,

Table 2. Hydrogen peroxide (H_2O_2) level [nmol · g^{-1} fresh weight] in leaves of pea pre-treated with selenium (Se) in the form of selenite or selenate and colonized by aphids

Colonization by aphids	Time from aphid colonization of pea								
	24 h			48 h			72 h		
	Selenite pre-treatment [μM Se]								
	0	10	20	0	10	20	0	10	20
Uncolonized	81.14 b ±7.92	117.10 c ±10.07	89.67 b ±8.45	61.20 a ±5.94	83.53 b ±8.20	121.66 c ±11.65	75.32 b ±7.21	97.43 bc ±10.21	90.54 bc ±9.23
Colonized	166.11 d ±16.89	119.03 c ±11.63	62.07 a ±6.31	75.31 b ±7.28	103.06 c ±9.96	72.21 ab ±7.05	73.39 ab ±7.31	76.56 b ±7.75	85.65 b ± 0.05
	Selenate pre-treatment [μM Se]								
	0	10	20	0	10	20	0	10	20
Uncolonized	81.14 b ±7.92	60.35 a ±5.97	61.77 a ±6.28	61.20 a ±5.94	68.94 ab ±6.32	82.44 b ±8.35	75.32 b ±7.21	76.69 b ±7.92	82.85 b ±8.54
Colonized	166.11 d ±16.89	134.09 cd ±17.29	105.55 c ±10.12	75.31 b ±7.28	67.50 ab ±6.44	106.84 c ±11.02	73.39 ab ±7.31	81.28 b ±8.34	92.11 bc ±9.67

Statistical analyses – three-way ANOVA, Duncan's test, $n = 3$. Results marked with the same letters do not differ significantly at $\alpha \leq 0.05$

Table 3. Hydroxyl radical ($^{\bullet}OH$) level [A_{540} · g^{-1} fresh weight] in leaves of pea pre-treated with selenium (Se) in the form of selenite or selenate and colonized by aphids

Colonization by aphids	Time from aphid colonization of pea								
	24 h			48 h			72 h		
	Selenite pre-treatment [μM Se]								
	0	10	20	0	10	20	0	10	20
Uncolonized	1.84 c ±0.27	2.26 c ±0.34	2.10 c ±0.31	0.19 a ±0.04	0.45 ab ±0.07	0.25 a ±0.04	0.19 a ±0.05	0.56 b ±0.08	0.32 ab ±0.06
Colonized	1.72 c ±0.44	2.13 c ±0.32	2.00 c ±0.30	0.31 ab ±0.05	0.24 a ±0.03	0.69 b ±0.07	0.31 ab ±0.04	0.41 ab ±0.06	0.28 a ±0.05
	Selenate pre-treatment [μM Se]								
	0	10	20	0	10	20	0	10	20
Uncolonized	1.84 c ±0.27	2.01 c ±0.28	1.84 c ±0.26	0.19 a ±0.04	0.45 ab ±0.06	0.43 ab ±0.07	0.19 a ±0.05	0.20 a ±0.03	0.18 a ±0.03
Colonized	1.72 c ±0.44	2.10 c ±0.30	1.88 c ±0.27	0.31 ab ±0.05	0.48 ab ±0.08	0.24 a ±0.05	0.31 ab ±0.04	0.24 a ±0.05	0.34 ab ±0.06

Statistical analyses – three-way ANOVA, Duncan's test, $n = 3$. Results marked with the same letters do not differ significantly at $\alpha \leq 0.05$

a significant decrease in the level of the studied radical was observed. A significantly higher •OH level was observed only at 48 h in plants infested by aphids and treated with 20 µM selenite than in uncolonized plants, and also at 72 h in plants uncolonized by aphids and treated with 10 µM selenite, than in controls without Se. No significant differences were found in the other combinations.

The selenite pre-treatment resulted in a significant decrease in SOD activity in the pea tissues uncolonized by aphids, compared to the untreated plants (Fig. 1). At all times, there was a trend towards higher enzyme activity in aphid-infested plants than in uncolonized ones. A significant increase in SOD activity as a result of colonization by aphids was observed at 48 h in plants treated with 20 µM selenite, while in the selenate-treated plants

