Abstract of Ph.D. thesis

INVESTIGATIONS ON OXIDATIVE STRESS CAUSED BY HEAVY METAL TREATMENTS IN GERMINATING SEEDS OF INDIAN MUSTARD (*BRASSICA JUNCEA* L.)

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Szeged

2012

INTRODUCTION

It has been reported in several studies that heavy metals because of their common presence in soil-water-plant- animal- human system may strongly affect development, growth, basic metabolisms etc. of living organisms. Essential heavy metals like copper (Cu) or zinc (Zn) which are often cofactors of various enzymes such as oxidases or components of photosynthetic apparatus, also might act as pollutants at higher concentrations in the environment due to industrial, municipial or agricultural activities. It is also well-known that not only non-essential (like cadmium, Cd) but essential heavy metals, mainly at higher levels, usually cause overproduction of reactive oxygen species (ROS) resulting in oxidative stress in plants, and consequently cell wall and membrane damage, lipid peroxidation or disorders in various metabolic processes and visible morphological and/or histological alterations. Numerous plants are not only capable of surviving and taking up Cd but can also allocate it to different plant parts, generally to the shoot, moreover can tolerate and accumulate this metal, such as Indian mustard (*Brassica juncea* L., Brassicaceae). These plants are called 'hyperaccumulators' because of amassing metals (e.g. cadmium) at levels even 100-fold higher than it is measured in nonaccumulator plants. Yet, most of the experiments so far have been concentrating on oxidative stress only in adult plants (including Indian mustard) after heavy metal stress, and there are not much data about the effects in the early stages of ontogenesis in *Brassica juncea* L.

exposed to heavy metal stress, since germination is a very sensitive period of development, effects of heavy metal stress are more expressed and visual than later.

AIMS

The aims of our study were to investigate potential oxidative stress and antioxidative defense mechanisms in germinating seeds of Indian mustard exposed to copper (Cu), zinc (Zn) and cadmium (Cd) excess. Although, this species is generally known to be cadmium-tolerant and hyperaccumulator, we wanted know whether copper or zinc as essential heavy metals can cause similar changes like cadmium which is basicly toxic for the plants.
In my thesis I present the results which intend to answer the following questions:

- Does heavy metal (Cu, Zn or Cd) treatment cause oxidative stress during germination?
- Which biochemical parameters are the most available to decribe this oxidative stress? Which parameters could be good indicators?
- How can duration and heavy metal concentration influence oxidative stress? How are the parameters affected by duration and concentration?
- How can oxidative stress be proved and visualized histochemically?

- What morphological and/or histological alterations do occur in the root tips after heavy metal exposure?

MATERIALS AND METHODS

Seeds of Indian mustard were germinated at 0, 50, 100, 200 mg L^{-1} Cu, Zn and Cd concentrations, in dark for 12, 24, 48 and 96 h, at 24±1°C. The real metal content in seeds was determined by AAS. For biochemical measurements 8 replicas of fresh material were homogenised and the supernatant was used for all assays.

The following parameters were evaluated: FRAP (ferric reducing ability of plasma), lipid peroxidation (LP), reduced glutathione content (GSH), total protein content and the activity of glutathione-S-transferase (GST), superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPOX) and glutathione reductase (GR). Moreover, we assessed the loss of plasma membrane integrity in the root tips *in vivo* using Trypan blue, Aniline blue was applied for callose staining, Schiff's reagent was used for the detection of lipid peroxidation, and phloroglucinol-HCl for visualization of the lignification of cell walls.

We also made cross sections of the root tips to investigate the potential alterations of the tissues caused by heavy metal stress.

RESULTS

Our study provides the following results in this topic:

1. Oxidative stress occured in the seeds due to heavy metal treatments, and all parameters were significantly affected by duration and metal concentration used. Increase of duration and/or metal concentration resulted in higher metal uptake in germinating seeds of Brassica juncea L.

