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Research Note

Quantification of fine root responses to selenium toxicity

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The morphological changes of lettuce and ryegrass roots in response to Se toxicity were quantified by image analysis. Based on the assumption that soil stresses increase endogenous ethylene production in plants, changes indicating higher construction costs for roots, such as decreased specific root length, were expected. As lettuce roots in soil without Se addition (control) became older, their width increased whereas their specific length, specific surface area and specific volume decreased. In younger lettuce, large Se additions induced changes resembling those identified in the control plant upon senescence. In older plants, Se fertilisation reversed these changes or made them smaller. Ryegrass roots were not as sensitive to added Se as lettuce roots; a lower dosage stimulated root growth but a higher one reduced it.

Key words: scanning, image analysis, lettuces, morphology, ryegrass, selenium, toxicity

Introduction

The background of this study is the low bioavailability of selenium (Se) in Finnish soils (Yläranta 1985) and the average daily Se intake that used to be below the recommended safe and adequate level (Ekholm et al. 1995). For this reason, multinutrient fertilizers have been supplemented with sodium selenate in Finland since 1984 to increase the Se content of agricultural products and to improve their nutritional value. Even if Se is an essential element for humans and animals, it is toxic at high intake levels. Although recent studies have shown that at low concentrations Se exerts beneficial effects also on plants (Hartikainen and Xue 1999, Xue and Hartikainen 2000, Hartikainen et al. 2000), agricultural plants are generally considered not to require Se and to have a low tolerance to it.

The toxicity mechanisms of excess Se have been discussed extensively in the literature (for

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references see e.g. Läuchli 1993), but to our knowledge no data on the effects of Se on root morphology have been published. However, it is known that the sulphur-containing amino acid methionine is a natural precursor of ethylene in plants (see Morgan and Drew 1997), and that the formation of selenoamino acids through replacement of sulphur by Se enhances ethylene production (Konze et al. 1978). Thus, it would be reasonable to expect the effects of Se toxicity on root morphology to be similar to those of other stresses with ethylene-mediated responses, such as increased root width and decreased specific root length.

Increased root width leads to inefficient use of photosynthates, as root systems with numerous fine roots containing only a few layers of cells produce more root surface area with less photosynthesised carbon than do larger roots (Eissenstat 1992). An altered root surface area in a soil volume may affect nutrient uptake by plants, e.g. for carrots (Pietola and Smucker 1998, Pietola and Salo 2000). Low values of specific root length (i.e. root length per dry weight) may also decrease water and nutrient uptake. The efficiency of roots however, depends also on additional factors such as the length density (i.e. root length per soil volume) and branching patterns of roots as well as the diffusion and adsorption properties of water and ions in the soil (Fitter 1996, de Willigen et al. 2000). Thus, the Se-induced changes in root morphology can be expected to affect the shoot yields.

Image analysis of washed roots permits measurement of their lengths and diameters which is essential for the evaluation of root morphological efficiency (Smucker 1993). Our aim was to apply image analysis to quantify the effects of Se toxicity on root widths, specific lengths, specific surface areas and the specific volumes of lettuce and ryegrass with a fibrous fine root (∅ < 1 mm) system. Quantitative data are needed to get detailed information on toxicity symptoms seen in root morphology. The results are also discussed in terms of root aging and plant adaptation to Se toxicity.

Material and methods

The material was collected from two greenhouse experiments where Se toxicity was studied with lettuce (*Lactuca sativa* L. cv. Australischer gelber) (unpublished) and ryegrass (*Lolium perenne* L. cv. Prego) (Hartikainen et al. 2000). The plants were cultivated in a sand soil (4 dm^3) without or with Se added at 0.1 or 1.0 mg kg^{-1}. As the range of total Se in Finnish mineral soils is 0.04–0.7 mg kg^{-1} (Yläranta 1985), fertilization markedly increased the Se reserves in soil. Se was added as H_2SeO_4 to avoid any possible effect of nutrient cations. Lettuce was cultivated in two sets of pots, one of which was harvested 48 and the other one 96 days after sowing. Ryegrass was grown in one pot set and harvested 96 days after sowing.

Lettuce and ryegrass roots were sampled from all the soil volume in each pot. After separating the mineral soil from the roots by hydropneumatic elutriation (Smucker et al. 1982), and removing the remaining organic debris and the lettuce roots over 1 mm in diameter, the roots were stored in 15% ethanol solution for later image analysis. Only subsamples (5–15%) of lettuce and ryegrass root samples were cleaned as they contained a lot of debris. Some roots of lettuce and ryegrass were partly stored in water, but these roots were used only to measure the total dry mass of roots.