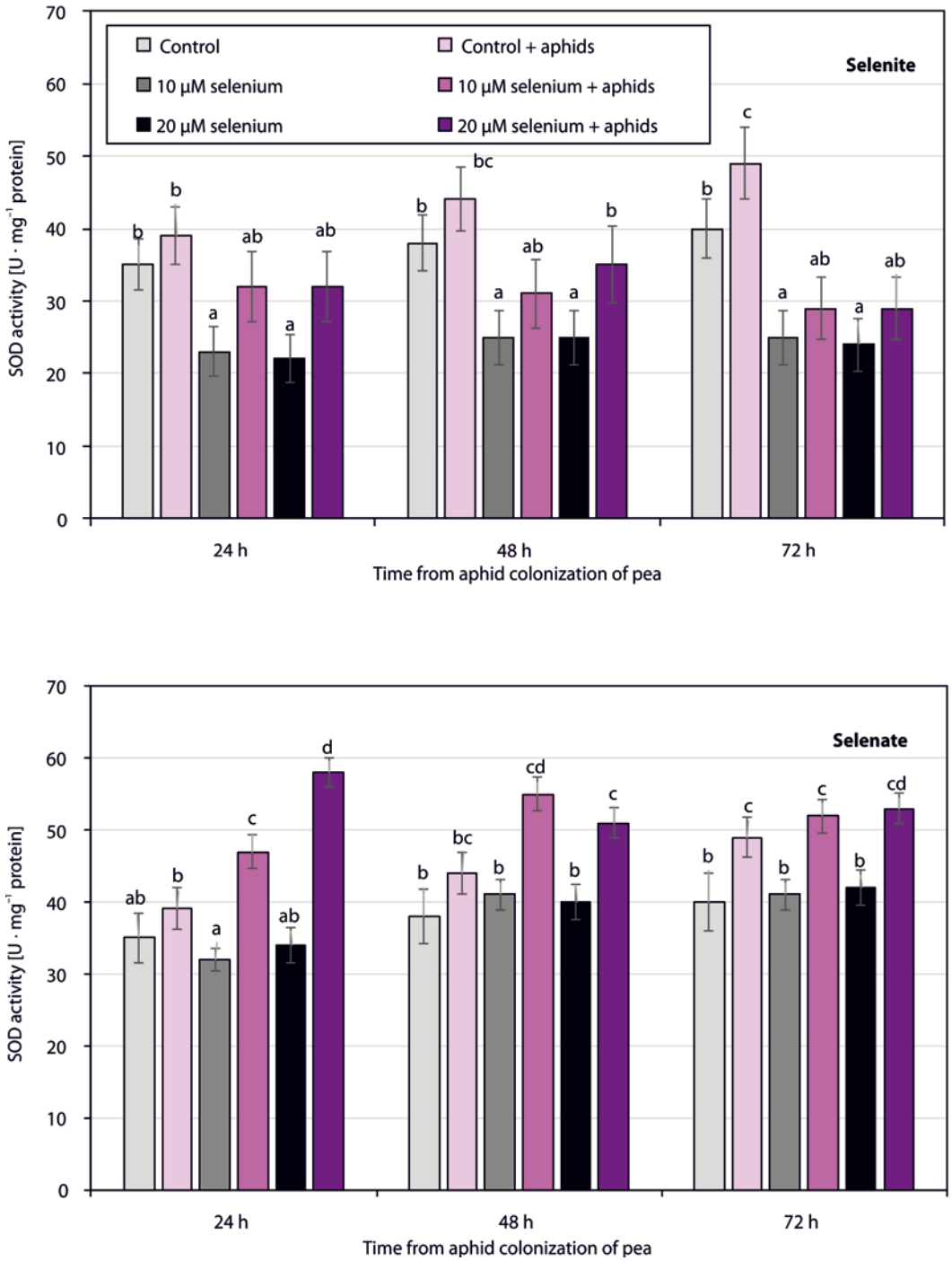

Fig. 1. Superoxide oxidase (SOD) activity in leaves of pea pre-treated with selenium (Se) in form of selenite or selenate and colonized by aphids. Statistical analyses (three-way ANOVA, Duncan's test, $n = 3$) was made for selenite and selenate individually. Bars marked with the same letters do not differ significantly at $\alpha < 0.05$

infested by aphids, the activity of SOD at all times was higher than in the uncolonized plants. After 72 h, such a relationship was also found in the control without Se.

The pretreatment with Se of plants affected the APX activity in pea leaves (Fig. 2). In the case of the selenite action in plants uncolonized by aphids, the APX activity increased proportionally to the concentration of Se, and it was similar at all times. At 72 h, in the Se-untreated plants, infested by aphids, there was an increase in APX activity by 112% compared to uncolonized plants. In the remaining variants, the APX activity was high at this time and no significant differences were observed between the plants colonized and uncolonized by aphids. While under the influence of selenate, there were no significant differences between the individual variants in APX activity after 24 and