2. FRAP showed to be a good and quick semi-quantitative parameter to prove that oxidative stress caused by heavy metals is followed by the quick activation of antioxidant defence system including e.g. ascorbic acid and phenolic components. Similarly, both essential heavy metals (Cu and Zn) and toxic Cd treatments were followed by GSH-depletion which was probably due to the increased activity of Halliwell-Asada cycle and the elavated synthesis of phytochelatins (PC).

3. The level of lipid peroxidation (LP) determined as malondialdehyde (MDA) content showed time- and dose-dependence. Generally, lipid peroxidation was higher at the beginning of germination at all concentrations, and then attenuated. Significant differences were observed between the control and the treated seeds after each period. We could detect LP histochemically using Schiff's reagent and LP seemed to be the highest in Cd-treated root tips. The reduction of LP in the second period of the

germination was probably due to the activation of antioxidants and the elimination of lipid peroxides.

4. The application of Cu and Zn increased the activity of SOD in time and dose-dependent manner since both metals are cofactors of cytoplasmatic Cu/Zn SOD. Adversely, Cd inhibited SOD activity after 48-96h treatment.

5. The activity of CAT and GPOX increased after 48-96h Cu and Zn excess while the activity of CAT, GPOX and GR decreased as a consequence of Cd exposure supporting that Cd induces oxidative stress indirectly in plant cells.

6. LP as one the markers of oxidative stress was detected after staining with Schiff's reagent in root tips treated by both essential heavy metals though morphological symptoms of metal toxicity occurred only in Cu-stressed plants (stunted, hooked-formed and brownish root tips). In the plants exposed to Cd LP occurred already after 12h in the meristematic zone and later in older differentiated regions but the intensity of Schiff-staining reduced due to probable scavenging of lipid peroxides. Production of callose could be visualized using Anilin blue while lignification of cell walls was detected by phloroglucinol-HCl, mainly in Cu-treated root tips.

7. Alterations of the cells and the tissues due to abnormal cell division and cell elongation occurred mainly in the cross sections of

Cu-stressed root tips after 48-96h and not after Zn or Cd excess. Production of callose was usually detected in the external tangential walls of rhizodermis cells while lignification occurred not only in rhizodermis but in hypodermis, as well, which can be an early adaptation of plant cells against heavy metal stress.

LIST OF PUBLICATIONS

Present thesis is based on the following articles:

Articles published in scientific journals:

1. Ilona Sz. Varga, **Réka Szőllősi**, Mária Bagyánszki (2000). Estimation of total antioxidant power in medicinal plants (adaptation of FRAP method). Current Topics in Biophysics 24:219-225.

2. **R. Szőllősi**, I. Varga-Szőllősi (2002). Total antioxidant power in some species of Labiatae (Adaptation of FRAP method), Acta Biologica Szegediensis 46: 125–127.

3. **Szőllősi R**, Varga I Sz, Erdei L, Mihalik E (2009). Cadmium-induced oxidative stress and antioxidative mechanisms in germinating Indian mustard (*Brassica juncea* L.) seeds. Ecotoxicology and Environmental Safety 72, 1337-1342. **IF: 2,133**

4. **Réka Szőllősi**, Erika Kálmán, Anna Medvegy, Andrea Pető, Sz. Ilona Varga (2011). Studies on oxidative stress caused by Cu and Zn excess in germinating seeds of Indian mustard (*Brassica juncea* L.) Acta Biologica Szegediensis 55:175-178.

Study published in a book:

Szollosi, R. (2011). Indian Mustard (*Brassica juncea* L.) seeds in health. In V. R. Preedy, R. R. Watson, V. B. Patel (Editors), Nuts & Seeds in Health and Disease Prevention (1st ed.) (pp. 671-676).

