

These of Ph.D. dissertation

The role of nitro-oxidative stress in selenium toxicity in different plant species

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Introduction

In the recent decades anthropogenic activities, like industry, agriculture or transportation increased the exposure of the environment to heavy metals and other elements. The contamination of surface waters and soil has become an environmental problem, which can significantly influence living organisms. Selenium belongs to the group of rare elements; however, anthropogenic activities can increase the environmental selenium content rapidly, leading to harmful effects in living organisms.

Selenium is an essential micronutrient for all organisms, with the exception of higher plants and some bacteria. It is found in all environmental phases and despite the incapability to utilize selenium as an essential micronutrient, land plants take up this element through phosphate and sulphate transporters. Selenium is incorporated into organic molecules with the enzymes of the sulfur metabolism. In small amounts selenium exerts beneficial effects on plant growth and development, has stress alleviating effects and inhibits plant senescence. Excess selenium leads to toxic effects, which include the formation of non-specific selenoproteins, the disturbance of hormonal homeostasis and carbon assimilation, it inhibits the uptake of several other nutrients. Recently selenium toxicity has been associated also with nitro-oxidative stress.

Nitro-oxidative stress includes the effects of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Most biotic and abiotic stresses disturb the metabolism of these molecules, leading to a modified homeostasis. These secondary stress processes exert their effects through macromolecule modifications, such as ROS-induced lipid peroxidation and RNS-triggered macromolecule nitration and *S*-nitrosation. Protein tyrosine nitration is considered as a biomarker of nitro-oxidative stress. The effect of this posttranslational protein modification is mostly protein inactivation, leading to an altered active protein pool. Previous studies have detected protein tyrosine nitration in response to selenium treatment; however, the exact relation between these processes and the metabolism of RNS molecules under selenium toxicity is still unclear.

Aims of this study

Previous research data suggested a connection between selenium toxicity and nitro-oxidative signaling; however, at the beginning of my PhD studies the background processes of the association of selenium and nitro-oxidative stress were unknown. In the present study, we evaluated the effect of excess selenium in three different experimental systems. We compared the toxicity of different selenium species on various selenium sensitive and tolerant plants. The experiments were carried out on the following plant species: selenium sensitive *Arabidopsis* (*Arabidopsis thaliana*), secondary selenium accumulator Indian mustard (*Brassica juncea*), selenium-sensitive *Astragalus membranaceus*, which is used in medicine and selenium hyperaccumulator *Astragalus bisulcatus*.

During this work we intended to answer the following questions:

1. What is the selenium uptake capacity of different plant species, how does selenium translocate between different organs and is significant selenium accumulation detectable?
2. How plant growth and development is affected by different selenium treatments ?
3. How do different plant species tolerate selenium and which tolerance mechanisms are activated?
4. How does the metabolism of ROS and RNS changes in response to selenium treatment and can nitro-oxidative be detectable?
5. Is there a connection between selenium tolerance or toxicity and nitro-oxidative stress?

Materials and methods

Plant growth conditions

Our experiments involved three separate plant growth systems, each presenting a different viewpoint on this topic.

In the first experimental system, Indian mustard (*Brassica juncea* L. Czern. cv. Negro Caballo) was examined. Plants were grown on aerated Hoagland solution containing 0 (control), 20, 50 or 100 μM sodium selenite (Na_2SeO_3) or sodium selenate (Na_2SeO_4).

The second experimental system compared the heavy metal tolerant Indian mustard (*Brassica juncea* L. Czern, cv. Negro Caballo) and the model plant *Arabidopsis* (*Arabidopsis thaliana* L. Heynh, Columbia-0). Plant growth was similar to the previous system; however, the treatment was carried out only with 0 (control) 20, 50 or 100 μM Na_2SeO_3 .

In the third experimental design, selenium sensitive *Astragalus membranaceus* L. Fisch, Bunge and hyperaccumulator *Astragalus bisulcatus* L. Hook A. Gray was examined. Plants were grown in sterile conditions on half strength Murashige-Skoog media, containing 0,8% agar. Treatment selenium was applied through the media in the form of 0 (control), 50 vagy 100 μM Na_2SeO_4 .