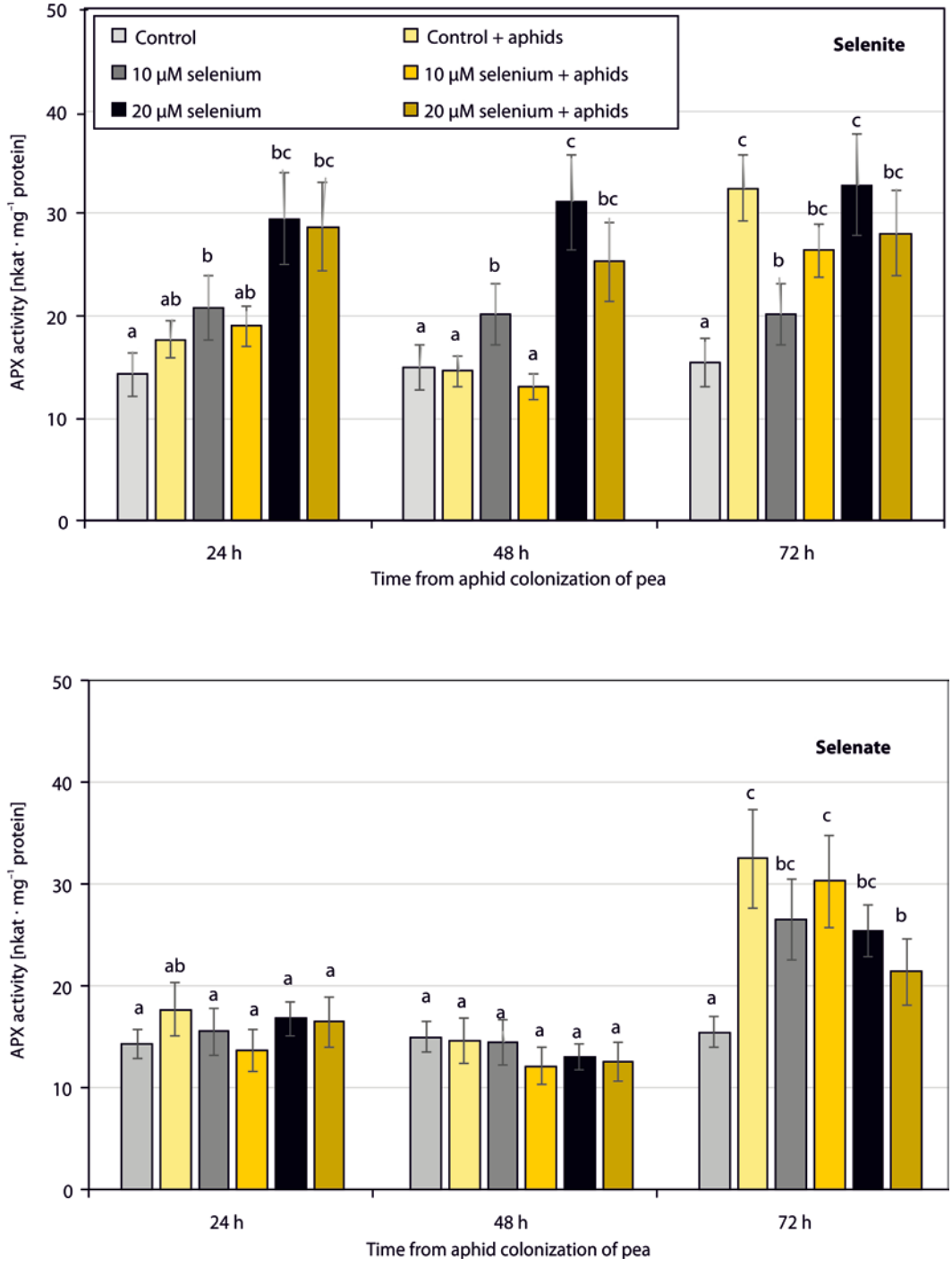

Fig. 2. Ascorbate peroxidase (APX) activity in leaves of pea pre-treated with selenium (Se) in form of selenite or selenate and colonized by aphids. Statistical analyses (three-way ANOVA, Duncan's test, $n = 3$) was made for selenite and selenate individually. Bars marked with the same letters do not differ significantly at $\alpha < 0.05$

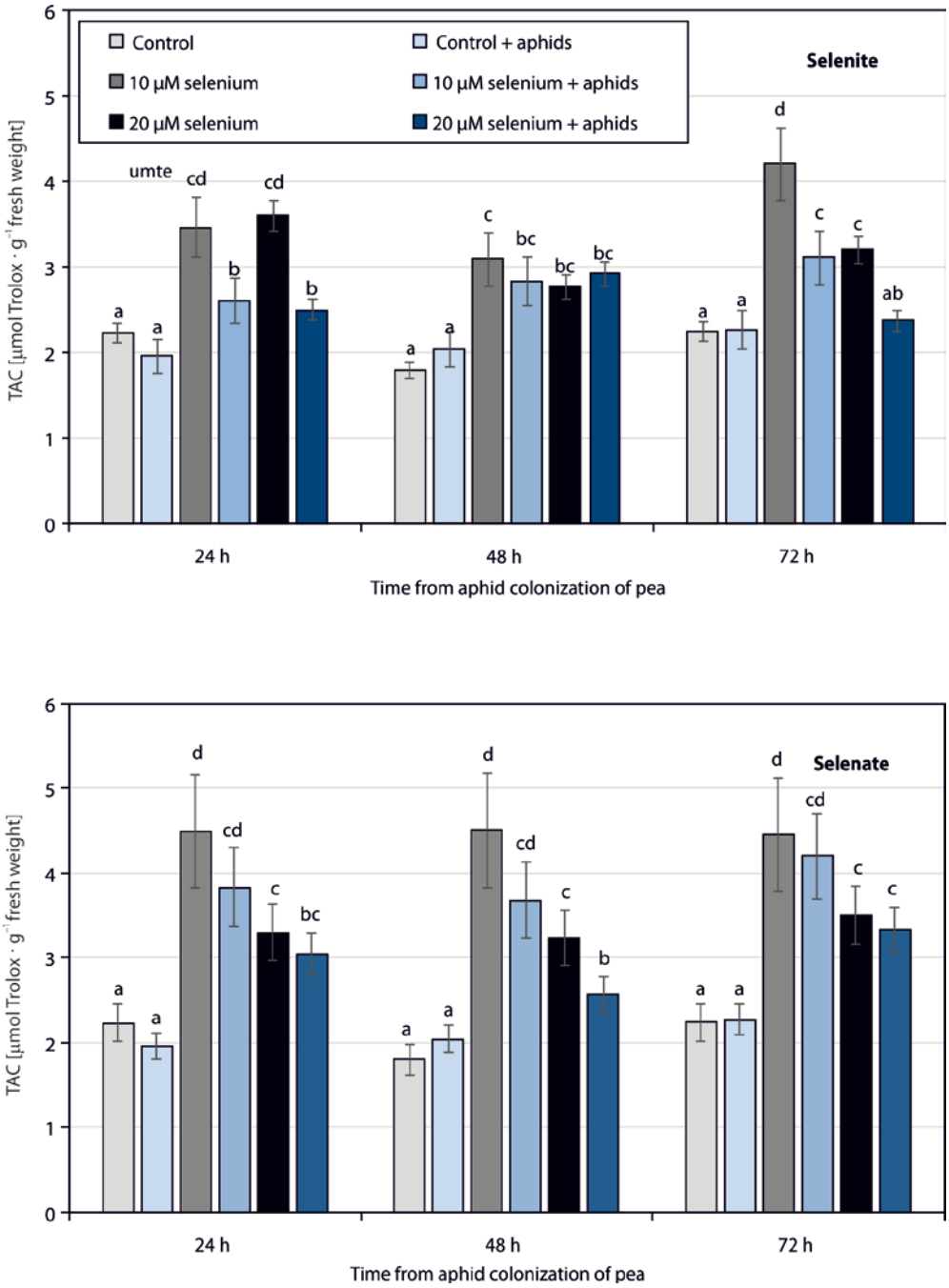

Fig. 3. Total antioxidant (TAC) activity in leaves of pea pre-treated with selenium (Se) in form of selenite or selenate and colonized by aphids. Statistical analyses (three-way ANOVA, Duncan's test, $n = 3$) was made for selenite and selenate individually. Bars marked with the same letters do not differ significantly at $\alpha < 0.05$

48 h. It was not until 72 h that the effect of both selenate pretreatment and aphid infestation on the activity of the studied enzyme was seen. The colonization by aphids resulted in a 2-fold increase in APX activity. Under the influence of selenate at a concentration of 20 µM in plants infested by aphids the enzyme activity decreased by 33%, compared to plants not treated with Se.