London, Burlington, San Diego: Academic Press is an imprint of Elsevier. ISBN: 9780123756886

Conference abstracts published in scientific journals:

R. Szőllősi, I. Varga Sz., E. Mihalik, L. Erdei (2006). Investigations on oxidative stress caused by Cd treatment in germinating *Brassica juncea* L. seeds. Poszter, XIIIth Biennial Meeting of the SFRRI. Davos, Switzerland, August 15-19, 2006. Free Rad Res (2006) 40, Suppl. 1. p. 98. **IF: 2,536**

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1. Ilona Sz. Varga, **Réka Szőllősi**, Mária Bagyánszki (2000). Estimation of total antioxidant power in medicinal plants (adaptation of FRAP method). Előadás, 5th Symposium (SFRR)- Free Radicals in Biology and Medicine, Łódź, 7-10 June

2. **R. Szőllősi**, E. Mihalik (2005). Estimation of Cu, Zn phytoextraction by weeds grown on contaminated sites. Poszter, XVIIth International Botanical Congress, Vienna, 17-23 July 2005

3. **Szőllősi R**., Sz. Varga I., Mihalik E., Erdei L (2005). Cd- kezelés hatására bekövetkező oxidatív stressz vizsgálata *Brassica juncea* L. magvakban. Poszter, 2005. szeptember 26. The 12th Symposium on Analytical and Environmental Problems. Szeged

4. **Szőllősi R.**, Sz. Varga I., Erdei L., Mihalik E. (2005). Oxidatív stressz és antioxidáns paraméterek vizsgálata Cd- kezelt *Brassica juncea* magvakban. Előadás, Magyar Szabadgyök-kutató Társaság III. Konferenciája, Debrecen, 2005. október 13-15.

5. **R. Szőllősi**, I. Varga Sz, E. Mihalik, L. Erdei (2006). Preliminary Investigations of Oxidative Stress in Cd Treated Germinating Indian Mustard (*Brassica Juncea* L.) Seeds. Poszter, International Symposium on Trace Elements in the Food Chain (TEFC). Budapest, Hungary, May 25-27, 2006

6. **Szőllősi R**., Mihalik E. (2006). Effects of cadmium on floral morphological traits of Indian Mustard (*Brassica juncea* L.) – Preliminary investigations. Poszter, THE 13th SYMPOSIUM ON ANALYTICAL AND ENVIRONMENTAL PROBLEMS. Szeged, Hungary, 25 September 2006

7. **Szőllősi R**, Kálmán E, Sz. Varga I, Medvegy A, Erdei L, Mihalik E (2010). A Cu-kezelés hatásai az indiai mustár (*Brassica juncea* L.) egyedfejlődésének korai szakaszában. Előadás- SZABAD GYÖKÖK ÉS MIKROELEMEK - miniszimpózium, 2010. szept. 17. Budapest, MTA Kémiai Kutatóközpont)

8. **Szőllősi R**, Kálmán E, Sz. Varga I, Medvegy A, Pető A, Erdei L (2010) The influence of copper excess on early development, lipid peroxidation and antioxidative system in germinating Indian mustard (*Brassica juncea* L.) seeds. Poszter, ISIRR, 2010. okt. 13-15. Szeged

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Articles published in scientific journals:

1. Then, M., Vásárhelyi-Perédi, K., **Szőllősi, R**., Szentmihályi, K. (2004). Polyphenol-, Mineral Element Content and Total Antioxidant Power Of Sage (*Salvia officinalis* L.) Extracts. Acta Horticulturae ISHS, 629: 123-129.

2. **Réka Szőllősi**, Anna Medvegy, Anikó Németh, Katalin Kálmán, Erzsébet Mihalik (2010). Intra-inflorescence variations in floral morphological and reproductive traits of *Iris sibirica* L. Acta Biologica Szegediensis 54:103-110.

3. Feigl, G., **Szollosi, R**., Mihalik, E. (2010). Studies on established *Acorus calamus* (L.) populations. Acta Biologica Szegediensis 54: 99-101.

4. **R. Szőllősi**, A. Medvegy, E. Benyes, A. Németh, E. Mihalik (2011). Flowering phenology, floral display and reproductive success of *Iris sibirica*. Acta Botanica Hungarica 53: 409-422.