Conditions in the greenhouse were the following: 150 $\mu\text{mol m}^{-2}/\text{s}$ photon flux density with 12 h/12 h light/dark cycle, relative humidity 55–60% and temperature 25 ± 2 °C.

Analysis of germination, plant growth and stomatal parameters:

To evaluate the growth and development we measured the fresh and dry weight of plant organs, lateral root number and root length. In the case of *Astragalus* species, germination and root tissue thickness was examined. The number of stomata and the stomatal opening was measured on microscopic samples prepared from leaf epidermis.

Element content analyses:

The content of selenium and other micronutrients were measured with ICP-MS (Agilent 7700 Series, Santa Clara, USA or Thermo Scientific XSeries II, Asheville, USA).

Microscopic methods:

For every method at least ten root tips (~0.5 cm long) were stained. Immunohistochemistry and Auramine O staining was carried out on 100 µm thick root sections, fixed in 4% paraformaldehyde prepared with the help of a vibratome. Microscopic analysis was carried out under a Zeiss Axiowert 200M inverted microscope (Carl Zeiss, Jena, Germany) with a digital camera (AxioCam HR, HQ CCD, Carl Zeiss, Jena, Germany). Pixel intensity was measured with Axiovision Rel. 4.8 software in circles with 100 µm radii. The applied fluorescent and non-fluorescent labellings are summarized in the following table:

Molecule	Stain	Buffer	Microscopic filter
Pectin	Ruthenium red	Distilled water	visible
Cell wall peroxidases	Pyrogallol	10 mM phosphate buffer	visible
Lipid peroxidation	Schiff reagent	K ₂ S ₂ O ₅	visible
Viability	FDA	10/50 mM MES/KCl	Zeiss Filter 10
Callose	Aniline-blue	Distilled water	Zeiss Filter 49
Lignin and szuberin	Auramin O	10 mM Tris-HCl	Zeiss Filter 9
NO	DAF-FM DA	10 mM Tris-HCl	Zeiss Filter 10
ONOO ⁻	DHR 123	10 mM Tris-HCl	Zeiss Filter 10
O ₂ ⁻	DHE	10 mM Tris-HCl	Zeiss Filter 9
H ₂ O ₂	Amplex Red	50 mM Na-phosphate buffer	Zeiss Filter 20
Glutathione	MBB	Distilled water	Zeiss Filter 49
GSNO	Antibody	TBSA-BSAT	Zeiss Filter 10
3-nitrotyrosine	Antibody	TBSA-BSAT	Zeiss Filter 10

Western blot and native gel electrophoresis methods:

Plant biomass was homogenized in double volume extraction buffer, the supernatant was treated with protease inhibitor cocktail and used in further experiments.

To detect protein tyrosine nitration, plant extracts were separated on 12% SDS polyacrylamide gels, and transferred overnight on PVDF membranes. Membranes were blocked in 5 % milk protein solution and labeled with 3-nitrotyrosine antibody (produced in rabbit, 1:2000). To develop the membranes secondary antibody is added (conjugated with alkaline phosphatase, produced in goat, 1:10000) and the labeled proteins were visualized in buffer containing NBT/BCIP.

To evaluate the activity of NADPH oxidase, plant extract was separated in native gel electrophoresis in 10% acrylamide gels. The gel was transferred to a buffer containing NBT and NADPH, in which NADPH oxidase enzyme activity formed purple discoloration.

Activity and isoforms of SOD enzyme were examined after similar native gel electrophoresis. Gels were transferred first into a NBT solution, after that a riboflavin and TEMED containing buffer followed. Visualization of SOD activity was due to the fact, that it will prevent the purple discoloration of treated gels in light.

To examine GSNOR enzyme activity, gels were treated with NADH and GSNO, and the reaction resulted in the loss of NADH fluorescence under UV light.

Spectrophotometric analysis:

To measure the amount of anthocyanin in leaves, plant material was homogenized and pigments were extracted with acetone. The solution was measured on 534 nm, 634 and 661 nm. From these data the total anthocyanin content was calculated.