In general, pretreatment with Se compounds increased the TAC in pea leaves (Fig. 3). There was a significant increase in TAC at all times as result of selenite treatment. At 24 h and 72 h, the studied parameter was significantly higher in plants treated with selenite and uncolonized by aphids than in the infested ones. When selenate was used, TAC was also higher, in both uncolonized and aphid-colonized plants than in the control without Se. The high values of the studied parameter did not change with time, and the effect of 10 µM selenate was stronger than 20 µM. There was also a trend towards lower TAC in selenate-treated and aphid-infested plants than in uncolonized.

Discussion

The fastest defensive response of plants to stress caused by e.g. insect feeding is the so-called oxidative burst resulting in a dramatic increase in the generation of ROS (Łukasik *et al.* 2012; Hossain *et al.* 2015). Due to the strong reactivity of ROS, their persistently high levels lead to lipid peroxidation, damage of protein and nucleic acid structures or inactivation of enzymes (Apel and Hirt 2004; Lehmann *et al.* 2015; Shao *et al.* 2019). Therefore, ROS must be continuously scavenged by non-enzymatic and enzymatic antioxidant systems (Saxena *et al.* 2016). Se with its antioxidant properties can counteract the effects of biotic and abiotic stresses, leading to increased ROS production (del Pino *et al.* 2019). The aim of our study was to establish if Se at low concentrations would alleviate the oxidative stress caused by aphid feeding on pea plants. To determine the occurrence of oxidative stress, the levels of ROS, such as $O_2^{\bullet-}$, H_2O_2 and $^{\bullet}OH$, the activity of antioxidant enzymes lowering the levels of ROS, i.e. SOD and APX, as well as TAC in pea leaves were compared in the Se-treated and untreated plants.

Numerous literature reports indicate the generation of ROS as an early response of plants to aphid feeding (Kuśnierczyk *et al.* 2008). Plant tissue damage caused by aphid feeding and saliva may induce the production of ROS (Łukasik and Goławska 2013; Dampc *et al.* 2020).

H_2O_2 plays an important role in the early response to stress factors. It is more stable and less reactive than other ROS and, additionally, easily penetrates the membranes. Therefore, it can act as a signaling molecule in the induction of resistance genes and in the phenomenon of cross-interaction (Yang and Poovaiah 2002; Quan *et al.* 2008; Łukasik *et al.* 2012; Hossain *et al.* 2015; Saxena *et al.* 2016). Moreover, H_2O_2 shows direct toxicity to herbivores (Łukasik *et al.* 2012; Hossain *et al.* 2015). The results obtained by other authors indicate the presence of the generation of H_2O_2 in response to aphid feeding (Kuśnierczyk *et al.* 2008; Mai *et al.* 2016). In our study, the highest level of H_2O_2 was observed within 24 h in plants not treated with Se and colonized by aphids (Table 2). It was twice as high than in non-colonized plants. Mai *et al.* (2013) also showed an increase in the level of H_2O_2 in 24 h of aphid feeding in the tissues of pea inhabited by *A. pisum*. In soybean leaves infested with *Aphis craccivora* Koch, a sudden increase in the generation of H_2O_2 was observed after 12 h of aphid feeding. The high level of the studied molecule in plant tissues persisted for 72 h (Mai *et al.* 2016). Czerniewicz *et al.* (2017) showed a significant increase in the level of H_2O_2 in the resistant cultivar of winter triticale after 24 h of feeding by *Sitobion avenae* (F.). In the research of Shao (2019), the highest level of H_2O_2 was observed at 48 h of *Melanaphis sacchari* (Zehntner) feeding in both resistant mutant sorghum and the line susceptible to feeding of aphids. In other research, a decrease in the generation of H_2O_2 was observed as a result of the foliar enrichment of barley and lettuce with Se (Ríos *et al.* 2009; Habibi 2013). Analyzing the generation of H_2O_2 in our study, it was found that the treatment with Se in both forms of plants infested by aphids lowered the level of the studied molecule.

Data found in the literature shows that the stress caused by aphid feeding causes the formation of other ROS such as $O_2^{\bullet-}$ and $^{\bullet}OH$. Mai *et al.* (2013) demonstrated the generation of $O_2^{\bullet-}$ in the leaves of pea infested by *A. pisum*, the intensity and duration of which depended on the degree of aphid infestation. In the case of plant colonization by 30 individuals, the level of $O_2^{\bullet-}$ continuously increased from 24 h until the end of the experiment at 96 h. In the case of 10 individuals, an increase in the generation of $O_2^{\bullet-}$ was observed for 48 h, while in the case of 20 individuals – from 24 h (Mai *et al.* 2013). In our research, an increased generation of $O_2^{\bullet-}$ was observed after 48 h as a result of colonization by 20 aphids (Table 1). However, no effect of Se treatment in both forms on the reduction of the $O_2^{\bullet-}$ level was found. In contrast, selenite showed a pro-oxidative effect causing the generation of the studied radical.

In the case of $^{\bullet}OH$, there was no influence of aphid feeding nor was there any effect of Se on the generation of this radical (Table 3).

