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Foliar application of selenium for protection against the first stages of mycotoxin infection of crop plant leaves

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Abstract

BACKGROUND: The aim of this study was to investigate whether the application of selenium (Se) ions directly to the leaf surface can protect plants against infection by the fungal toxin zearalenone (ZEA). The experiments were performed for the most common and agronomically important crops such as wheat, oat, and barley (both tolerant and sensitive varieties) because mycotoxin accumulation in plants is the cause of many diseases in animals and people.

RESULTS: ZEA at a concentration of 10 μmol L^{-1} either alone or in combination with Se (5 μmol L^{-1} Na_2SeO_4) was applied to the second leaf of seedlings. Visualization of leaf temperature profiles by infrared thermography demonstrated a decrease in temperature at the location of ZEA infection that was more noticeable in sensitive genotypes. The presence of Se significantly suppressed changes at the site of ZEA application in all tested plants, especially the tolerant genotypes. Microscopic observations confirmed that foliar administration of ZEA resulted in its penetration to deeper localized cells and that damage induced by ZEA (mainly to chloroplasts) decreased after Se application. Analyses of antioxidant enzymes demonstrated the involvement of Se in antioxidation mechanisms, in particular by activating SOD and CAT under ZEA-induced stress conditions.

CONCLUSION: The foliar application of Se to seedling leaves may be a non-invasive method of protecting crops against the first steps of ZEA infection.

Keywords: selenium; mycotoxin stress; crops; foliar treatment; cellular changes; enzyme activities

INTRODUCTION

Selenium (Se) supplementation of plants is currently being investigated, not only to possibly increase its accumulation in plant cells – it is a very important element in the diets of humans and animals – but also as potential protection against environmental stresses.[1] Se was used in previous studies to diminish cell damage caused by heavy metals,[2] UV radiation,[3] low and high temperatures,[4] drought,[5] and desiccation.[6] Its protective properties against mycotoxin stress were also recently verified.[7,8]

The mechanism of Se ions in cell protection is not fully explained, but their influence on the activation of antioxidative enzymes has been observed[1,9] suggesting that Se may control the intensity of oxidative stress. Se-induced activity changes in antioxidative enzymes, particularly superoxide dismutases (SOD), were studied under stressful conditions[10] because these enzymes are responsible for deactivation of one of the most reactive oxygen species (ROS), O^{-}, into H_2O_2.[11] Other enzymes such as catalases (CAT) and/or peroxidases (POX), which can degrade excess hydrogen peroxide to water[12] were also analysed in the context of Se-protection.[13] Filek *et al.*[8] showed that Se-application (at 10 μmol L^{-1}) to wheat grains infected with zearalenone (ZEA), the toxin produced by *Fusarium* fungi, changed the activities of antioxidative enzymes to the levels observed under non-stressful conditions. These Se-induced reversible changes were more marked in tolerant than in sensitive plants. The participation of Se ions in protection against oxidative stress caused by mycotoxin was therefore confirmed. Moreover, model studies suggest that the mechanism of Se protection involves ZEA-Se interactions that depend on genotype-specific structural differences in membranes.[14] Infection of plants by ZEA is an important agronomical problem because *Fusarium* fungi can be adsorbed on various plant organs (grains, roots, leaves).[15–17] When humidity is high and temperatures relatively low, as in spring in temperate climate zones, the leaves of young seedlings are especially exposed to the action of fungi.

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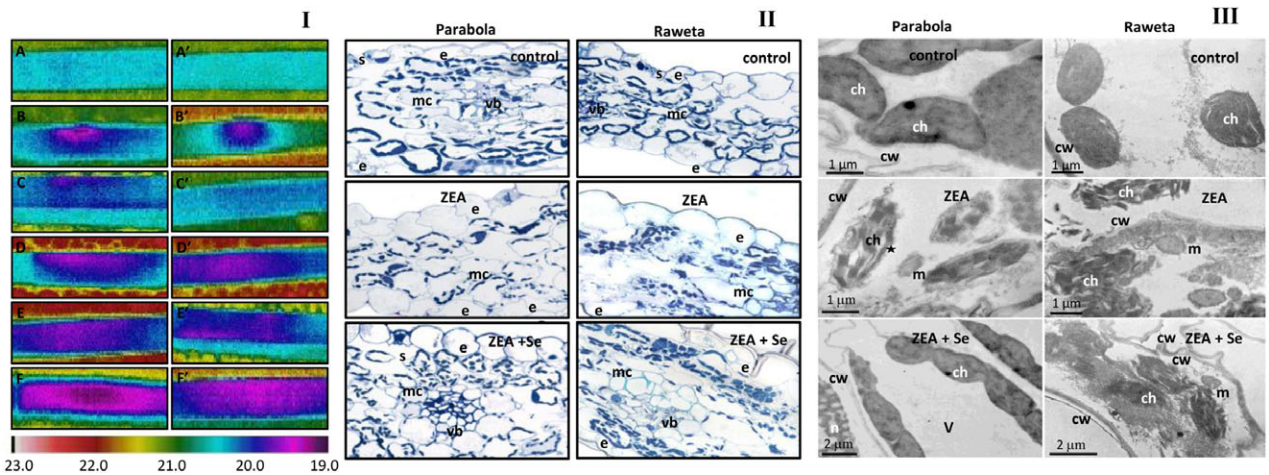