Total SOD activity was measured due to the fact, that it will inhibit the photochemical reduction of NBT in light.

Statistical analysis:

To analyze the data, Microsoft Excel 2010, Systat Sigmaplot 12 and Statistica 9 software was used.

Summary of the results

The research data can be summarized as follows:

- I. Selenium is a non-essential micronutrient for plants; however, as the effect of the treatments **all plant species were capable to accumulate** it. Selenate had much larger translocation rate, than selenite, most likely due to its slower metabolism and incorporation in seleno-amino acids in roots. The accumulated selenium disturbed the homeostasis of micronutrients, notably iron, zinc, manganese, boron and molybdenum in sensitive *Astragalus* plants.
- II. The accumulated **selenium changed the growth and biomass of plants**. Small amounts of selenium could be beneficial, and in tolerant plant species, like Indian mustard and *Astragalus bisulcatus* it had beneficial effects on growth. Compared to this, selenium sensitive plants (*Arabidopsis thaliana* and *Astragalus membranaceus*) showed diminished growth and biomass, accompanied by the significant decrease in cell viability and tolerance index. Tolerant plant species suffered slight growth reduction in response to high concentrations of selenium. These plants showed milder reduction in meristem cell viability compared to other species. It is notable, that despite the large amount of accumulated selenium in the shoot, no visible symptoms like necrosis or chlorosis appeared on leaves.
- III. Plant tolerance and detoxification mechanisms include alterations in cell wall structure and composition. **Sensitive plant species synthesized callose** in response to selenium stress, which was not observable in tolerant species. The latter species **altered the amount of lignin and pectin in the cell walls**, probably effectively alleviating the effects of stress. Selenium-treated *Brassica juncea* leaves contained increased number of opened stomata suggesting Se detoxification *via* volatilization.
- IV. As other abiotic stresses, also selenium can disturb the natural homeostasis of reactive oxygen species (ROS), resulting in oxidative signal transduction and oxidative macromolecule damage. **Treatments altered the levels of $O_2^{\cdot-}$ and H_2O_2** in all plant species, compared to control. These changes were more intense in sensitive species, resulting in increased macromolecule damage. Lipid peroxidation was used as a marker to evaluate ROS-induced macromolecule damage, and selenium increased it in a concentration dependent manner. NADPH oxidase is

capable of producing large amounts of $O_2^{\cdot-}$, resulting in an oxidative burst. In *Astragalus membranaceus*, treatments increased the activity of **NADPH oxidase and several new isoenzymes were activated**. Superoxide radical is quenched by SOD. In almost all plant species and experimental systems, **changes in SOD activity were remarkable**, especially with respect of the isoenzymes. Even if the total SOD activity was similar to control, the activity of SOD isoenzymes changed significantly in response to selenium. Cell wall peroxidases were also induced in response to the stress. Glutathione levels were altered in both *Arabidopsis* and *Brassica juncea*.

V. The homeostasis of reactive nitrogen species (RNS) has been less examined in selenium stress compared to the oxidative counterpart. In our study, **NO levels were control-like** in species of *Brassicaceae* family and increased in sensitive *A. membranaceus*. Moreover, *A. bisulcatus* cotyledons showed an increase in NO levels, but in roots no significant differences were detected. **Peroxynitrite production was associated with selenium toxicity** in all three experimental designs. In *Astragalus membranaceus*, a significant concentration-dependent $ONOO^-$ accumulation was observable contributing to selenium toxicity. Selenite treatment significantly increased $ONOO^-$ levels compared to selenate, where no notable differences were detected. S-nitrosoglutathione is a mobile NO storage being capable of nitrosative signalization in plant cells through posttranslational protein modifications. **The levels of GSNO decreased** in both organs of *A. bisulcatus* as the effect of selenium, in contrast **cotyledons of *A. membranaceus* accumulated GSNO**. Decomposition of GSNO is catalyzed by GSNOR enzyme. Its activity decreased in *A. bisulcatus*, in contrast *A. membranaceus* showed slightly increased GSNOR activity in the cotyledons.