Numerous studies have shown that the increase in the activity of peroxidases is part of the defense response of plants to insect feeding. The activity of peroxidases allows plants to remove excessive amounts of ROS, generated in response to oxidative stress caused by aphid feeding. Studies on soybean lines that are resistant and susceptible to feeding of *Aphis glycines* Matsumura have shown a correlation between the activity of peroxidases and the expression of their genes, and their tolerance to aphid feeding (Prochaska 2011; Pierson *et al.* 2011; Prochaska *et al.* 2013; Marchi-Werle *et al.* 2014; Mai *et al.* 2016). Prochaska (2011), analyzing transcriptional changes in aphid-resistant and susceptible soybean plants, found in resistant plants two genes encoding peroxidases that are involved in plant response to aphid feeding, which were not present in susceptible plants. Increased peroxidase activity was also demonstrated in wheat in lines resistant to feeding of aphid *Diuraphis noxia* (Mordw.) (Ni *et al.* 2001), and in barley (Coppola *et al.* 2013).

An important enzyme which forms the first line of defense in the case of oxidative stress in plants is SOD (Moloi and van der Westhuizen 2008; Shao *et al.* 2019). This enzyme catalyzes the disproportionation of very reactive $O_2^{\bullet-}$ to less reactive H_2O_2 and water (del Río *et al.* 2002; Maffei *et al.* 2007). In our study, at

72 h, a higher SOD activity was observed in the leaves of plants colonized by aphids, than in the uncolonized ones (Fig. 1). Selenite decreased the activity of the studied enzyme, which would explain the high level of $O_2^{\bullet-}$ both in plants colonized and uncolonized by aphids at 48 h and 72 h. Selenate increased the activity of SOD in plants colonized by aphids, which undoubtedly resulted in lower $O_2^{\bullet-}$ values, than in selenite treated plants. Shao *et al*. (2019) found an increasing tendency of SOD activity in sorghum, as a result of aphid *M. sacchari* feeding. Moloi and van der Westhuizen (2008) showed a relationship between the activity of SOD and the defensive strategy of wheat against the aphid *D. noxia*. The highest SOD activity was found in the resistant cultivar. Ni and Quinsberry (2003) also observed the effect of *D. noxia* feeding on the increase of SOD activity in resistant oat and wheat cultivars, and in sensitive barley cultivar. Mai *et al*. (2013) observed an increase in SOD activity in pea plants as a result of their colonization with a different number of *A. pisum* individuals. In the case of the variant inhabited with 30 individuals, the SOD activity increased from 24 h and reached its maximum value at 48 h, while in the case of 10 and 20 individuals, the maximum SOD activity was observed at 72 h of aphid feeding (Mai *et al*. 2013). A similar result was obtained in our experiment, where the effect of colonization by 20 aphids on a significant increase in SOD activity was found only after 72 h. Our studies showed a decrease in SOD activity in plants treated with selenite compared to control. A similar effect of selenite on the activity of this enzyme was also observed by Pereira *et al.* (2018) in *Pfaffia glomerata* (Spreng). Cartes *et al.* (2010) also found that 2 µM Se in the form of selenite significantly reduced the activity of SOD in seedlings of rye treated with aluminum at toxic concentrations. On the other hand, in the variant with selenate, our experiment showed an increase in SOD activity at 24 and 48 h in plants inhabited by aphids. The increase in SOD activity under the influence of selenate was observed in various rice genotypes treated with a toxic dose of Se (Gouveia *et al.* 2020), as well as in rice subjected to water deficiency (Andrade *et al.* 2018). A determining factor of SOD activity may be the varied concentrations of this enzyme's cofactors, i.e. iron, manganese, copper and zinc. It was found in earlier research that the treatment of pea plants with 10 and 20 µM selenite decreased the accumulation of these micronutrients (Łukaszewicz *et al.* 2018).

APX, due to its high affinity to H_2O_2, plays a key role in the reduction of H_2O_2 at the toxic level in cytosol and chloroplasts (Gill and Tuteja 2010; Das and Roychoudhury 2014; Shao *et al.* 2019). In our study, APX activity in pea leaves doubled after 72 h from the beginning of colonization by aphids (Fig. 2). This would explain the fact that the H_2O_2 level was reduced at the same time to the value observed in uncolonized plants. Shao *et al*. (2019) found higher APX activity in resistant mutant sorghum inhabited by *M. sacchari,* than the activity in the line susceptible to aphid feeding. Łukasik *et al.* (2012) showed an increase in APX activity in the tissues of two triticale cultivars at 24 h of *S. avenae* and *Rhopalosiphum padi* (L.) feeding. Moloi and van der Westhuizen (2008) observed an increase in the activity of this enzyme as a result of *D. noxia* feeding in resistant wheat plants. In plants treated with Se compounds, an increase in the activity of H_2O_2 removing enzymes, including APX, was found (Ríos *et al.* 2009; Habibi 2013). Our research showed high APX activity in plant tissues treated with 10 µM selenite at all times. In the case of plants treated with selenate, an increase in APX activity was not observed until 72 h.

SOD and APX are just two of numerous enzymes in the antioxidant system. Plants have efficient complex enzymatic and non-enzymatic antioxidant defense systems to avoid–toxic effects of ROS. Non-enzymatic systems consist of low molecular weight antioxidants (ascorbic acid, glutathione, proline, carotenoids, phenolic acids, flavonoids, etc.) and high molecular weight secondary metabolites such as tannins (Kasote *et al.* 2015). Under oxidative stress conditions, Se stimulates the synthesis of non-enzymatic antioxidants, increasing the antioxidant capacity of plants (Guardado-Félixa *et al.* 2017; Shalaby *et al*. 2017). Our research showed an increase in TAC in both selenite and selenate-treated plants (Fig. 3). Typical antioxidants (ascorbate, glutathione) react very rapidly with $ABTS^{\bullet+}$. Other substances react more slowly (Re *et al.* 1999; Bartosz 2013). The very short period of time (10 s) used in our experiment to measure the reduction of $ABTS^{\bullet+}$ indicates that fast-acting antioxidants are responsible for the increase in TAC under the influence of Se. The highest values which were maintained at all times were observed in the variant with 10 µM selenate. Generally, there were no significant changes in TAC due to colonization by aphids.