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6. Kolbert Zs, Pető A, **Szőllősi R,** Erdei L, Tari I (2011). Nitric oxide (NO) generation during vegetative/generative transition of the apical meristem in wheat. Acta Biologica Szegediensis 55:95-97.

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1. Then, M., **Szőllősi, R.,** Szentmihályi, K. (2002). Polyphenol-, Mineral Element Content and Total Antioxidant Power Of Sage (Salvia officinalis L.) Extracts. Poszter, XXVI International Horticultural Congress: The Future for Medicinal and Aromatic Plants, August 11-17, Toronto, Canada

2. **Szőllősi, R.,** Mihalik, E. (2004). Asteraceae-fajok nehézfém-felvételének összehasonlító vizsgálata. Poszter, Aktuális flóra- és vegetációkutatás a Kárpát-medencében VI. konferencia összefoglalói. Keszthely, 2004. febr. 26-29. p. 114.

3. Mihalik E., Németh A., **Szőllősi R**., Medvegy A., Kálmán K. (2005). Ex situ *Adonis vernalis* populáció pollen életképesség és pollenszám diverzitása. Előadás, Lippay-Ormos-Vas Tudományos Ülésszak, Budapest. 2005. október 19-20.

4. Mihalik E, Németh A, **Szőllősi R,** Medvegy A, Kálmán K (2006). A morfológiai bélyegek és a reproduktív kapacitás virágzaton belüli variabilitása egy telepített *Iris sibirica* populációban. Poszter, Az Aktuális flóra- és vegetációkutatás a Kárpát-medencében VII. c. konferencia összefoglalói. Kitaibelia 11, 66.

5. Mihalik E, Németh A, **Szőllősi R**, Medvegy A, Kálmán K, Radvánszky A (2006). Védett növényfajok ex situ populációinak hosszú távú fenntartása. Az Aktuális flóra- és vegetációkutatás a Kárpát-medencében VII. konferencia összefoglalói. Kitaibelia 11, 33.

6. **Szőllősi, R.,** Mihalik, E. (2006). Preliminary investigations of pollen viability in Zn treated Indian mustard (*Brassica juncea* L.). Poszter, XIXth International Congress on Sexual Plant Reproduction. Budapest, Hungary, July 11-15, 2006.

7. **Szőllősi Réka**, Mihalik Erzsébet (2007). Pollenprodukció- és fertilitás vizsgálata kadmiummal kezelt indiai mustár (*Brassica juncea* L.) növényeknél. Poszter, 10. Magyar Magnézium Szimpózium, 2007. április 12-13. Szeged, Hungary

8. **Szőllősi R**, Varga Sz I, Mihalik E (2008). Egy „elfelejtett" gyógynövény, az orvosi kálmos (*Acorus calamus* L.) mint antioxidáns-forrás. MSZKT és MTA MIKROELEM MUNKABIZOTTSÁG munkaértekezlete, Előadás, 2008. szeptember 26., Budapest, MSD Centrum

9. **Szőllősi R**, Medvegy A, Mihalik E (2008). Virágbiológiai vizsgálatok az *Iris sibirica* L. egy telepített populációjában. "Molekuláktól a globális folyamatokig" - V. Magyar Természetvédelmi Biológiai Konferencia, Program és absztrakt-kötet: 145. (2008. november 6-9. Nyíregyháza, poszter).

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12. Medvegy A, **Szőllősi R,** Mihalik E (2009). Florális szex allokáció időbeli variációja a protogyniás *Adonis vernalis* L. populációjában. (Poszter, 8. Magyar Ökológus Kongresszus, Szeged, 2009. augusztus 26-28. p. 148.)

13. Németh A, Makra O, Mihalik E, **Szőllősi R** (2009). Studies on temporal changes in several reproductive traits in an ex situ

population of *Dianthus diutinus* Kit. (Poszter, 2nd European Congress of Conservation Biology, Book of Abstracts, Prague, 2009. szept. 1-5., p.196.)

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