VI. Protein tyrosine nitration is widely used as a biomarker of nitro-oxidative stress. To our understanding nitrated proteins are most likely inactivated, resulting in damage to the active protein pool. **Selenite more intensively increased protein tyrosine nitration compared to selenate**. In shoot, newly appeared nitrated protein band was observable in response to selenite treatment. **Both *Arabidopsis thaliana* and *Brassica juncea* showed selenium-triggered increase in protein nitration**, without significant changes to the pattern itself. *Astragalus membranaceus* **suffered intense nitration**, with several newly appeared nitrated protein bands on the membrane, suggesting a significant stress. The hyperaccumulator *A. bisulcatus* managed to decrease the number of nitrated protein bands, most likely *via*

proteosomal degradation of malformed proteins. It is important to note that proteolysis could contribute to selenium tolerance by degradation of nonspecific selenoproteins. **Our results suggest that protein tyrosine nitration and nitro-oxidative stress strongly contribute to selenium toxicity, supporting the importance of nitrosative posttranslational modifications in plant defense reactions.**

Using different experimental designs and multiple examined species I demonstrated the importance of the process during stress and the results provide insight into the highly complex abiotic stress responses as well as the ROS and RNS homeostasis.

These data are new in international literature, and in my opinion those contributed to the better understanding of nitro-oxidative stress processes in plants. However, we should keep in mind that other RNS-dependent macromolecule modifications (e.g. lipid and nucleic acid nitration) may be involved, therefore their investigation is well-founded in the future.

List of publications

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Publications associated with the Ph.D. dissertation

- Árpád Molnár, Gábor Feigl, Vanda Trifán, Attila Ördög, Réka Szöllősi, László Erdei, Zsuzsanna Kolbert (2018) The intensity of tyrosine nitration is associated with selenite and selenate toxicity in *Brassica juncea* L. *Ecotoxicology and Environmental Safety*, 147: 93–101, IF: 4,527
- Árpád Molnár, Zsuzsanna Kolbert, Krisztina Kéri, Gábor Feigl, Attila Ördög, Réka Szöllősi, László Erdei (2018) Selenite-induced nitro-oxidative stress processes in *Arabidopsis thaliana* and *Brassica juncea* *Ecotoxicology and Environmental Safety*, 148: 664–674, IF: 4,527
- Zsuzsanna Kolbert, Árpád Molnár, Réka Szöllősi, Gábor Feigl, László Erdei, Attila Ördög (2018) Nitro-Oxidative Stress Correlates with Se Tolerance of *Astragalus* Species *Plant and Cell Physiology*, pcy099, 59 : 9 pp. 1827-1843, IF: 3.929

Other scientific publications:

- Gábor Feigl, Devanand Kumar, Nóra Lehotai, Nóra Tugyi, **Árpád Molnár**, Attila Ördög, Ágnes Szepesi, Katalin Gémes, Gábor Laskay, László Erdei, Zsuzsanna Kolbert (2013) Physiological and morphological responses of the root system of Indian mustard (*Brassica juncea* L. Czern.) and rapeseed (*Brassica napus* L.) to copper stress. *Ecotoxicology and Environmental Safety*, 94 179–189
- Gábor Feigl, Nóra Lehotai, **Árpád Molnár**, Attila Ördög, Marta Rodríguez-Ruiz, José M. Palma, Francisco J. Corpas László Erdei, Zsuzsanna Kolbert (2015) Zinc induces distinct changes in the metabolism of reactive oxygen and nitrogen species (ROS and RNS) in the roots of two *Brassica* species with different sensitivity to zinc stress, *Annals of Botany*, 116: 613-625
- Gábor Feigl, Devanand Kumar, Nóra Lehotai, **Árpád Molnár**, Éva Rácz, Attila Ördög, László Erdei, Zsuzsanna Kolbert, Gábor Laskay (2015) Comparing the effects of excess copper in the leaves of *Brassica juncea* (L. Czern) and *Brassica napus* (L.) seedlings: growth inhibition, oxidative stress and photosynthetic damage, *Acta Biologica Hungarica*, 66 (2): 205-221
- Gábor Feigl, Zsuzsanna Kolbert, Nóra Lehotai, **Árpád Molnár**, Attila Ördög, Ádám Bordé, Gábor Laskay, László Erdei (2016) Different zinc sensitivity of *Brassica* organs is accompanied by distinct responses in protein nitration level and pattern *Ecotoxicology and Environmental Safety*, 125: 141–152
- Kolbert Zsuzsanna, Lehotai Nóra, **Molnár Árpád**, Feigl Gábor (2016) "The roots" of selenium toxicity: a new concept. *Plant Signaling and Behavior* 11:(10) Paper e1241935.
- Lehotai Nóra, Feigl Gábor, Koós Ágnes, **Molnár Árpád**, Ördög Attila, Petó Andrea, Erdei László, Kolbert Zsuzsanna (2016) Nitric oxide-cytokinin interplay influences selenite sensitivity in *Arabidopsis*. *Plant Cell Reports* 35:(10) pp. 2181-2195
- Zsuzsanna Kolbert, Gábor Feigl, Ádám Bordé, **Árpád Molnár**, László Erdei (2017) Protein tyrosine nitration in plants: Present knowledge, computational prediction and future perspectives. *Plant Physiology and Biochemistry* 113 pp. 56-63
- Gábor Feigl, Ádám Bordé, **Árpád Molnár**, Zsuzsanna Kolbert (2018) Exogenous ascorbic acid is a pro-nitrant in *Arabidopsis thaliana*. *ACTA BIOLOGICA SZEGEDIENSIS* 62 : 2 pp. 115-122.
- Gábor Feigl, Edit Horváth, **Árpád Molnár**, Dóra Oláh, Péter Poór, Zsuzsanna Kolbert (2019) Ethylene-Nitric Oxide Interplay During Selenium-induced Lateral Root Emergence in *Arabidopsis* *Journal of Plant Growth Regulation* 38 pp. 1481-1488

- Kolbert Zsuzsanna, **Molnár Árpád**, Feigl Gábor, van Hoewyk Doug (2019) Plant selenium toxicity: Proteome in the crosshairs
JOURNAL OF PLANT PHYSIOLOGY 232 pp. 291-300.
- Feigl Gábor, **Molnár Árpád**, Szöllősi Réka, Ördög Attila, Töröcsik Kitti, Oláh Dóra, Bodor Attila, Perei Katalin, Kolbert Zsuzsanna (2019) Zinc-induced root architectural changes of rhizotron-grown *B. napus* correlate with a differential nitro-oxidative response
NITRIC OXIDE-BIOLOGY AND CHEMISTRY 90 pp. 55-65.
- Kolbert Zsuzsanna, **Molnár Árpád**, Oláh Dóra, Feigl Gábor, Horváth Edit, Erdei László, Ördög Attila, Rudolf Eva, Barth Teresa, Lindermayr Christian (2019) S-Nitrosothiol Signalling Is Involved In Regulating Hydrogen Peroxide Metabolism Of Zinc-Stressed *Arabidopsis*
PLANT AND CELL PHYSIOLOGY 60: 11 pp. 2449-2463.
- **Molnár Árpád**, Papp Márk, Zoltán Kovács Dávid, Béteky Péter, Oláh Dóra, Feigl Gábor, Szöllősi Réka, Rázga Zsolt, Ördög Attila, Erdei László, Rónavári Andrea, Kónya Zoltán, Kolbert Zsuzsanna (2020) Nitro-oxidative signalling induced by chemically synthesized zinc oxide nanoparticles (ZnO NPs) in *Brassica* species
CHEMOSPHERE 251 Paper: 126419
- Vollár Martin, Feigl Gábor, Oláh Dóra, Horváth Attila, **Molnár Árpád**, Kúsz Norbert, Ördög Attila, Csupor Dezső, Kolbert Zsuzsanna (2020) Nitro-Oleic Acid in Seeds and Differently Developed Seedlings of *Brassica napus* L.
Plants 9:406.