Conclusions

Colonization by aphids caused oxidative stress in pea leaves, which was manifested by increased generation of H_2O_2 (24 h) and $O_2^{\bullet-}$ (48 h). In general, Se pretreatment was effective in alleviating oxidative stress by reducing the levels of H_2O_2 and $O_2^{\bullet-}$, increasing the activity of antioxidant enzymes – SOD and APX, and increasing TAC dependent on fast-acting antioxidants. The effect depended on the form of Se, its concentration and how much time passed since the beginning of the colonization by aphids. Neither the influence of aphid infestation, nor the effect of Se on the changes of $^{\bullet}OH$ level were found. The Se effect was

not always favorable. Pre-treatment with selenite acted pro-oxidatively, causing increased generation of $O_2^{\bullet-}$ in both aphid infested and uninfested plants. Lower SOD activity was demonstrated in plants treated with selenite than in untreated plants.

References

Andrade F.R., da Silva G.N., Guimarães K.C., Barreto H.B.F., de Souza K.R.D., Guilherme L.R.G., Faquin V., Reis A.R. 2018. Selenium protects rice plants from water deficit stress. Ecotoxicology and Environmental Safety 164: 562–570. DOI: https://doi.org/10.1016/j.ecoenv.2018.08.022

Apel K., Hirt H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annual Review of Plant Biology 55: 373–399. DOI: https://doi.org/10.1146/annurev.arplant.55.031903.141701

Bartosz G. 2013. Druga twarz tlenu. Wolne rodniki w przyrodzie. [Second Face of Oxygen. Free Radicals in Nature]. Wydawnictwo Naukowe PWN, Warszawa, Poland, 447 pp. (in Polish)

Beauchamp C., Fridovich I. 1971. Superoxide dismutase, improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry 44 (1): 276–287. DOI: https://doi.org/10.1016/0003-2697(71)90370-8

Bradford M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72 (1–2): 248–254. DOI: https://doi.org/10.1016/0003-2697(76)90527-3

Cartes P., Jara A., Pinilla L., Rosas A., Mora M. 2010. Selenium improves the antioxidant ability against aluminium-induced oxidative stress in ryegrass roots. Annales of Applied Biology 156: 297–307. DOI: https://doi.org/10.1111/j.1744-7348.2010.00387.x

Coppola V., Coppola M., Rocco M., Digilio M.C., D'Ambrosio C., Renzone G., Renzone G., Martinelli R., Scaloni A., Pennacchio F., Rao R., Corrado G. 2013. Transcriptomic and proteomic analysis of a compatible tomato-aphid interaction reveals a predominant salicylic acid-dependent plant response. BMC Genomics 14: 515–532. DOI: https://doi.org/10.1186/1471-2164-14-515

Czerniewicz P., Sytykiewicz H., Durak R., Borowiak-Sobkowiak B., Chrzanowski G. 2017. Role of phenolic compounds during antioxidative responses of winter triticale to aphid and beetle attack. Plant Physiology and Biochemistry 118: 529–540. DOI: https://doi.org/10.1016/j.plaphy.2017.07.024

Dampc J., Kula-Maximenko M., Molon M., Durak R. 2020. Enzymatic defense response of apple aphid *Aphis pomi* to increased temperature. Insects 11 (7): 436. DOI: https://doi.org/10.3390/insects11070436

Das K., Roychoudhury A. 2014. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Frontiers in Environmental Science 2: 53. DOI: https://doi.org/10.3389/ fenvs.2014.00053

Dat J., Vandenabeele S., Vranová E., Van Montagu M., Inzé D., van Breusegem F. 2000. Dual action of the active oxygen species during plant stress responses. Cellular and Molecular Life Sciences 57: 779–795. DOI: https://doi: 10.1007/s000180050041

del Pino A.M., Guiducci M., D'Amato R., Di Michele A., Tosti G., Datti A., Palmerini C.A. 2019. Selenium maintains cytosolic Ca^{2+} homeostasis and preserves germination rates of maize pollen under H_2O_2-induced oxidative stress. Scientific Reports 9 (1): 1–9. DOI: https://doi.org/1038/s41598-019-49760-3

del Río L.A., Corpas F.J., Sandalio L.M., Palma J.M., Gómez M., Barroso J.B. 2002. Reactive oxygen species, antioxidant systems and nitric oxide in peroxisomes. Journal of Experimental Botany 53: 1255–1272. DOI: https://doi.org/10.1093/jexbot/53.372.1255

Doke N. 1983. Involvement of superoxide anion generation in the hypersensitive response of potato tuber tissues to infection with an incompatible race of *Phytophthora infestans* and to the hyphal wall components. Physiological Plant Pathology 23 (3): 345–357. DOI: https://doi.org/10.1016/0048-4059(83)90019-X

Feng R., Wei C., Tu S. 2013. The roles of selenium in protecting plants against abiotic stresses. Environmental and Experimental Botany 87: 58–68. DOI: https://doi.org/10.1016/j.envexpbot.2012.09.002

Foyer C.H., Rasool B., Davey J.W., Hancock R.D. 2016. Cross-tolerance to biotic and abiotic stresses in plants: a focus on resistance to aphid infestation. Journal of Experimental Botany 67 (7): 2025–2037. DOI: https://doi.org/10.1093/jxb/erw079.