The roots were dyed with malachite green oxalate for 2 days before root scanning by injecting 3 ml of 1% dye into the plastic storage container containing 15% ethanol. Stained roots were rinsed with water on a fine nylon screen. The roots were then placed uniformly on a clear glass tray (18 × 28 cm), covered with 3 mm of water. Samples which had lot of roots were split into subsamples to avoid overlapping on the tray. The tray was placed on a flatbed scanner (HP Scan Jet 4c, equipped with a transparency adapter), and roots were then scanned, creating high resolution (600 dpi, brightness 30) images of the sampled roots (Pietola 1998, Simojoki 2000). Digital output from the scanner was stored by the microcomputer in the form of a TIFF file. The dry weights of roots were recorded after scanning and drying (at 70°C for 48 h). The scanned images of roots were analysed by the ROOTEDGE image analysis program (Ewing and Kaspar 1995), which measures the lengths and widths of roots in binary images. Surface area (A) was calculated based on the cylindrical nature of roots and the formula $A = 2 \pi r L$, where r is root radius and L root length. The root volume (V) was calculated as $V = \pi r^2 L$.

The specific lengths, specific surface areas and specific volumes of the roots were calculated by summing the lengths, areas and volumes, respectively, of all objects of the sample and dividing by the dry mass of the scanned roots. The average root width was calculated by dividing the total projection area of the roots by their total length.

Lettuce and ryegrass roots were sampled only from one replicate pot. The splits of scanned root samples (n = 6–22) were used for calculating the standard deviation.

Results and discussion

The addition of Se at 1.0 mg kg^{-1} to the soil was toxic to lettuce, as shown by the decreased dry mass of shoots and fine roots in both harvests (Table 1). In fact, root dry mass was decreased already by the lower dose of Se (0.1 mg kg^{-1}), although shoot growth was not much affected. The toxicity was also indicated by an increase in thick roots (∅ > 1 mm) originating from the hypocotyl (data not shown). In the Se-treated lettuce the dry weight of these thick roots at the latter harvest was doubled when Se addition increased from 0.1 to 1.0 mg kg^{-1}. Ryegrass was not as sensitive to Se toxicity as lettuce: the root dry mass of ryegrass was decreased by the higher Se concentration, but shoot growth was somewhat increased. In general, Se toxicity tended to decrease the root-to-shoot ratio.

The restricted root growth caused by Se toxicity led to a variation in lettuce fine root morphology (Tables 1 and 2). Se fertilisation decreased the specific length of lettuce roots in the young lettuce (48 d), and the width and specific volume of roots were increased by the lower Se dose (Table 2). Such responses can be induced by large concentrations of ethylene, the production of which can be expected to increase due to Se-evoked enhancement due to the synthesis of its precursor (see Konze et al. 1978). However, in senesced lettuce (96 d old) the effects of Se fertilisation were nearly reversed. This can be interpreted in terms of root aging. The changes in the control treatment (no added Se) reveal that as the roots became older, their width increased and specific length decreased (Table 2). In the young plants the Se-induced changes in root morphology resembled those induced by root aging in the control plants. Upon senescence, Se fertilisation made these changes smaller or reversed them. Thus, added Se promoted senescence in the younger lettuce plants but prevented it in the older plants. The responses in specific root surface area and specific root volume were less clear but resembled those in specific root length.

In the control treatment, the root length per plant decreased from 56 m to 47 m upon aging, but increased from 27 m to 31 m at the lower Se addition and from 7 m to 16 m at the higher Se level (data in Tables 1 and 2). Nevertheless, it is obvious that the size of the root system was crucially decreased by the higher Se fertilisation. Futhermore, the results suggest that the Se toxicity found in the younger lettuce plants was later partly alleviated by the more efficient use of photosynthates in constructing the root system. They also agree with the recent finding that Se is able to delay plant senescense by counteracting the oxidative stress involved (Xue, Hartikainen and Piironen, unpublished).

The morphology of ryegrass roots was not much affected by Se fertilisation (Tables 1 and 2). Added Se tended to induce a small increase in the specific root length. At the highest

Table 1. Number of seedlings, total dry mass and width of lettuce and ryegrass roots[A] at various Se-addition levels.