Oral presentations:

1. Gábor Feigl, Devanand Kumar, Andrea Pető, Nóra Lehotai, Attila Ördög, **Árpád Molnár**, Zsuzsanna Kolbert, László Erdei (2012) The effect of zinc on the microelement homeostasis and the metabolism of reactive signal molecules in *Brassica juncea* and *Brassica napus*.

Third Annual Workshop of COST Action FA 0905 – Mineral improved crop production for healthy food and feed, Lisbon, Portugal, 23-26 October 2012

2. Lehotai Nóra, Pető Andrea, Feigl Gábor, Ördög Attila, Tugyi Nóra, **Molnár Árpád**, Lyubenova L, Schröder Peter, Kolbert Zsuzsanna, Erdei László (2013) Selenium induced stress responses in *Arabidopsis thaliana* and *Pisum sativum* plants: the possibilities of biofortification.

Societas Biologiae Plantarum Hungarica, Conference of Young Biologists, Szeged, Hungary. 25 January 2013

3. Ördögné Dr. Kolbert Zsuzsanna, Feigl Gábor, **Molnár Árpád**, Prof Dr. Erdei László (2015) Growth responses induced by microelement excess: the role of reactive nitrogen species.

Joint development of higher education and training programmes in plant biology in support of knowledge-based society opening conference, Szeged, Hungary, 20-21 April 2015

4. **Molnár Árpád**, Feigl Gábor, Bordé Ádám, Erdei László, Kolbert Zsuzsanna (2015),

Különböző *Brassica* szervek cinkérzékenységének és eltérő protein nitrációjának kapcsolata.

A Magyar Szabadgyök-Kutató Társaság VIII. Kongresszusa, Budapest, 2015. november 5-6.

5. Gábor Feigl, Ádám Bordé, **Árpád Molnár**, Zsuzsanna Kolbert (2016) Disturbance in RNS or ascorbate metabolism affects protein tyrosine nitration in *Arabidopsis*

6th Plant Nitric Oxide International Meeting. 78 p. Granada, Spanyolország, 2016 szeptember 14-16.

6. **Molnár Árpád**, Feigl Gábor, Szöllősi Réka, Erdei László, Kolbert Zsuzsanna (2017) Fehérje tirozin nitráció, mint a növényi szelénérzékenység markere

Magyar Növénybiológiai Társaság Fiatal Növénybiológusok Előadássorozata, Debrecen, Magyarország, 2017. június 9.

7. **Molnár Árpád**, Feigl Gábor, Ördög Attila, Szöllősi Réka, Erdei László, Kolbert Zsuzsanna (2017) A fehérje nitráció szerepe a növényi szelén-érzékenységben

12th Congress of the Hungarian Society of Plant Biology, Szeged, Hungary, 30. August-1. September 2017

8. **Molnár Árpád** (2018) A szelén fitotoxicitás háttér folyamatai különböző szeléntoleranciájú növényekben
XXI. Tavasz Szél Konferencia, Győr, Magyarország, 2018. május 4-6.
9. Zsuzsanna Kolbert, **Árpád Molnár**, Dóra Oláh, Attila Ördög, Eva Rudolf, Christian Lindermayr, Gábor Feigl (2018) Exogenous zinc modifies reactive nitrogen species metabolism in *Arabidopsis*
7th Plant Nitric Oxide International Meeting, Nice, France, 24-26 October 2018
10. **Molnár Árpád** (2019) Cink oxid nanopartikulumok hatása keresztesvirágú növényekre
XXII. Tavasz Szél Konferencia, Debrecen, Magyarország, 2018. május 3-5.
11. Feigl Gábor, **Molnár Árpád**, Oláh Dóra, Kolbert Zsuzsanna (2019) A fehérje tirozin nitráció mint az elemtöbblet által indukált nitro-oxidatív stressz indikátora növényekben
Magyar Szabadgyök-Kutató Társaság X. Kongresszusa, Szeged, Magyarország, 2019. augusztus 29-30.
12. **Molnár Árpád**, Feigl Gábor, Papp Márk, Béteky Péter, Rónavári Andrea, Kolbert Zsuzsanna (2019) Cink-oxid nanopartikulum-indukált nitro-oxidatív stressz Brassica növényekben
Magyar Szabadgyök-Kutató Társaság X. Kongresszusa, Szeged, Magyarország, 2019. augusztus 29-30.