Figure 1. I) Thermographs visualizing the decrease in temperature at sites where zearalenone (ZEA) (A–F) or a ZEA + Se (Na_2SeO_4) mixture (A′–F′) was applied to the surface of wheat (A:Parabola; B: Raweta), oat (C: Bingo; D: Siwek), or barley (E: CAM/B1; F: Maresi) leaves. II) Micrographs of sections of wheat leaves: mc: mesophyll cell; vb: vascular bundle; e: epidermis; s: stroma. III). Ultrastructure of chloroplasts: cw: cell wall; v: vacuole; n: nucleus; m: mitochondrion).

The aim of the present experiments was to determine whether the application of Se ions together with ZEA on the surface of leaves diminishes the penetration of this toxin into cells. Foliar application of Se is considered a better and more efficient method of Se incorporation than application of Se fertilizers to soil because of losses in the latter case due to Se accumulation in the root system.[18] Se incorporation *via* cuticle and mesophyll cells into the internal tissue structure is genotype-dependent, and therefore the experiments were performed on wheat, oat, and barley as the most common crops in agronomy. Measurement of temperature changes at the site of administration accompanied by microscopic examination should indicate the changes caused by mycotoxin treatment.[19] To check the intensity of ROS generation under stress conditions, the activation of antioxidative enzymes was analysed. If spraying Se onto the leaves can be shown to protect against ZEA, this may be a relatively easy way to ameliorate the effects of mycotoxin infection on crop plants.

MATERIAL AND METHODS

Two spring wheat genotypes with different stress tolerances (tolerant: cv. Parabola; sensitive: cv. Raweta) were chosen on the basis of earlier experiments.[20] Oat genotypes (tolerant: cv. Bingo; sensitive: cv. Siwek) were selected based on the observations of Łabanowska *et al.*,[21] and barley (tolerant: cv. CAM/B1; sensitive: cv. Maresi) as described by Filek *et al.*[22] All genotypes were cultured under the light and temperature conditions described by Grzesiak *et al*[20] until the appearance of the 4th leaf. All experiments were performed on the second well-developed leaf.

Aqueous solutions of ZEA alone (10 µmol L^{-1}) or mixed with Se (Na_2SeO_4, 5 µmol L^{-1}) were placed on leaves as 3 µL drops (five drops on each leaf). Three h after treatment, by which time the droplets of the applied solutions had dried, infrared thermography measurements were performed on intact leaves. For microscopic analyses, treated leaf fragments were fixed and samples for subsequent biochemical measurements immediately frozen with liquid N_2 and stored at −80 °C. Leaves treated with deionized water were used as controls.

Enzymes (superoxide dismutases, SOD; catalases, CAT; and peroxidases, POX) were analysed as described in detail by Grzesiak *et al.*[20] All enzymes were examined spectrophotometrically (UV-Vis Evolution 220**,** Thermo Scientific, Waltham, USA) using KINLAB software to determine the reaction kinetics. The amount of protein was determined using BSA as a standard. Measurements for each enzyme were replicated eight times in three independent experiments.

For microscopic observation, wheat leaves fixed in Carnoy's solution (100% ethanol:glacial acetic acid; 3:1 v/v), were saturated with LR GOLD resin (Fluka LR Gold embedding kit for microscopy Germany). Documentation of the results from leaf sections was performed using a BX50 microscope (Olympus Tokyo, Japan) with NIS Elements AR 3.00 NIKON software.[22]

Leaf temperature profiles were visualized with an FLIR E50 infrared camera (Wilsonville, USA) with a spectral range of 3.5–5.0 m and a sensitivity of 0.07 °C, and presented in the form of pseudocolour infrared images. From the 20–30 photographs taken, representative samples were selected for presentation of the observed effects.