Gill S.S., Tuteja N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry 48 (12): 909–930. DOI: https://doi.org/10.1016/j.plaphy.2010.08.016

Gouveia G.C.C., Galindo F.S., Lanza M.G.D.B., Silva A.C.R., Mateus M.P.B., Silva M.S., Tavanti R.F.R., Tavanti T.R., Lavres J., Reis A.R. 2020. Selenium toxicity stress-induced phenotypical, biochemical and physiological responses in rice plants: Characterization of symptoms and plant metabolic adjustment. Ecotoxicology and Environmental Safety 202: e110916. DOI: https://doi.org/10.1016/j.ecoenv.2020.110916

Guardado-Félixa D., Serna-Saldivarb S.O., Cuevas-Rodrígueza E.O., Jacobo-Velázquezb D.A., Gutiérrez-Uribeb J.A. 2017. Effect of sodium selenite on isoflavonoid contents and antioxidant capacity of chickpea (*Cicer arietinum* L.) sprouts. Food Chemistry 226: 69–74. DOI: https://doi.org/10.1016/j.foodchem.2017.01.046

Gupta M., Gupta S. 2017. An overview of plant selenium uptake, metabolism and toxicity in plants. Frontiers in Plant Science 7: e2074. DOI: https://doi.org/10.3389/fpls.2016.02074

Habibi G. 2013. Effect of drought stress and selenium spraying on photosynthesis and antioxidant activity of spring barley. Acta Agriculturae Slovenica 101: 31–39. DOI: https://doi.org/10.2478/acas-2013-0004

Hartikainen H., Xue H., Piironen V. 2000. Selenium as an antioxidant. Plant and Soil 225: 193–200. DOI: https://doi.org/10.1023/A:1026512921026

He J., Chen F., Chen S., Lv G., Deng Y., Fang W., Guan Z., He C. 2011. Chrysanthemum leaf epidermal surface morphology and antioxidant and defence enzyme activity in response to aphid infestation. Journal of Plant Physiology 168 (7): 687–693. DOI: https://doi.org/10.1016/j.jplph.2010.10.009

Holman J. 2009. Host Plant Catalog for Aphids. Palearctic Region. Springer Science + Business Media B.V., Berlin/Heidelberg, Germany, 1216 pp.

Hossain M.A., Bhattacharjee S., Armin S.M., Qian P., Xin W., Li H.Y., Burritt D.J., Fujita M., Tran L.-S.P. 2015. Hydrogen peroxide priming modulates abiotic oxidative stress tolerance: insights from ROS detoxification and scavenging. Frontiers in Plant Science 6: e420. DOI: https://doi.org/10.3389/ fpls.2015.00420

Kasote D.M., Katyare S.S., Hegde M.V., Bae H. 2015. Significance of antioxidant potential of plants and its relevance to therapeutic applications. International Journal of Biological Sciences 11 (8): 982–991. DOI: https://doi:10.7150/ijbs.12096

Kuśnierczyk A., Winge P., Jorstad T.S., Troczyńska J., Rossiter J.T., Bunes A.M. 2008. Towards global understanding of plant defence against aphids timing and dynamics of early *Arabidopsis* defence responses to cabbage aphid (*Brevicoryne brassicae*) attack. Plant, Cell and Environment 31 (8): 1097–1115. DOI: https://doi.org/10.1111/j.1365-3040.2008.01823.x

Lehmann S., Serrano M., L'Haridon F., Tjamos S.E., Metraux J.P. 2015. Reactive oxygen species and plant resistance to fungal pathogens. Phytochemistry 112: 54–62. DOI: https://doi.org/10.1016/j.phytochem.2014.08.027

Łukasik I., Goławska S., Wójcicka A. 2012. Effect of cereal aphid infestation on ascorbate content and ascorbate peroxidase activity in triticale. Polish Journal of Environmental Studies 21 (6): 1937–1941.

Łukasik I., Goławska S. 2013. Effect of host plant on levels of reactive oxygen species andantioxidants in the cereal aphids *Sitobion avenae* and *Rhopalosiphum padi*. Biochemical Systematic and Ecology 51: 232–239. DOI: https://doi.org/10.1016/j.bse.2013.09.001

Łukaszewicz S., Politycka B., Smoleń S. 2018. Effect of selenium on the content of essential micronutrients and their translocation in garden pea. Journal of Elementology 23 (4): 1307–1317. DOI: https://doi.org/10.5601/jelem.2017.22.4.1577.

Maffei M.E., Mithöfer A., Boland W. 2007. Insects feeding on plants: Rapid signals and responses preceding the induction of phytochemical release. Phytochemistry 68 (22–24): 2946–2959. DOI: https://doi.org/10.1016/j.phytochem.2007.07.016

Mai V.C., Bednarski W., Borowiak-Sobkowiak B., Wilkaniec B., Samardakiewicz S., Morkunas I. 2013. Oxidative stress in pea seedling leaves in response to *Acirthosiphon pisum* infestation. Phytochemistry 93: 49–62. DOI: https://doi.org/10.1016/j.phytochem.2013.02.011

Mai V.C., Tran N.T., Nguyen D.S. 2016. The involvement of peroxidases in soybean seedlings' defence against infestation of cowpea aphid. Arthropod-Plant Interactions 10: 283–292. DOI: https://doi.org/10.1007/s11829-016-9424-1

Marchi-Werle L., Heng-Moss T.M., Hunt T.E., Baldin E.L.L., Baird L.M. 2014. Characterization of peroxidase changes in tolerant and susceptible soybeans challenged by soybean aphid (Hemiptera: Aphididae). Journal of Economic Entomology 107 (5): 1985–1991. DOI: https://doi.org/10.1603/EC14220

Mechora Š., Ugrinović K. 2015. Can plant-herbivore interaction be affected by selenium? Austin Journal of Environmental Toxicology 1 (1): e5.