Se added mg kg^{-1} soil	Seedlings no.	Shoot dry mass g	Root dry mass g	Width µm	(s)	n
Lettuce 48 d old						
0	17	22.2	2.34	386	(23)	10
0.1	12	17.6	0.97	479	(36)	6
1.0	12	3.4	0.28	384	–	1
Lettuce 96 d old						
0	11	50.6	3.02	448	(25)	17
0.1	16	59.6	2.02	424	(25)	7
1.0	12	30.3	0.53	335	(20)	8
Ryegrass 96 d old						
0	509	21.4	8.25	302	(28)	22
0.1	450	22.3	13.0	301	(19)	13
1.0	474	26.9	1.19	278	(17)	18

[A] Fine roots (∅ < 1 mm), s = standard deviation of root width, n = number of scanned subsamples

Table 2. Specific length, surface area and volume of lettuce and ryegrass roots[A] at various Se-addition levels.

Se added	Specific length		Specific surface area		Specific volume		n
mg kg^{-1} soil	m g^{-1}	(s)	cm^2 g^{-1}	(s)	cm^3 g^{-1}	(s)	
Lettuce 48 d old							
0	404	(101)	4850	(980)	54.0	(5.3)	10
0.1	336	(68)	5040	(990)	66.8	(15.1)	6
1.0	306	–	3690	–	44.3	–	1
Lettuce 96 d old							
0	157	(29)	2190	(320)	27.0	(3.3)	17
0.1	246	(39)	3290	(660)	40.4	(10.6)	9
1.0	355	(38)	3720	(360)	33.5	(3.9)	8
Ryegrass 96 d old							
0	212	(51)	1990	(410)	16.0	(3.4)	22
0.1	225	(32)	2120	(310)	17.0	(2.8)	13
1.0	233	(35)	2030	(230)	15.0	(1.3)	18

[A] Fine roots (∅ < 1 mm), s = standard deviation, n = number of scanned subsamples

Se level the roots tended to be thinner than in the other treatments. Specific root area of ryegrass remained around 2000 cm^2 g^{-1} for all treatments. Se dose of 1.0 mg kg^{-1} crucially decreased the total length, surface area, and volume of roots, whereas the Se dose of 0.1 mg kg^{-1} clearly stimulated root growth: the total lengths per seedling in the Se treatments of 0, 0.1, and 1.0 mg kg^{-1} were 3.4, 6.5, and 0.6 m, respectively.

Conclusions

Image analysis of lettuce and ryegrass roots provided evidence that a high Se fertilisation can adversely affect the efficiency of root systems for water and nutrient uptake through morphological changes. The effects of Se fertilisation depend however on plant species, and they change with time. Se fertilisation enhanced senescence in lettuce plants younger than 48 days, but prevented it in older plants. Some root morphological changes in the younger plants could be explained by the assumption that Se fertilisation increase ethylene production in roots. In the older plants, however, the response seemed rather to be related to the antioxidative properties of Se than to ethylene. The root system of ryegrass was less sensitive to Se. The addition of 0.1 mg kg^{-1} to the soil stimulated the root growth of ryegrass, but not that of lettuce.

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SELOSTUS

Seleenin myrkytysoireet juurissa

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Juurten kasvua voivat rajoittaa maan fysikaaliset tekijät, kuten vedenpuute ja suuri mekaaninen vastus. Myös kemialliset tekijät, kuten eräiden mikroravinteiden korkeat pitoisuudet, voivat heikentää juurten kasvuedellytyksiä. Seleeni (Se) on ihmiselle ja eläimille välttämätön alkuaine, joka on kuitenkin myrkyllinen jo verraten pieninä pitoisuuksina. Suomessa moniravinteisiin lannoitteisiin on vuodesta 1984 lähtien lisätty seleeniä rehun ja ruoan ravitsemuksellisen arvon parantamiseksi. Viljelykasvien ei ole katsottu välttämättä tarvitsevan seleeniä, mutta myös kasvien on vastikään havaittu hyötyvän pienestä seleenilisästä. Suuret lisäykset ovat selvästi haitallisia ja rajoittavat kasvin kasvua.

Seleenin myrkytysoireiden ilmenemistä juurissa tutkittiin astiakokeessa, jossa salaattia ja raiheinää kasvatettiin melko suurilla, nousevilla seleenimäärillä (0, 0,1 ja 1,0 mg kg^{-1}) lannoitetussa hiekkamaassa. Vertailusalaatin (Se ei lisätty) juuret tulivat vanhetessaan paksummiksi, kun taas niiden ominaispituus, -pinta-ala ja -tilavuus pienenivät. Suuri seleenilisäys aiheutti nuoren salaatin juurissa samanlaisia muutoksia kuin vanheneminen vertailusalaatissa. Kasvin vanhetessa seleenilannoituksen vaikutukset pienenivät tai muuttuivat vastakkaisiksi. Raiheinän juuret eivät reagoineet seleenin lisäykseen yhtä herkästi kuin salaatin juuret, mutta suurin seleenilisäys vähensi juurten kuivamassaa.