The presented results are the mean values ± standard errors. The data from the biochemical experiments were statistically analysed with Duncan's multiple range test using the SAS ANOVA procedure (PC SAS 8.0). A probability of $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

ZEA application to the surface of leaves resulted in visible temperature changes at the location of the toxin (Fig. 1). The effects of ZEA were more evident in sensitive varieties of the cereals studied, with the smallest changes being observed in the tolerant wheat genotype, Parabola. The lowering of the leaf temperature at the site of toxin action was similar to that previously described by Kuźniak *et al.*[19] for plants infected by *Botytis cinera*. Differences in the infrared images of temperature changes in the leaves of the various genotypes studied were caused by differences in intensity of transpiration/evaporation and by structural variations in mesophyll cells that affect the incorporation of aqueous toxin solution. The specific ultrastructure of mesophyll cells of oat, barley, and wheat has been described previously,[23–25] and the observed structural differences shown to be associated with variations in

Table 1. Activities of antioxidative enzymes (SOD: superoxide dismutase; CAT: catalase; and POX: peroxidases) of wheat, oat and barley leaves after foliar treatment with zearalenone (ZEA) or a mixture of ZEA and Se (Na_2SeO_4)

	Antioxidative enzymes									
	SOD [U mg^{-1} protein]			CAT [U mg^{-1} protein × 10^{-3}]			POX [U mg^{-1} protein]			
Object	Control	ZEA	ZEA + Se	Control	ZEA	ZEA + Se	Control	ZEA	ZEA + Se	
Wheat										
Parabola	0.094 ± 0.004^{a}	0.097 ± 0.003^{a}	0.090 ± 0.004^{a}	3.102 ± 0.005^{b}	3.502 ± 0.004^{a}	3.097 ± 0.003^{b}	0.0338 ± 0.0006^{a}	0.0224 ± 0.0005^{c}	0.0249 ± 0.0007^{b}	
Raweta	0.078 ± 0.003^{a}	0.053 ± 0.002^{c}	0.060 ± 0.003^{b}	2.940 ± 0.004^{a}	1.642 ± 0.003^{c}	2.715 ± 0.004^{b}	0.0320 ± 0.0006^{a}	0.0163 ± 0.0005^{c}	0.0196 ± 0.0006^{b}	
Oat										
Bingo	0.079 ± 0.005^{a}	0.066 ± 0.004^{b}	0.084 ± 0.007^{a}	3.661 ± 0.003^{a}	2.433 ± 0.002^{c}	3.054 ± 0.003^{b}	0.0326 ± 0.0008^{a}	0.0207 ± 0.0006^{c}	0.0235 ± 0.0006^{b}	
Siwek	0.068 ± 0.004^{a}	0.046 ± 0.003^{c}	0.057 ± 0.003^{b}	2.702 ± 0.002^{a}	1.155 ± 0.002^{c}	2.246 ± 0.003^{b}	0.0298 ± 0.0007^{a}	0.0172 ± 0.0005^{b}	0.0181 ± 0.0006^{b}	
Barley										
CAM/B1	0.085 ± 0.004^{a}	0.072 ± 0.002^{b}	0.078 ± 0.003^{a}	2.988 ± 0.003^{a}	2.830 ± 0.002^{c}	2.951 ± 0.002^{b}	0.0279 ± 0.0005^{a}	0.0244 ± 0.0004^{c}	0.0255 ± 0.0005^{b}	
Maresi	0.073 ± 0.002^{a}	0.053 ± 0.003^{c}	0.062 ± 0.003^{b}	2.772 ± 0.003^{a}	2.525 ± 0.002^{c}	2.632 ± 0.003^{b}	0.0248 ± 0.0005^{a}	0.0181 ± 0.0004^{c}	0.0199 ± 0.0004^{b}	

Data represent the mean from three independent experiments (eight repetition for each independent experiment) ± standard error (SE). Different letters indicate significant differences between treatments; $P \leq 0.05$.