Poster presentations:

1. Feigl G, Pető A, Lehotai N, **Molnár Á**, Erdei L, Kolbert Zs (2013) Comparison of the effect of copper and zinc in Brassica juncea and Brassica napus roots: Microelement homeostasis, metabolism of reactive signal molecules and morphological adaptation.
Biomedica Miniconference, 13 December 2013, Szeged, Hungary.
2. Feigl Gábor, Lehotai Nóra, **Molnár Árpád**, Erdei László, Kolbert Zsuzsanna (2014) Detection of protein tyrosine nitration in zinc-treated Brassica plants.
11th Congress of the Hungarian Society of Plant Biology, 27-29. August 2014, Szeged, Hungary
3. **Molnár Árpád**, Feigl Gábor, Lehotai Nóra, Erdei László, Kolbert Zsuzsanna (2014) Microscopic study of zinc localization in Brassica roots.
11th Congress of the Hungarian Society of Plant Biology, 27-29. August 2014, Szeged, Hungary
4. **Molnár Árpád**, Trifán Vanda, Feigl Gábor, Erdei László, Kolbert Zsuzsanna (2016) Selenite treatment induces nitro-oxidative stress and decreases viability in indian mustard
22nd International Symposium on Analytical and Environmental Problems, Szeged, Hungary 2016. október 10.
5. Zsuzsanna Kolbert, **Árpád Molnár**, Gábor Feigl (2017) Selenium-influenced root growth: the involvement of ethylene
VISCEA International Conference: Plant Nutrition, Growth & Environment Interactions III., Bécs, Ausztria, 2017. február.20-21.
6. **Molnár Árpád**, Szöllősi Réka, Ördög Attila, Feigl Gábor, Kolbert Zsuzsanna (2017) In vivo fehérje tirozin nitráció szelén-kezelt Astragalus fajokban.
XV. Magyar Növényanatómiai Szimpózium, Budapest, Magyarország, 2017. szeptember 7.
7. Szöllősi Réka, Ördög Attila, **Molnár Árpád**, Feigl Gábor, Kolbert Zsuzsanna (2017) Anatómiai változások szelén-kezelt Astragalus fajok gyökerében.
XV. Magyar Növényanatómiai Szimpózium, Budapest, Magyarország, 2017. szeptember 7.
8. Feigl Gábor, **Molnár Árpád**, Krajner Jolán, Töröcsik Kitti, Kolbert Zsuzsanna (2017) Adaptation or inhibition – a rhizotron study on zinc-affected root growth of Brassica napus L.
12th Congress of the Hungarian Society of Plant Biology, Szeged, Hungary, 30. August-1. September 2017
9. Kolbert Zsuzsanna, **Molnár Árpád**, Poór Péter, Szöllősi Réka, Feigl Gábor (2017) The involvement of ethylene, stigolactones and nitric oxide in selenium-induced root morphogenic response (SIMR)
12th Congress of the Hungarian Society of Plant Biology, Szeged, Hungary, 30. August-1. September 2017