Messner B., Boll M. 1994. Cell suspension of spruce (*Picea abies*): inactivation of extracellular enzymes by fungal elicitor-induced transient release of hydrogen peroxide. Plant Cell Tissue Organ and Culture 39: 69–78. DOI: https://doi.org/10.1007/BF00037594

Moloi M.J., van der Westhuizen A.J. 2008. Antioxidative enzymes and the Russian wheat aphid (*Diuraphis noxia*) resistance response in wheat (*Triticum aestivum*). Plant Biology 10 (3): 403–407. DOI: https://doi.org/10.1111/j.1438-8677.2008.00042.x

Nakano Y., Asada K. 1981. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant Cell Physiology 22 (5): 867–880. DOI: https://doi.org/10.1093/oxfordjournals.pcp.a076232

Ni X., Quinsberry S.S. 2003. Possible roles of esterase, glutathione S-transferase, and superoxide dismutase activities in understanding aphid–cereal interactions. Entomologia Experimentalis et Applicata 108: 187–195. DOI: https://doi.org/10.1046/j.1570-7458.2003.00082.x

Ni X., Quisenberry S.S., Heng-Moss T.M., Markwell J., Sarath G., Klucas R., Baxendale F. 2001. Oxidative responses of resistant and susceptible cereal leaves to symptomatic and nonsymptomatic cereal aphid (Hemiptera: Aphididae) feeding. Journal of Economic Entomology 94: 743–751. DOI: https://doi.org/10.1603/0022-0493-94.3.743

Pereira A.S., Dorneles A.O.S., Bernardy K., Sasso V.M., Bernardy D., Possebom G., Rossato L.V., Dressler V.L., Tabaldi L.A. 2018. Selenium and silicon reduce cadmium uptake and mitigate cadmium toxicity in *Pfaffia glomerata* (Spreng.) Pedersen plants by activation antioxidant enzyme system. Environmental Science and Pollution Research 25: 18548–18558. DOI: https://doi.org/10.1007/s11356-018-2005-3

Pierson L.M., Heng-Moss T.M., Hunt T.E., Reese J. 2011. Physiological responses of resistant and susceptible reproductive stage soybean to soybean aphid (*Aphis glycines* Matsumura) feeding. Arthropod-Plant Interactions 5: 49–58. DOI: https://doi.org/10.1007/s11829-010-9115-2

Prochaska T.J. 2011. Characterization of the Tolerance Response in the Soybean KS4202 to *Aphis glycines* Matsumura. M.Sc. Thesis, University of Nebraska, Lincoln, USA.

Prochaska T.J., Pierson L.M., Baldin E.L.L., Hunt T.E., Heng-Moss T.M., Reese J.C. 2013. Evaluation of late vegetative and reproductive stage soybeans for resistance to soybean aphid (Hemiptera: Aphididae). Journal of Economic Entomology 106 (2): 1036–1044. DOI: https://doi.org/10.1603/EC12320

Quan L.J., Zhang B., Shi W.W., Li H.Y. 2008. Hydrogen peroxide in plants: a versatile molecule of the reactive oxygen species network. Journal od Integrative Plant Biology 50: 2–18. DOI: https://doi.org/10.1111/j.1744-7909.2007.00599.x

Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C. 1999. Antioxidant activity applying and improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 26: 1231–1237. DOI: https://doi.org/10.1016/s0891-5849(98)00315-3

Ríos J.J., Blasco B., Cervilla L.M., Rosales M.A., Sanchez-Rodriguez E., Romero L., Ruiz J.M. 2009. Production and detoxification of H_2O_2 in lettuce plants exposed to selenium. Annals of Applied Biology 154: 107–116. DOI: https://doi.org/10.1111/j.1744-7348.2008.00276.x

Saxena I., Srikanth S., Chen Z. 2016. Cross talk between H_2O_2 and interacting signal molecules under plant stress response. Frontiers in Plant Science 7: e570. DOI: https://doi.org/10.3389/fpls.2016.00570

Shalaby T., Bayoumi Y., Alshaal T., Elhawat N., Sztrik A., El-Ramady H. 2017. Selenium fortification induces growth, antioxidant activity, yield and nutritional quality of lettuce in salt-affected soil using foliar and soil applications. Plant Soil 421: 245–258. DOI: https://doi.org/10.1007/s11104-017-3458-8

Shao Y., Guo M., He X., Fan Q., Wang Z., Jia J., Guo J. 2019. Constitutive H_2O_2 is involved in sorghum defense against aphids. Brazilian Journal of Botany 42 (2): 271–281. DOI: https://doi.org/10.1007/s40415-019-00525-2

Sieprawska A., Kornaś A., Filek M. 2015. Involvement of selenium in protective mechanisms of plants under environmental stress conditions – review. Acta Biologica Cracoviensia. Series Botanica 57 (1): 9–20. DOI: http://dx.doi.org/10.1515/abcsb-2015-0014

van Breusegem F., Vranová E., Dat J.F., Inzé D. 2001. The role of active oxygen species in plant signal transduction. Plant Science 161 (3): 405–416. DOI: https://doi.org/10.1016/S0168-9452(01)00452-6

von Tiedemann A.V. 1997. Evidence for a primary role of active oxygen species in induction of host cell death during infection of bean leaves with *Botrytis cinerea*. Physiological and Molecular Plant Pathology 50 (3): 151–166. DOI: https://doi.org/10.1006/pmpp.1996.0076

Walz C., Juenger M., Schad M., Kehr J. 2002. Evidence for the presence and activity of a complete defence system in mature sieve tubes. The Plant Journal 31 (2): 189–197. DOI: https://doi.org/10.1046/j.1365-313X.2002.01348.x

Wu J., Baldwin I.T. 2010. New insights into plant responses to the attack from insect herbivores. Annual Review of Genetics 44: 1–24. DOI: https://doi.org/10.1146/annurev-genet-102209-163500

Yang T., Poovaiah B. W. 2002. Hydrogen peroxide homeostasis: activation of plant catalase by calcium/calmodulin. Proceedings of the National Academy of Sciences of the United States of America 99 (6): 4097–4102. DOI: https://doi.org/10.1073/pnas.052564899