membrane permeability.[26] In additional experiments, electrolyte leakage was measured for the intact leaf surfaces of control plants using a conductometer (Elmetron, Poland), and the results related to leaf area. The leaf surface conductivity values obtained were 0.15 ± 0.02, 0.20 ± 0.03, and 0.41 ± 0.05 μS cm^{-1} for Parabola, Bingo, and CAM, respectively, and 0.24 ± 0.03, 0.31 ± 0.05, and 0.52 ± 0.03 μS cm^{-1} for Raweta, Siwek, and Maresi, respectively. The greater temperature changes observed in the ZEA-treated areas of sensitive varieties compared with tolerant plants are therefore presumably related to the greater penetration of the toxin into the cells at the application site. Representative photomicrographs showing the differences between ZEA action on tolerant and sensitive wheat plant cells are presented in Fig. 1 (part II). For the Parabola variety, no significant changes in leaf architecture were found compared with the control: numerous chloroplasts were still present in oval mesophyll cells; only in subepidermal cells were chloroplasts smaller. In the Raweta variety, cells were 'flattened', indicating loss of turgor. In the vacuole-deprived cytoplasm, irregularly structured chloroplasts were visible with their interiors clearly divided. The greater changes in chloroplast structure observed after ZEA treatment in a susceptible variety were confirmed by electron microscopic analysis (Fig. 1, part III). These observations indicate that ZEA adsorbed on the leaf surface may penetrate deeper cells and bring about more significant changes in sensitive varieties than in tolerant plants. The effect of ZEA on tolerant plant was (i) loss of turgor in epidermal cells, resulting in wilting, and (ii) changes to plastid structure. Wilting of leaves was caused by the outflow of vacuole water, while changes in plastid structure included 'condensation' of thylakoid membranes (visible as a division into light and dark coloured parts) and their lamellae, and chloroplast degeneration. Similar microscopic changes have been reported in barley (CAM and Maresi) plants after water stress.[22]

Selenium supplementation resulted in significant decreases both in the surface (thermography) and cellular (microscopy) changes caused by ZEA in all studied genotypes, with the greatest effect in tolerant plants (Fig. 1, parts I, II, and III). Moreover, measurements of total chlorophyll content[27] in the leaves of the tolerant genotypes indicated that Se applied together with ZEA increased the level of these pigments by about 10–12% compared with treatment with ZEA alone. (ZEA alone decreased chlorophyll concentrations by about 10%, 17%, and 25% *versus* control for Parabola, Bingo, and CAM/B1, respectively). For the sensitive genotypes, applying Se along with ZEA increased chlorophyll accumulation by about 5–7% compared with ZEA alone. (ZEA alone reduced chlorophyll by 25%, 30%, and 38% *versus* control for Raweta, Siwek, and Maresi, respectively.) Protection by Se was accompanied by activation of the antioxidative enzymes in the leaves whose activities were partially blocked by ZEA treatment (Table 1). This is in agreement with the general observation that the genetically determined higher expression of antioxidant enzymes in tolerant plants is more effective in reducing the concentration of reactive radicals than the more limited expression of these enzymes in sensitive genotypes.[20,28] For the cereal plants in our study under non-stressed conditions, all analysed enzymes in the tolerant genotypes (Parabola, Bingo, and CAM/B1) had higher activities than those in the sensitive genotypes (Raweta, Siwek, and Maresi). ZEA-infection decreased enzyme activities, with this effect being stronger in the sensitive genotypes. The decline in activity of antioxidant enzymes under stress conditions is considered to be a result of inhibition of the genes directing their synthesis and/or damage to the enzyme proteins by ROS.[29]

This could explain the significant decrease in enzyme activity in the sensitive genotypes in response to ZEA. Se application seems to reduce the negative effects of ZEA, increasing enzyme activity, especially that of CAT, the enzyme responsible for H_2O_2 deactivation. Se-mediated protection against ROS generation in ZEA-stressed plants therefore seems to operate *via* increasing the activities of the SOD and CAT enzymes throughout the treatment. The reduction of O^- and H_2O_2 levels by Se addition lessens the effects of the destructive chain reaction caused by ROS, which diminishes damage to the membranes of plant organelles.[2] This could explain the reduced disruption to cell structures, especially the chloroplasts (visible in microscopic observation), in ZEA-treated plants when Se is present.

From the results presented here, it can be concluded that ZEA applied to the leaf surface initiates changes in water relations in cells that are associated with the generation of ROS. Plant genotypes conferring tolerance to oxidative stressors also promote resistance to ZEA stress. Se-induced activation of antioxidants is involved in protection against cell damage caused by ZEA. The foliar application of Se ions may be a non-invasive method for protecting plant cells against penetration of ZEA adsorbed on the leaf surface.

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