10. Véseiné Szöllősi Réka, Ördög Attila, **Molnár Árpád**, Feigl Gábor, Kolbert Zsuzsanna (2017) Szelén-kezelés okozta anatómiai változások *Astragalus* fajok gyökerében
12th Congress of the Hungarian Society of Plant Biology, Szeged, Hungary, 30. August-1. September 2017
11. Gábor Feigl, Nóra Lehotai, **Árpád Molnár**, Ádám Bordé, Zsuzsanna Kolbert (2017) Excess element-induced protein tyrosine nitration as the marker of nitrosative stress in plants
8th International Symposium on Root Development, Umeå, Svédország, 29. May-01. June 2017
12. Gábor Feigl, **Árpád Molnár**, Jolán Krajner, Dóra Oláh, Zsuzsanna Kolbert (2018) The effect of different vertical zinc distribution on the early root development and zinc accumulation of *Brassica napus*
24th International Symposium on Analytical and Environmental Problems, Szeged, Hungary
October 8-9, 2018
13. **Árpád Molnár**, Gábor Feigl, Réka Szöllősi, Dóra Oláh, Attila Ördög, Zsuzsanna Kolbert (2018) The background processes of selenium-induced protein tyrosine nitration in *Astragalus* roots
7th Plant Nitric Oxide International Meeting, Nice, France, 24-26 October 2018
14. Gábor Feigl, **Árpád Molnár**, Réka Szöllősi, Kitti Törőcsik, Dóra Oláh, Zsuzsanna Kolbert (2018) Distinct nitrosative response behind zinc-induced root architectural changes in *Brassica napus* L.
7th Plant Nitric Oxide International Meeting, Nice, France, 24-26 October 2018
13. **Árpád Molnár**, Gábor Feigl, Márk Papp, Dóra Oláh, Zsuzsanna Kolbert (2019) THE EFFECT OF ZINC OXIDE NANOPARTICLES ON ROS AND RNS METABOLISM OF BRASSICA ROOTS
14th International Conference on Reactive Oxygen and Nitrogen Species in Plants, München, Németország
2019. július 10-12.
14. Dóra Oláh, **Árpád Molnár**, Réka Szöllősi, Gábor Feigl, Zsuzsanna Kolbert (2019) NICKEL-INDUCED ROS AND RNS IMBALANCE IN BRASSICACEAE
14th International Conference on Reactive Oxygen and Nitrogen Species in Plants, München, Németország
2019. július 10-12.
15. Gábor Feigl, **Árpád Molnár**, Dóra Oláh, Ádám Czifra, Zsuzsanna Kolbert (2019) COMBINED HEAVY METAL TREATMENT AFFECTS NITRO-OXIDATIVE STATUS OF RAPESEED AND SUNFLOWER ROOTS DIFFERENTLY
14th International Conference on Reactive Oxygen and Nitrogen Species in Plants, München, Németország
2019. július 10-12.
16. Zsuzsanna Kolbert, **Árpád Molnár**, Dalma Ménesi, Ildikó Valkai, Taras Pasternak, Attila Fehér (2019) SIGNAL INTERACTION BETWEEN NITRIC OXIDE (NO) AND RHO GTPASES DURING ROOT GROWTH
14th International Conference on Reactive Oxygen and Nitrogen Species in Plants, München, Németország
2019. július 10-12.
17. Varga Viktória, **Molnár Árpád**, Kolbert Zsuzsanna, Feigl Gábor (2019) Két *Alyssum lesbiacum* populáció nikkelle adott válaszainak összehasonlítása nitrozatív paraméterek felhasználásával
Magyar Szabadgyök-Kutató Társaság X. Kongresszusa, Szeged, Magyarország, 2019. augusztus 29-30.

Statement

As the author of the following scientific publications I certify, that Árpád Molnár predoctoral student contributed significantly in the creation of these articles and the data evaluated in his dissertation are not used in other Ph.D. dissertations.

Árpád Molnár, Gábor Feigl, Vanda Trifán, Attila Ördög, Réka Szöllősi, László Erdei, Zsuzsanna Kolbert (2018) The intensity of tyrosine nitration is associated with selenite and selenate toxicity in *Brassica juncea* L.

Ecotoxicology and Environmental Safety, 147: 93–101, IF: 4,527

Szeged, 4. August 2020



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Árpád Molnár, Zsuzsanna Kolbert, Krisztina Kéri, Gábor Feigl, Attila Ördög, Réka Szöllősi, László Erdei (2018) Selenite-induced nitro-oxidative stress processes in *Arabidopsis thaliana* and *Brassica juncea*

Ecotoxicology and Environmental Safety, 148: 664–674, IF: 4,527

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Zsuzsanna Kolbert, **Árpád Molnár**, Réka Szöllősi, Gábor Feigl, László Erdei, Attila Ördög (2018) Nitro-Oxidative Stress Correlates with Se Tolerance of *Astragalus* Species
Plant and Cell Physiology, pcy099, 59 : 9 pp. 1827-1843, IF: 3.929

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